NOTICE OF RETRACTION

The following two articles previously published in the Journal have been retracted because the authors failed to obtain ethics approval required for the studies and made false statements in their submission documents and in the manuscripts indicating that ethics reviews and approvals were obtained.


EDITORIALS

Comment on notice of retraction
The Editors

Being mothers too early
Ricardo Gomez, Joaquin Santolaya
Puente Alto, Chile, Bethesda, Md, and Detroit, Mich

EDITORS’ CHOICE

Maternal-perinatal morbidity and mortality associated with adolescent pregnancy in Latin America: Cross-sectional study
Agustin Conde-Agudelo, MD, MPH, José M. Belizán, MD, PhD,
Cristina Lammers, MD, MPH
Cali, Colombia, and Montevideo, Uruguay

In Latin America, adolescent pregnancy is independently associated with increased risks of adverse maternal and perinatal outcomes.

Commentary
This landmark paper from South America compares maternal and neonatal outcomes for 344,626 pregnant adolescents, including 33,498 who were <15 years of age to outcomes in 509,751 women aged 20 to 24. See the accompanying editorial by Dr Ricardo Gomez, p 340.
Cervicovaginal fibronectin improves the prediction of preterm delivery based on sonographic cervical length in patients with preterm uterine contractions and intact membranes

Ricardo Gomez, MD, Roberto Romero, MD, Luis Medina, MD, Jyh Kae Nien, MD, Tinnakorn Chaiworapongs, MD, Mario Carstens, MD, Rogelio González, MD, Jimmy Espinoza, MD, Jay D. Iams, MD, Sam Edwin, PhD, Iván Rojas, MD
Puente Alto, Chile, Bethesda, Md, Detroit, Mich, and Columbus, Ohio

The prediction of preterm delivery in patients with preterm uterine contractions is improved by adding vaginal fetal fibronectin determinations to the sonographic measurements of the cervical length.

Commentary
This article by Romero and colleagues addresses the value of measuring cervical length by trans-vaginal ultrasound and the detection of fetal fibronectin from the cervix in patients with preterm uterine contractions. Both tests are known to have high negative predictive value but weak positive predictive value. This new study demonstrates that this latter limitation is substantially improved by the combination of the two tests. Subsequent studies would be useful to address the next question, the possibility that using the tests in sequence, and only performing the second test if the first is positive, would be a potentially practical way to utilize the information provided in this study.

Plurality-dependent risk of respiratory distress syndrome among very-low-birth-weight infants and antepartum corticosteroid treatment

Isaac Blickstein, MD, Eric S. Shinwell, MD, Ayala Lusky, MSc, Brian Reichman, MBChB, in collaboration with the Israel Neonatal Network Jerusalem, Tel Hashomer, and Tel Aviv, Israel

Preterm (24-32 weeks of gestation) very-low-birth-weight twins and triplets have an excess risk of respiratory distress syndrome, despite complete antenatal corticosteroid therapy.

Commentary
Though antenatal corticosteroids are recommended for preterm delivery in multifetal gestation, their effectiveness in these patients remains a topic of discussion. This paper by Blickstein et al adds to previous discussions of the effectiveness of antenatal steroids in twins in AJOG. See Murphy DJ, et al. Cohort study of the neonatal outcome of twin pregnancies that were treated with prophylactic or rescue antenatal corticosteroids. Am J Obstet Gynecol 2002;187:483-8; and Jobe AH. Antenatal steroids in twins. Am J Obstet Gynecol 2003;188:856.

CLINICAL OPINION

Estimation of iatrogenic monozygotic twinning rate following assisted reproduction: Pitfalls and caveats

Isaac Blickstein, MD Jerusalem, Israel

Zygotic splitting following assisted reproduction is underestimated, mainly because dichorionic monozygotic twins, embryonic/fetal deaths, and higher-order monozygotic pregnancies are not counted.
REVIEW ARTICLE

Asthma controller therapy during pregnancy
Joan C. Gluck, MD, Paul A. Gluck, MD
Miami, Fla

The safety and efficacy of asthma controller medications in pregnancy and Food and Drug Administration pregnancy ratings for controller medications are reviewed.

GENERAL OBSTETRICS AND GYNECOLOGY: GYNECOLOGY

Pregnancy outcome after the use of an aromatase inhibitor for ovarian stimulation
Mohamed F. Mitwally, MD, Marinko M. Biljan, MD, Robert F. Casper, MD
Toronto, Ontario, Canada, Detroit, Mich, and Montreal, Quebec, Canada

Pregnancies achieved after the use of an aromatase inhibitor for ovarian stimulation are associated with favorable outcomes, particularly a lower risk for a multiple gestation.

Estrogen therapy and risk of cognitive decline: Results from the Women’s Estrogen for Stroke Trial (WEST)
Catherine M. Viscoli, PhD, Lawrence M. Brass, MD, Walter N. Kernan, MD, Philip M. Sarrel, MD, Samy Suissa, PhD, Ralph I. Horwitz, MD
New Haven, Conn, Montreal, Canada, and Cleveland, Ohio

A randomized trial for secondary prevention of stroke fails to find a benefit for estradiol therapy in reducing the risk of cognitive decline in elderly women.

Feasibility and clinical outcome of laparoscopic colorectal resection for endometriosis
Emile Darai, MD, PhD, Isabelle Thomassin, MD, Emmanuel Barranger, MD, Romain Decheves, MD, Annie Cortez, MD, Sydney Houry, MD, Marc Bazot, MD
Paris, France

Laparoscopic segmental colorectal resection for endometriosis is feasible and significantly improves gynecologic and digestive symptoms. However, women must be informed of the risk of de novo urinary and digestive adverse effects and rectovaginal fistula when vaginal resection is required.

Ultrasonography and color Doppler-based triage for adnexal masses to provide the most appropriate surgical approach
Stefano Guerriero, MD, Silvia Ajossa, MD, Nicoletta Garau, MD, Bruno Piras, MD, Anna Maria Paoletti, MD, Gian Benedetto Melis, MD
Cagliari, Italy

The evaluation of vessel distribution by color Doppler permits, safely, to treat by laparoscopy more than 90% of benign masses.

Population characteristics in cervical cancer trials: Search for external validity
Annie Yessaian, MD, Alberto A. Mendivil, MD, Wendy R. Brewster, MD, PhD
Irvine, Calif

The results of cooperative group trials in cervical cancer may be generalized to the disease population in the United States without being significantly confounded by age or ethnic distribution.
Current cervical neoplasia screening practices of obstetrician/gynecologists in the US  
Mona Saint, MD, MPH, Ginny Gildengorin, PhD, George F. Sawaya, MD  
San Francisco, Calif  
Most US obstetrician/gynecologists screen women at low risk for cervical neoplasia often and indefinitely despite national guidelines designed to minimize screening harms caused by overtesting.

A randomized trial that compared povidone iodine and chlorhexidine as antiseptics for vaginal hysterectomy  
Patrick J. Culligan, MD, Kari Kubik, MD, Miles Murphy, MD, MSPH, Linda Blackwell, RN, BSN, James Snyder, PhD  
Louisville, Ky  
Chlorhexidine gluconate is more effective than povidone iodine in decreasing the bacterial colony counts that are found in the operative field during vaginal hysterectomy.

The expression and function of the endothelin system in contractile properties of vaginal myofibroblasts of women with uterovaginal prolapse  
Sébastien Poncet, MSc, Sylvain Meyer, MD, Christelle Richard, BSc, John-David Aubert, MD, Lucienne Juillerat-Jeanneret, PhD  
Lausanne, Switzerland  
The endothelin system is expressed in contractile myofibroblasts of women with uterovaginal prolapse but decreases their contractile properties, which is contrary to skin myofibroblasts.

GENERAL OBSTETRICS AND GYNECOLOGY: OBSTETRICS  
The combined maternal administration of magnesium sulfate and aminophylline reduces intraventricular hemorrhage in very preterm neonates  
Gian Carlo Di Renzo, MD, PhD, Marcela Mignosa, MD, Sandro Gerli, MD, Liliana Burnelli, MD, Giuseppe Luzi, MD, Graziano Clerici, MD, Fabiana Taddei, MD, Doretra Marinelli, MD, Patrizia Bragetti, MD, Daniele Mezzetti, MD, Benedetta Della Torre, MD, Alessandra Fantauzzi, MD, Maria Serena Lungarotti, MD, PhD  
Perugia, Italy  
Maternal administration of magnesium sulfate and aminophylline was associated with a significant decrease in the rate of intraventricular hemorrhage in very preterm neonates (less than 30 weeks).

Delayed interval delivery in twin pregnancies in the United States: Impact on perinatal mortality and morbidity  
Yinka Oyelese, MD, Cande V. Ananth, PhD, MPH, John C. Smulian, MD, MPH, Anthony M. Vintzileos, MD  
New Brunswick, NJ  
Delayed delivery of a second twin was associated with improved outcomes when the first twin was delivered at 22 to 23 weeks and the delivery interval was ≥1 week and ≥3 weeks.
A randomized comparison of oral mifepristone to intravenous oxytocin for labor induction in women with prelabor rupture of membranes beyond 36 weeks' gestation

Deborah A. Wing, MD, Cristiane Guberman, MD, Michael Fassett, MD
Los Angeles, Calif

Oral mifepristone administration did not improve labor stimulation in women with prelabor rupture of membranes near term and was associated with more adverse fetal outcomes than oxytocin infusion.

Severe fetal placental vascular lesions in term infants with neurologic impairment

Raymond W. Redline, MD
Cleveland, Ohio

Severe fetal vascular lesions were identified in more than one half of placentas from neurologically impaired term infants who are referred for medicolegal consultation.

Progesterone enhances the tocolytic effect of ritodrine in isolated pregnant human myometrium

Boonsri Chanrachakul, MD, Fiona Broughton Pipkin, DPhil, Averil Y. Warren, MPhil, Sabaratnam Arulkumaran, MD, PhD, Raheela N. Khan, PhD
Derby, Nottingham, and London, United Kingdom

Natural progesterone enhances the relaxant effect of ritodrine in pregnant human myometrium, most likely through nongenomic mechanisms.


Anthony M. Vintzileos, MD, Cande V. Ananth, PhD, MPH, Eftichia Kontopoulos, MD, John C. Smulian, MD, MPH
New Brunswick, NJ

In the United States, cesarean delivery is associated with the lowest neonatal and infant mortality rates among triplet births.

Is bacterial vaginosis a stronger risk factor for preterm birth when it is diagnosed earlier in gestation?

Mark A. Klebanoff, MD, MPH, Sharon L. Hillier, PhD, Robert P. Nugent, PhD, Cora A. MacPherson, PhD, John C. Hauth, MD, J. Christopher Carey, MD, Margaret Harper, MD, MS, Ronald J. Wagner, MD, Wayne Trout, MD, Atef Moawad, MD, Kenneth J. Leveno, MD, Menachem Miodovnik, MD, Baha M. Sibai, MD, J. Peter VanDorsten, MD, Mitchell P. Dombrowski, MD, Mary J. O’Sullivan, MD, Michael Varner, MD, Oded Langer, MD, and the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network
Bethesda, Md, Pittsburgh, Pa, Rockville Md, Birmingham, Ala, Oklahoma City, Okla, Winston-Salem, NC, Philadelphia, Pa, Columbus, Ohio, Chicago, Ill, Dallas, Tex, Cincinnati, Ohio, Memphis, Tenn, Charleston, SC, Detroit, Mich, Miami, Fla, Salt Lake City, Utah, and San Antonio, Tex

Among 12,937 pregnant women who were screened for bacterial vaginosis, earlier gestational age at screening and diagnosis was not associated with an increased relative risk of preterm birth.

Contents continued on page 8A
Antenatal maternal administration of phenobarbital for the prevention of exchange transfusion in neonates with hemolytic disease of the fetus and newborn

Thomas N. Trevett, Jr, MD, Karen Dorman, RN, MS, Georgine Lamvu, MD, MPH, Kenneth J. Moise, Jr, MD
Chapel Hill, NC

Maternal oral administration of phenobarbital in pregnancies complicated by hemolytic disease of the fetus and newborn infant significantly decreased the need for neonatal exchange transfusion.

The cost-effectiveness of routine antenatal screening for maternal herpes simplex virus–1 and –2 antibodies

Stephen F. Thung, MD, William A. Grobman, MD, MBA
Chicago, Ill

Routine antenatal herpes simplex virus type 1 and 2 antibody screening to reduce the incidence of neonatal herpes infections is not cost-effective.

Combination of vaginal pH with vaginal sialidase and prolidase activities for prediction of low birth weight and preterm birth

Sabina Cauci, PhD, James McGregor, MD, Poul Thorsen, MD, PhD, Jakob Grove, PhD, Secondo Guaschino, MD
Tucson, Ariz, Aarhus, Denmark, and Udine and Trieste, Italy

Increased vaginal pH combined with elevated vaginal sialidase and/or prolidase enzyme activities is predictive for low birth weight and early prematurity.

The relationship between an aortic isthmus blood flow velocity index and the postnatal neurodevelopmental status of fetuses with placental circulatory insufficiency

Jean-Claude Fouron, MD, Julie Gosselin, PhD, Marie-Josée Raboisson, MD, Julie Lamoureux, MSc, Claudine-Amiel Tison, MD, Catherine Fouron, MA, Linda Hudon, MD
Montreal, Quebec, Canada, and Paris, France

In fetuses with abnormal umbilical artery Doppler velocity waveforms, there is a significant negative relationship between aortic isthmus blood flow index and neurodevelopmental outcome.

Maternal nonpregnant vascular function correlates with subsequent fetal growth

Marc E. A. Spaanderman, MD, PhD, Christine Willekes, MD, PhD, Arnold P. G. Hoeks, PhD, Timo H. A. Ekhart, Robert Aardenburg, MD, Dorette A. Courtar, MD, Hugo W. F. van Eijndhoven, MD, Louis L. H. Peeters, MD, PhD
Nijmegen, The Netherlands

In nonpregnant normotensive formerly preeclamptic women, arterial function is associated with subsequent fetal growth.

Markers of periodontal infection and preterm birth

Karim Jarjoura, DMD, MS, Patricia C. Devine, MD, Annette Perez-Delboy, MD, Miriam Herrera-Abreu, MS, Mary D’Alton, MD, Panos N. Papapanou, DDS, PhD
New York, NY

The severity of periodontitis was found to be significantly associated with preterm birth and low birth weight after adjustment for established risk factors.
Vitamin C and E supplementation in women at high risk for preeclampsia: A double-blind, placebo-controlled trial
Dorothy Beazley, Robert Ahokas, Jeffrey Livingston, Mary Griggs, Baha M. Sibai, MD
Boston, Mass, Memphis, Tenn, Roanoke, Va, and Cincinnati, Ohio
A randomized trial of vitamins C and E to prevent preeclampsia that was terminated early revealed that a sample size of more than 1000 subjects is required to study this intervention in high-risk pregnancies.

The onset of postpartum depression: Implications for clinical screening in obstetrical and primary care
Zachary N. Stowe, MD, Amy L. Hostetter, BA, D. Jeffrey Newport, MD, MS, MDiv
Atlanta, Ga
One-third of subjects evaluated for postpartum depression experienced onset outside the recognized 6-week window, suggesting that perinatal depression surveillance guidelines should be revised and expanded.

Implementing prenatal screening for cystic fibrosis in routine obstetric practice
Melissa H. Fries, Col, USAF MC, Michael Bashford, Lt Col, USAF MC, Mark Nunes, MD
Bethesda, Md, Kessler, Miss, and Fairfax, Va
Audio-visual counseling is an effective means to educate patients about genetic screening and does not require a trained genetics professional to administer; partner testing in mobile populations may prove problematic.

Fetal transabdominal anatomy scanning using standard views at 11 to 14 weeks’ gestation
Constantin S. von Kaisenberg, MD, PhD, Heidi Kuhling-von Kaisenberg, MD, Elfriede Fritzer, Sandra Schemm, MD, Ivo Meinhold-Heerlein, MD, Walter Jonat, MD, PhD
Kiel and Gettorf, Germany
The transabdominal visualization of fetal anatomy, using standard planes, simultaneous to the measurement of nuchal translucency, may now be possible in good quality.

Fetal and neonatal alloimmune thrombocytopenia in pregnancies involving in vitro fertilization: A report of four cases
Brian R. Curtis, MS, James B. Bussel, MD, Marilyn J. Manco-Johnson, MD, Richard H. Aster, MD, Janice G. McFarland, MD
Milwaukee, Wis, New York, NY, and Aurora, Colo
Neonatal alloimmune thrombocytopenia can occur in pregnancies achieved with assisted reproductive technology, and prepregnancy typing of surrogate mothers for the platelet antigen HPA-1a should be performed.

Role of Helicobacter pylori infection in iron deficiency during pregnancy
Maria Weyermann, PhD, Dietrich Rothenbacher, MD, Lydia Gayer, Gønter Bode, PhD, Guido Adler, MD, Dieter Grab, MD, Felix Flock, MD, Hermann Brenner, MD
Heidelberg and Ulm, Germany
In a large group of mothers after delivery, we found a possible moderate, but still relevant, independent role of Helicobacter pylori infection in iron deficiency during pregnancy.

Contents continued on page 10A
<table>
<thead>
<tr>
<th>Title</th>
<th>Page</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronic maternal and fetal <em>Porphyromonas gingivalis</em> exposure during pregnancy in rabbits</td>
<td>554</td>
</tr>
<tr>
<td>Kim A. Boggess, MD, Phoebus N. Madianos, DDS, PhD, John S. Preisser, Kenneth J. Moise, Jr, MD, Steven Offenbacher, DDS, PhD Chapel Hill, NC</td>
<td></td>
</tr>
<tr>
<td>In a rabbit model of infection, chronic maternal exposure to the oral pathogen <em>Porphyromonas gingivalis</em> can disseminate to the placenta and fetus.</td>
<td></td>
</tr>
<tr>
<td>Reduced flow-mediated vasodilation is not due to a decrease in production of nitric oxide in preeclampsia</td>
<td>558</td>
</tr>
<tr>
<td>Tamao Yamamoto, MD, Yoshikatsu Suzuki, MD, PhD, Kazuhisa Kojima, MD, PhD, Kaoru Suzumori, MD, PhD Nagoya, Japan</td>
<td></td>
</tr>
<tr>
<td>Reduction of endothelial nitric oxide activity might not be due to a decrease in the production of nitric oxide in preeclampsia.</td>
<td></td>
</tr>
<tr>
<td>Risk factors for <em>Toxoplasma gondii</em> infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening</td>
<td>564</td>
</tr>
<tr>
<td>Kenneth M. Boyer, MD, Ellen Holfels, BS, Nancy Roizen, MD, Charles Swisher, MD, Douglas Mack, PhD, Jack Remington, MD, Shawn Withers, RN, Paul Meier, PhD, Rima McLeod, MD, the Toxoplasmosis Study Group Chicago, Ill, Stanford, Calif, and Syracuse and New York, NY</td>
<td></td>
</tr>
<tr>
<td>Fewer than one half of North American mothers of infants with congenital toxoplasmosis had recognized risk factors or a typical illness that was consistent with acute, acquired toxoplasmosis during gestation.</td>
<td></td>
</tr>
<tr>
<td>Remodeling of myometrial radial arteries in preeclampsia</td>
<td>572</td>
</tr>
<tr>
<td>Stephen S. Ong, MRCOG, Philip N. Baker, DM, Terry M. Mayhew, PhD, William R. Dunn, PhD Nottingham and Manchester, United Kingdom</td>
<td></td>
</tr>
<tr>
<td>In preeclampsia, myometrial radial arteries are altered such that a similar amount of media tissue is remodeled around a smaller lumen compared with normal pregnancy. This reduced vascular diameter could increase uterine vascular resistance in preeclamptic women.</td>
<td></td>
</tr>
<tr>
<td>Prevalence of seat belt use among reproductive-aged women and prenatal counseling to wear seat belts</td>
<td>580</td>
</tr>
<tr>
<td>Laurie F. Beck, MPH, Brenda Colley Gilbert, PhD, Ruth A. Shults, PhD Atlanta, Ga</td>
<td></td>
</tr>
<tr>
<td>Fewer than 50% of pregnant women receive recommended prenatal counseling about the use of seat belts during pregnancy.</td>
<td></td>
</tr>
<tr>
<td>Use of the placental perfusion model to evaluate transplacental passage of <em>Trypanosoma cruzi</em></td>
<td>586</td>
</tr>
<tr>
<td>Stuart H. Shippey, III, MD, Christopher M. Zahn, MD, Margaret M. Cisar, BS, T. John Wu, PhD, Andrew J. Satin, MD Bethesda, Md</td>
<td></td>
</tr>
<tr>
<td>Using a placental perfusion model, transplacental passage of <em>Trypanosoma cruzi</em> is demonstrated as soon as 24 hours after perfusion with a solution containing parasites.</td>
<td></td>
</tr>
</tbody>
</table>

Stephen J. Bacak, MPH, William M. Callaghan, MD, MPH, Patricia M. Dietz, DrPH, Chadd Crouse, MSc
Atlanta, Ga

Pregnancy-associated hospitalizations declined in the United States during the 1990s; however, significant disparities remain among subpopulations.

Resolution of hypertension during pregnancy in familial hyperkalemia and hypertension with the WNK4 Q565E mutation

Haim Mayan, MD, Meir Mouallem, MD, Miriam Shaharabany, PhD, Rachel Pauzner, MD, Zvi Farfel, MD
Tel Hashomer, Israel

In 4 pregnancies of 2 women with familial hyperkalemia and hypertension and Q565E WNK4 mutation, hypertension resolved; however, hyperkalemia and hypercalciuria persisted (documented in 2 pregnancies).

Persistent elevation of cell-free fetal DNA levels in maternal plasma after selective laser coagulation of chorionic plate anastomoses in severe midgestational twin-twin transfusion syndrome

Tuangsit Wataganara, MD, Eduard Gratacos, MD, Jacques Jani, MD, Jorge Becker, MD, Liesbeth Lewi, MD, Lisa M. Sullivan, PhD, Diana W. Bianchi, MD, Jan A. Deprest, MD, PhD
Boston, Mass, Barcelona, Spain, and Leuven, Belgium

Persistent elevation of fetal DNA levels in maternal plasma after laser surgery for severe twin-twin transfusion syndrome supports the placenta as its tissue of origin.

Maternal and fetal amino acid concentrations in normal pregnancies and in pregnancies with gestational diabetes mellitus

Irene Cetin, MD, Maria S. Nobile de Santis, MD, Emanuela Taricco, MD, Tatjana Radaelli, MD, Cecilia Teng, PhD, Stefania Ronzoni, MD, Elena Spada, PhD, Silvano Milani, PhD, Giorgio Pardi, MD
Milano, Italy, and Denver, Colo

Placental exchange and fetal metabolism of amino acids is altered in gestational diabetes mellitus pregnancies with normal fetal weights but lower fetal/placental weight ratios.

Soluble factors released by placental villous tissue: Interleukin-1 is a potential mediator of endothelial dysfunction

Corinne Rusterholz, PhD, Anurag K. Gupta, MSc, Berthold Huppertz, PhD, Wolfgang Holzgreve, MD, Sinuhe Hahn, PhD
Basel, Switzerland, and Aachen, Germany

Interleukin-1 that is released by cultured villous explants from term placentae alters endothelial cell proliferation and induces an endothelial cell inflammatory response.

Effects of vasoactive agents on intracellular calcium and force in myometrial and subcutaneous resistance arteries isolated from preeclamptic, pregnant, and nonpregnant woman

Ruwan C. Wimalasundera, MRCOG, Simon A. McG. Thom, FRCP, Lesley Regan, FRCOG, Alun D. Hughes, PhD
London, United Kingdom

Preeclampsia is associated with a loss of the endothelium-dependent effects of acetylcholine (but not substance P) on intracellular Ca2+ and force, but vasoconstrictor reactivity is not altered.
Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy

Timothy S. Tracy, PhD, Raman Venkataramanan, PhD, Douglas D. Glover, MD, Steve N. Caritis, MD, for the National Institute for Child Health and Human Development Network of Maternal-Fetal-Medicine Units

Minneapolis, Minn, Morgantown, WV, and Pittsburgh, Pa

Drug metabolizing capacity is altered during pregnancy with the activity of cytochrome P450 1A2, which is decreased, although the activities of cytochrome P450 2D6 and 3A are increased.

EDUCATION

Abortion education in medical schools: A national survey

Eve Espey, MD, MPH, Tony Ogburn, MD, Alice Chavez, MD, Clifford Qualls, PhD, Mario Leyba

Albuquerque, NM

This survey of clerkship directors indicates that abortion education is limited in the curricula of US medical schools.

Attitudes of faculty and students toward case-based learning in the third-year obstetrics and gynecology clerkship

Wendy F. Hansen, MD, Kristi J. Ferguson, PhD, Christopher S. Sipe, MD, Joel Sorosky, MD

Iowa City, Iowa

Faculty favored lecture format, whereas student participants favored case-based presentations during the obstetrics and gynecology clerkship.

IMAGING

Uterine tissue development in healthy women during the normal menstrual cycle and investigations with magnetic resonance imaging

Caroline L. Hoad, PhD, Nick J. Raine-Fenning, MD, Jonathan Fulford, PhD, Bruce K. Campbell, PhD, Ian R. Johnson, MD, Penelope A. Gowland, PhD

Nottingham, UK

Developmental changes in endometrial and junctional zone volume and thickness have been quantified throughout the normal menstrual cycle with the use of high-resolution magnetic resonance imaging.

LETTERS TO THE EDITORS

Who should be the guardians of women’s “sacred space”??

Susan Bewley, MD, FRCOG, Janice Rymer, MD, MRCOG, FRANZCOG, ILTM

London, United Kingdom

Reply

L. Lewis Wall, MD, DPhil, Douglas Brown, PhD

St Louis, Mo

Oxidative stress in women with preeclampsia

Mehmet Harma, MD, Muge Harma, MD, Ozcan Erel, MD

Ankara, Turkey
Reply
Michael Phillips, MD, Michael Moretti, MD, Renee N. Cataneo, MA, Joel Greenberg, BS
Fort Lee, NJ

Randomized study on surgical treatment for vaginal prolapse
Peter K. Thompson, MD
Houston, Tex

Reply
Christopher Maher, FRANZCOG, CU
Brisbane, Australia

Accuracy of cervical cancer staging needs improvement
Sven Ackermann, MD, Matthias W. Beckmann, MD, PhD
Erlangen, Germany

Reply
Mitchel S. Hoffman, MD
Tampa, Fla

Unnecessary—The mother of invention?
Russell S. Kirby, PhD, MS, FACE
Birmingham, Ala

Reply
William C. Steinmann, MD
New Orleans, La

CORRECTION
“Comparing McRoberts’ and Rubin’s maneuvers for initial management of shoulder dystocia: An objective evaluation”
Gurewitsch et al
Why is nuchal translucency on the cover of the American Journal of Obstetrics and Gynecology?

Prenatal diagnosis can begin in the first trimester of pregnancy (11-14 weeks). Risk assessment for chromosomal abnormalities in the first trimester is possible with the combined use of ultrasound imaging of the embryo/fetus and the determination of maternal serum analytes (e.g. PAPP-A and free β-hCG). A key component of risk assessment is the measurement of nuchal translucency between 11 and 14 weeks. Compelling evidence now indicates than an increased nuchal translucency is a risk factor for trisomy 21, 18 and 13, and that fetuses with this finding and a normal karyotype have an increased risk for congenital heart diseases that may not be detected until the second trimester or until the time of birth. Moreover, an increased nuchal translucency can also be an indication of other congenital anomalies as well as genetic syndromes.

While first trimester sonographic examination for risk assessment is being offered in some centers in the United States, it is anticipated that the results of studies funded by the National Institute of Child Health and Human Development (NICHD/NIH) will bring these risk assessment tools to more women in the near future. We are committed to share with our readers clinically relevant information, and wish to bring to your attention the following articles that have or will be published on this subject.

1. A comprehensive review of nuchal translucency by Dr Kypros Nicolaides, who performed pioneering work in this area, was published in the American Journal of Obstetrics and Gynecology in July 2004 (AJOG 2004;191:45-67). This article is freely available to both subscribers and non-subscribers of the journal (“open access” doi:10.1016/j.ajog.2004.03.090). The article contains two important features: (1) a PowerPoint presentation that can be downloaded for teaching purposes by subscribers and non-subscribers of the American Journal of Obstetrics and Gynecology worldwide at no cost. This may be particularly helpful to residents, fellows and faculty; and (2) supplemental materials on the journal’s website which include nomograms and additional relevant clinical information (also available via “open access”).

2. A second article by Dr Nicolaides’ group entitled “Increased nuchal translucency with normal karyotype” will be published in April 2005 (Souka et al.). This article will also be available via “open access”.

3. Results of the BUN trial which provides evidence of an association between an enlarged nuchal translucency and congenital heart disease will be published in the May issue of the Journal (Bahado-Singh et al. “An enlarged first-trimester nuchal translucency increases the risk of congenital heart defects.”).

The Editors of the American Journal of Obstetrics and Gynecology believe that a first trimester sonographic examination is likely to become the initial encounter between the obstetrician and the pregnant woman and her embryo/fetus. Thus, incorporating this technique into clinical practice will require further education, training and research. Although nuchal translucency is a powerful and informative parameter, we believe it is only the first step. This is why we have selected this picture for the cover of the Journal for the next six months. In-depth examination of the phenotype of the embryo/fetus in early pregnancy may reveal clues about both health and disease (e.g. presence or absence of nasal bone). The application of “discovery techniques” such as genomics, proteomics and metabolomics can also revolutionize obstetrical care in the first trimester. We encourage our readers to explore the novel features offered by the Journal, and invite authors of relevant material to consider the American Journal of Obstetrics and Gynecology as a venue for publication.

The Editors
The Journal is the Official Publication of the following Societies

American Gynecological and Obstetrical Society
Association of Professors of Gynecology and Obstetrics
Central Association of Obstetricians and Gynecologists
South Atlantic Association of Obstetricians and Gynecologists
Pacific Coast Obstetrical and Gynecological Society
American Board of Obstetrics and Gynecology
Society for Maternal-Fetal Medicine
Society of Gynecologic Surgeons

New York Obstetrical Society
Obstetrical Society of Philadelphia
Brooklyn Gynecological Society
St. Louis Gynecological Society
Greater New Orleans Gynecological Society
Obstetrical and Gynecological Society of Maryland
Chicago Gynecological Society
Cincinnati Obstetrical and Gynecological Society
Washington Gynecological Society
Pittsburgh Obstetrical and Gynecological Society
Obstetrical Society of Boston
Louisville Obstetrical and Gynecological Society
Seattle Gynecological Society
Alabama Association of Obstetricians and Gynecologists
Akron Obstetrical and Gynecological Society
Kansas City Gynecological Society
Central New York Association of Gynecologists and Obstetricians
New Jersey Obstetrical and Gynecological Society
Texas Association of Obstetricians and Gynecologists
Oklahoma City Obstetrical and Gynecological Society
Memphis Obstetrical and Gynecological Society
Utah Obstetrical and Gynecological Society
Rochester Obstetrical and Gynecological Society
Arkansas Obstetrical and Gynecological Society
Tennessee State Obstetrical and Gynecological Society
New York Gynecological Society
Pacific Northwest Gynecological and Obstetrical Association
Buffalo Gynecologic and Obstetric Society
San Francisco Gynecological Society
Jackson Gynecic Society
Minnesota Obstetrical and Gynecological Society
Columbus Obstetric and Gynecologic Society
Greater Hartford Obstetrical and Gynecological Society
Cleveland Society of Obstetricians and Gynecologists
Comment on notice of retraction

The Editors

American Journal of Obstetrics and Gynecology

Notice of retraction

The article titled “A prospective randomized trial comparing tension-free vaginal tape and transobturator suburethral tape for surgical treatment of stress urinary incontinence” was published in March 2004 (Am J Obstet Gynecol 2004;190:602-8). On July 15, 2004, the authors, Renaud de Tayrac and Hervé Fernandez, submitted a written request for retraction of their article acknowledging a failure in their duty to obtain proper ethics review and approval before conducting the study. In the submission documents that accompanied the manuscript and in the manuscript itself, the authors stated “Ethical approval was obtained from the local committee,” when in fact their study had not been approved by the only French committee (Consultative Committee for Patients Protection in Biomedical Research) authorized to give ethics approval. Their statements misled our reviewers, editors, and readers. In accordance with the published guidelines of the Journal, the initial inquiry process was conducted.

The inquiry committee concluded that the authors’ violation of the ethical standards for conducting human research did represent an inappropriate act in the publication process. The Committee also found that the authors committed the same violation in the second article recently published (Risk of urge and stress urinary incontinence at long-term follow-up after vaginal hysterectomy. Am J Obstet Gynecol 2004;191:90-4). Again, the authors stated in the manuscript and submission materials that ethics approval was obtained for this postsurgical survey study. Again, their failure to obtain appropriate ethical review and approval (or formal notice of exemption) as required by French law represents a serious breach of the basic principles of scientific human research and warrants the retraction of this article also.

Human research and the integrity of the scientific process demand the unbiased review and approval by a certified ethical review committee on human investigation before research is conducted and, nearly always, fully informed consent by the study participant before she/ he is enrolled in the study. Failure to fulfill either of these requirements disallows the research and therefore, these two articles published by the authors must be retracted.

To their credit the authors volunteered this information to the Journal and requested retraction of the articles. However, this was well after publication and their acts still undermine the integrity of the scientific publication process. The authors are reprimanded for their inappropriate acts in the research and publication process. We cannot overemphasize the responsibility and duty of all scientific investigators when conducting human research. We hope readers will look upon this notice and these retractions as evidence of the Journal’s commitment to the safety of and respect for study participants who volunteer for the benefit of science and future patients.

References

EDITORIAL

Being mothers too early

Ricardo Gomez, a,b Joaquin Santolaya b,c

Center for Perinatal Diagnosis and Research (CEDIP), Sotero del Rio Hospital, P. Universidad Catolica de Chile, Puente Alto, Chile, a Perinatology Research Branch, National Institutes of Child Health and Human Development, NIH, DHHS, Bethesda, Md, b and Department of Obstetrics and Gynecology, Wayne State University, Detroit, Mich c

Mortality for adolescent mothers ranges from 1 in 7 women in Niger to 1 in 28,800 in Sweden. Complications from pregnancy and childbirth are the leading causes of death in young women in developing countries and kill approximately 70,000 adolescent mothers worldwide each year worldwide.1 Major causes of maternal mortality include hemorrhage, sepsis, eclampsia, and obstructed labor. A recent report1 has shown that one-third of pregnant women from developing countries are younger than 20 years of age. These patients and their neonates are more likely to die from complications associated with pregnancy.1 Furthermore, a recent study found that teenage mothers are at risk for subsequent premature death caused by heart disease, cancer, suicide, inflicted violence, and alcohol-related conditions.3 According to the National Center for Disease Control (CDC), the United States has a teenage birth rate of 43 per 1000, which is one of the highest in industrialized countries, including the United Kingdom, Germany, France, The Netherlands, Canada, or Japan.

The study of Conde-Agudelo and associates published in this issue of the Journal,5 provides additional insights for our understanding of adolescent pregnancy. They examined a large hospital-based database, which is used in several South American countries (SIP, perinatal information system) to study the association between maternal age, several related factors, and complications in mothers and newborn infants. Because they analyzed 854,377 pregnant women (344,626 teen pregnancies), they resolved the common limitation of sample size that has been noted in previous studies addressing the relationship between maternal age and pregnancy outcome. Moreover, this study examined the effect of 16 covariates on 21 outcomes using logistic regression analysis. Overall, the investigators confirmed that adolescent pregnancy is associated with a variety of maternal, fetal, and neonatal complications. Indeed, the adjusted odds ratios and 95% CIs demonstrated that adolescent pregnant women are more likely to have anemia, operative vaginal delivery, episiotomy, postpartum hemorrhage, puerperal endometritis, preterm delivery, small-for-gestational age, and low birth weight infants. Evaluation of the group with ages 16 years and under demonstrated the emergence of a greater frequency of maternal and neonatal death.5

Although the study period lasted from 1985 to 2003, the information provided is a valuable reference point to address current public health care issues in South America and is comparable with other reports from the region. These data demonstrate that teenage pregnancy is a problem that relentlessly crosses social and political boundaries worldwide. Conde-Agudelo et al5 point out the seriousness of this health care dilemma. Whether this problem is purely biologic or the consequence of the complex interplay of age and various social, economical and educational factors may be a matter of controversy for some, but the fact of the matter is that in South America, adolescent pregnant women and their children are 4 times more likely to die than 20- to 25-year-old women.

The research effort of Conde-Agudelo et al5 proves that pregnancies in young women are problematic and consequently, there is a need for more effective interventions aiming at reducing unintended pregnancy in adolescents worldwide. According to the American

For more information, please review www.teenpregnancy.org; www.savethechildren.org or write to adolhlth@acog.org.

0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.
College of Obstetrics and Gynecology, many teens say they are concerned about pregnancy, but still think, “it can’t happen to me.” The number one reason teens give for not using contraception is that they were not planning to have sex and that it “just happened.” Moreover, a recent systematic review of randomized trials about interventions to reduce unintended pregnancies among adolescents showed that primary prevention strategies did not delay the initiation of sexual intercourse or improve the use of birth control, with no reduction in the pregnancy rate and even an increase in those using an abstinence-based program. Thus, the challenge of preventing unintended pregnancies among women is not being successfully addressed by most countries. It is clear that actions aimed at preventing teenage pregnancies in years to come are educational and political in nature, and should focus on addressing the needs of young people and gaining a deeper understanding of the problem.

References

Maternal-perinatal morbidity and mortality associated with adolescent pregnancy in Latin America: Cross-sectional study

Agustin Conde-Agudelo, MD, MPH,a,b José M. Belizán, MD, PhD,b Cristina Lammers, MD, MPHb

Department of Obstetrics and Gynecology, Fundacion Clinica Valle del Lili, Cali, Colombia,a and Latin American Center for Perinatology and Human Development (CLAP), Division of Health Promotion and Protection, Pan American Health Organization, World Health Organization, Montevideo, Uruguayb

Received for publication June 1, 2004; revised October 1, 2004; accepted October 6, 2004

Objective: This study was undertaken to determine whether adolescent pregnancy is associated with increased risks of adverse pregnancy outcomes.

Study design: We studied 854,377 Latin American women who were younger than 25 years during 1985 through 2003 using information recorded in the Perinatal Information System database of the Latin American Center for Perinatology and Human Development, Montevideo, Uruguay. Adjusted odds ratios were obtained through logistic regression analysis.

Results: After an adjustment for 16 major confounding factors, adolescents aged 15 years or younger had higher risks for maternal death, early neonatal death, and anemia compared with women aged 20 to 24 years. Moreover, all age groups of adolescents had higher risks for postpartum hemorrhage, puerperal endometritis, operative vaginal delivery, episiotomy, low birth weight, preterm delivery, and small-for-gestational-age infants. All adolescent mothers had lower risks for cesarean delivery, third-trimester bleeding, and gestational diabetes.

Conclusion: In Latin America, adolescent pregnancy is independently associated with increased risks of adverse pregnancy outcomes.

OBJECTIVE: This study was undertaken to determine whether adolescent pregnancy is associated with increased risks of adverse pregnancy outcomes.

STUDY DESIGN: We studied 854,377 Latin American women who were younger than 25 years during 1985 through 2003 using information recorded in the Perinatal Information System database of the Latin American Center for Perinatology and Human Development, Montevideo, Uruguay. Adjusted odds ratios were obtained through logistic regression analysis.

RESULTS: After an adjustment for 16 major confounding factors, adolescents aged 15 years or younger had higher risks for maternal death, early neonatal death, and anemia compared with women aged 20 to 24 years. Moreover, all age groups of adolescents had higher risks for postpartum hemorrhage, puerperal endometritis, operative vaginal delivery, episiotomy, low birth weight, preterm delivery, and small-for-gestational-age infants. All adolescent mothers had lower risks for cesarean delivery, third-trimester bleeding, and gestational diabetes.

CONCLUSION: In Latin America, adolescent pregnancy is independently associated with increased risks of adverse pregnancy outcomes.

© 2005 Elsevier Inc. All rights reserved.

Adolescents aged 15 to 19 years gave birth to 17 million infants in 1997, of which 16 million were born in the developing world where 15% to 20% of all births are to adolescent mothers.1 Moreover, 85% of adolescent women are in the developing world2 and 25% of all maternal deaths occur in such age group.1 Although birth rates have dropped for adolescents in most developed countries, in sub-Saharan Africa, Latin America, and the Caribbean, only modest declines have been reported.2 Thereby, adolescent pregnancy continues to be a challenging public health issue around the world, mainly in developing countries.

Adolescent pregnancy has been associated with an increased incidence of several adverse maternal and perinatal outcomes such as low birth weight (LBW), preterm delivery, small-for-gestational-age (SGA) infants, perinatal death, eclampsia, operative vaginal...
delivery, and maternal death. Nevertheless, research in this area has many methodologic limitations such as small sample size, especially that of mothers aged 15 years or younger, studies not designed specifically to test associations between both maternal and perinatal complications and young maternal age, no uniform definitions for complications evaluated, and lack of control for potential confounding factors that may be related to both young maternal age and adverse pregnancy outcomes. In addition, few studies have addressed this topic among Latin American pregnant adolescents.

The aim of this study was to determine whether young maternal age was associated with increased risks of adverse pregnancy outcomes, adjusting for many potentially confounding factors.

**Material and methods**

This research is based on the Perinatal Information System database in Montevideo, Uruguay, which was designed by the Latin American Center for Perinatology and Human Development (CLAP) in 1983, and consists in the basic perinatal clinical record, its complementary forms and charts, the perinatal card, and a software package for personal computers. Detailed descriptions of the database have been published elsewhere. From 1985 through 2003, the Perinatal Information System database has recorded pregnancies of women from Uruguay (25.3%), Argentina (24.1%), Peru (9.4%), Colombia (8.6%), Honduras (8.2%), Paraguay (6.9%), El Salvador (4.2%), Chile (2.8%), Bolivia (2.3%), Costa Rica (2.2%), Panama (1.4%), Dominican Republic (1.3%), Nicaragua (1.2%), Bolivia (0.6%), Brazil (0.8%), Ecuador (0.6%), Mexico (0.4%), Belize (0.1%), and Venezuela (0.1%).

Inclusion in the study group was restricted to women aged between 10 and 24 years who had a singleton birth of at least 20 weeks' gestation or at least 400 g birth weight.

Maternal age was defined as the age of the mother in completed years at time of delivery and was categorized into 4 groups: less than 16, 16 to 17, 18 to 19, and 20 to 24 years. This last group was the reference group in all comparisons. Gestational age at birth was defined as the number of completed weeks’ gestation from the first day of the last menstrual period to delivery date. Parity was defined as the number of previous births, including stillbirths. The mother’s education was categorized into none, and appropriate or inappropriate for her age. Mothers older than 19 years were considered to have an age-appropriate educational level if they had completed at least the secondary education (11 or more years), whereas younger mothers had to have completed the minimal number of years for their age. Marital status was dichotomized between those who did and did not have partner. Maternal height and prepregnancy weight were recorded by recall at the woman’s first antenatal visit in centimeters and kilograms, respectively. The prepregnancy body mass index (weight [kg]/height[m]²) was categorized as follows: underweight (body mass index <19.8), normal (19.8-26.0), overweight (26.1-29.0), and obese (>29.0). Weight gain during pregnancy (kg/wk) was grouped into slow (<0.25), average (0.25-0.44), and rapid (≥0.45). Information on cigarette smoking was also recorded at the time of first attendance for antenatal care, and categorized into non-smoker and smoker. For parous women, interpregnancy interval was defined as the time elapsed between the woman’s last delivery and the date of the last menstrual period for the index pregnancy. Interpregnancy intervals were categorized as less than 12, 12 to 23, 24 to 47, and 48 months or more.

Adverse maternal outcomes were classified according to the International Classification of Diseases, tenth revision (ICD-10). Preeclampsia and eclampsia were coded O14 and O15, respectively. Third-trimester bleeding including placenta previa with hemorrhage (code O44.1) and placental abruptio (code O45). Cesarean delivery was coded O82 and operative vaginal delivery as code O81. Episiotomy was coded O70.9. Anemia, urinary tract infection, premature rupture of membranes, gestational diabetes, postpartum hemorrhage, and puerperal endometritis were coded O99.0, O23, O42, O24.4, O72, and O85, respectively. Maternal death was defined as the death of a woman while pregnant or within 42 days after delivery from any cause related to or aggravated by the pregnancy or its management, but not from accidental or incidental causes.

Perinatal outcomes evaluated were LBW (live infant weighing <2500 g at birth), very LBW (live infant weighing <1500 g at birth), preterm delivery (live infant delivered at <37 weeks’ gestation), very preterm delivery (live infant delivered at <32 weeks’ gestation), SGA (live infant with birth weight below the 10th percentile for the gestational age and gender, according to the Williams et al reference curve), fetal death (delivery of a dead infant at or after 20 weeks’ gestation), early neonatal death (neonatal death occurring during the first 7 days of life), and low Apgar scores at 5 minutes (<7).

Rates of adverse pregnancy outcomes were calculated for each maternal age group. Estimates of crude odds ratio (OR) with 95% CI were computed as measures of association between each maternal age group and adverse pregnancy outcomes considered. Adjusted ORs were derived through logistic regression models. The estimates were adjusted for the following potential confounding factors: parity, mother’s education, marital status, cigarette smoking, interpregnancy interval, prepregnancy body mass index, weight gain during pregnancy, history of miscarriage, LBW, perinatal death, and chronic hypertension, gestational age at first attendance.
<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Maternal age (y)</th>
<th>All adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤15 (n = 33,498)</td>
<td>16-17 (n = 119,723)</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>94.6</td>
<td>83.8</td>
</tr>
<tr>
<td>1</td>
<td>4.4</td>
<td>14.9</td>
</tr>
<tr>
<td>≥2</td>
<td>1.0</td>
<td>1.3</td>
</tr>
<tr>
<td>Mother's education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>4.6</td>
<td>3.9</td>
</tr>
<tr>
<td>Age inappropriate</td>
<td>48.2</td>
<td>51.0</td>
</tr>
<tr>
<td>Age appropriate</td>
<td>47.2</td>
<td>45.1</td>
</tr>
<tr>
<td>Marital status</td>
<td></td>
<td></td>
</tr>
<tr>
<td>With partner</td>
<td>45.1</td>
<td>61.5</td>
</tr>
<tr>
<td>Without partner</td>
<td>54.9</td>
<td>38.5</td>
</tr>
<tr>
<td>Cigarette smoking</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>7.5</td>
<td>9.4</td>
</tr>
<tr>
<td>No</td>
<td>92.5</td>
<td>90.6</td>
</tr>
<tr>
<td>Interpregnancy interval (mo)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>45.6</td>
<td>42.4</td>
</tr>
<tr>
<td>12-23</td>
<td>41.8</td>
<td>40.1</td>
</tr>
<tr>
<td>24-47</td>
<td>11.4</td>
<td>15.9</td>
</tr>
<tr>
<td>≥48</td>
<td>1.2</td>
<td>1.6</td>
</tr>
<tr>
<td>Weight gain (kg/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;0.25</td>
<td>20.6</td>
<td>19.8</td>
</tr>
<tr>
<td>0.25-0.44</td>
<td>63.6</td>
<td>65.3</td>
</tr>
<tr>
<td>≥0.45</td>
<td>15.8</td>
<td>14.9</td>
</tr>
<tr>
<td>Prepregnancy body mass index (kg/m²)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;19.8</td>
<td>10.9</td>
<td>10.4</td>
</tr>
<tr>
<td>19.8-26.0</td>
<td>72.2</td>
<td>68.1</td>
</tr>
<tr>
<td>26.1-29.0</td>
<td>10.9</td>
<td>14.6</td>
</tr>
<tr>
<td>&gt;29.0</td>
<td>6.0</td>
<td>6.9</td>
</tr>
<tr>
<td>History of miscarriage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>2.5</td>
<td>4.9</td>
</tr>
<tr>
<td>No</td>
<td>97.5</td>
<td>95.1</td>
</tr>
<tr>
<td>History of LBW</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.9</td>
<td>1.6</td>
</tr>
<tr>
<td>No</td>
<td>99.1</td>
<td>98.4</td>
</tr>
<tr>
<td>History of perinatal death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.5</td>
<td>1.6</td>
</tr>
<tr>
<td>No</td>
<td>99.5</td>
<td>98.4</td>
</tr>
<tr>
<td>History of chronic hypertension</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>0.4</td>
<td>0.5</td>
</tr>
<tr>
<td>No</td>
<td>99.6</td>
<td>99.5</td>
</tr>
<tr>
<td>Gestational age at first antenatal visit (wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-13</td>
<td>19.6</td>
<td>20.8</td>
</tr>
<tr>
<td>14-26</td>
<td>43.8</td>
<td>47.1</td>
</tr>
<tr>
<td>≥27</td>
<td>36.6</td>
<td>32.1</td>
</tr>
<tr>
<td>Number of antenatal visits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>23.5</td>
<td>23.6</td>
</tr>
<tr>
<td>1-4</td>
<td>37.5</td>
<td>35.1</td>
</tr>
<tr>
<td>≥5</td>
<td>39.0</td>
<td>41.3</td>
</tr>
</tbody>
</table>

Values are percentage of women.
for antenatal care, number of antenatal visits, geographic area, hospital type, and year of delivery. Early neonatal death and low Apgar scores at 5 minutes were additionally adjusted for birth weight and gestational age. The effects of maternal age on adverse pregnancy outcomes were evaluated separately for nulliparous and parous women. All analyses were performed with the SPSS 8.0 program package (SPSS Inc, Chicago, Ill).

### Results

Over the 19-year period, 2,073,968 pregnancies were recorded in our database, of which we excluded 36,917 multiple pregnancies, 1,039,863 to mothers older than 24 years, and 142,811 for whom information on adverse pregnancy outcomes or interpregnancy intervals was missing. The remaining 854,377 women constituted the study population of which 344,626 were adolescents. There were no significant differences between the women excluded because of incomplete information and those with complete information with regard to maternal age, parity, education, and marital status. Overall, adolescents accounted for 18.4% of all deliveries in our database.

Table I shows the maternal demographic and obstetric characteristics of the study groups. Compared with women aged 20 to 24 years, adolescent mothers were more likely to be nullipara, without permanent partner, to have lower body mass index before pregnancy and shorter interpregnancy intervals, to begin prenatal care later, to have lower number of prenatal visits, and to have a lower proportion of previous miscarriages, LBW infants, perinatal deaths, and chronic hypertension.

There were no striking differences with regard to weight gain during pregnancy, cigarette smoking, and mother’s education.

Table II Rates of adverse maternal outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Maternal age (y)</th>
<th>All adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤ 15 (n = 33,498)</td>
<td>16-17 (n = 119,723)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>5.9</td>
<td>4.9</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>1.1</td>
<td>0.6</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>0.9</td>
<td>1.0</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>4.3</td>
<td>4.4</td>
</tr>
<tr>
<td>Premature rupture of membranes</td>
<td>4.9</td>
<td>6.4</td>
</tr>
<tr>
<td>Third-trimester bleeding</td>
<td>0.2</td>
<td>0.5</td>
</tr>
<tr>
<td>Anemia</td>
<td>8.8</td>
<td>7.2</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>15.3</td>
<td>14.0</td>
</tr>
<tr>
<td>Operative vaginal delivery</td>
<td>4.1</td>
<td>3.8</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>75.7</td>
<td>71.0</td>
</tr>
<tr>
<td>Postpartum hemorrhage</td>
<td>7.0</td>
<td>5.6</td>
</tr>
<tr>
<td>Puerperal endometritis</td>
<td>16.7</td>
<td>9.7</td>
</tr>
<tr>
<td>Maternal death*</td>
<td>18.5</td>
<td>4.0</td>
</tr>
</tbody>
</table>

Values are percentage of women unless stated otherwise. * Rate per 10,000 women.

Table II Rates of adverse maternal outcomes

There was a clear trend toward increasing rates of preeclampsia, eclampsia, anemia, operative vaginal delivery, episiotomy, postpartum hemorrhage, and puerperal endometritis as maternal age decreased (Table II). Rates of gestational diabetes, third-trimester bleeding, and premature rupture of membranes, increased progressively with increasing maternal age. Overall, the rate of cesarean delivery was lower in adolescents than in women aged 20 to 24 years. There were 397 maternal deaths in the study population. The youngest adolescents (≤ 15 years) had the highest maternal fatality rate, whereas the rates for older adolescents were similar to that of adult women.

The rates of LBW, very LBW, preterm delivery, very preterm delivery, SGA, and early neonatal death consistently increased with decreasing maternal age and were always highest among infants born to mothers aged 15 years or younger (Table III). Adolescents did not differ from reference group in the rates of fetal death and low Apgar scores at 5 minutes.

Adjusted ORs for the association between maternal age and adverse maternal outcomes are shown in Table IV. Compared with mothers aged 20 to 24 years, adolescent mothers had higher risks of operative vaginal delivery, episiotomy, postpartum hemorrhage, and puerperal endometritis. The youngest mothers faced the highest risks, whereas mothers aged 16 to 17 and 18 to 19 years had smaller increases in risks. Mothers aged 15 years or younger had about a 40% increased risk of anemia compared with women aged 20 to 24 years. When adolescents were considered as a whole, they were
adolescent mothers under 16 years were 4 times more likely to die than mothers aged 20 to 24 years. With respect to gestational diabetes, third-trimester bleeding, and cesarean delivery, all age groups of adolescents had decreased risks compared with adult women. There were no significant differences in the effect of adolescent pregnancy on preeclampsia, eclampsia, urinary tract infection, and premature rupture of membranes.

Table V depicts adjusted ORs for the association between maternal age and adverse perinatal outcomes. Risks of LBW, very LBW, preterm delivery, very preterm delivery, and SGA increased with decreasing maternal age. Compared with infants of mothers aged 20 to 24 years, those born to women aged 15 years or younger faced a 50% increase in risk of early neonatal death. Notwithstanding, this association disappeared when it was adjusted for birth weight and gestational age (OR 1.16, 95% CI 0.95-1.43) suggesting the increased risk of early neonatal death among the youngest adolescents was almost entirely explained by the high rates of preterm delivery and LBW in this group. We found no statistically significant differences in the effect of low maternal age on fetal death and low Apgar scores at 5 minutes.

When the data were analyzed separately for nulliparous and parous women, the results were similar to those obtained in the analyses of the entire population. However, the adjusted ORs of adverse pregnancy outcomes were slightly higher in nulliparous adolescents compared with parous ones (data not shown).

**Table III** Rates of adverse perinatal outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Maternal age (y)</th>
<th>All adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤15 (n = 33,498)</td>
<td>16-17 (n = 119,723)</td>
</tr>
<tr>
<td>LBW</td>
<td>12.8</td>
<td>10.3</td>
</tr>
<tr>
<td>Very LBW</td>
<td>1.7</td>
<td>1.6</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>14.6</td>
<td>11.0</td>
</tr>
<tr>
<td>Very preterm delivery</td>
<td>2.8</td>
<td>2.3</td>
</tr>
<tr>
<td>SGA</td>
<td>17.0</td>
<td>15.9</td>
</tr>
<tr>
<td>Fetal death*</td>
<td>17.0</td>
<td>15.0</td>
</tr>
<tr>
<td>Early neonatal death*</td>
<td>15.2</td>
<td>12.0</td>
</tr>
<tr>
<td>Low Apgar scores at 5 min</td>
<td>1.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

Values are percentage of infants unless stated otherwise.

* Rate per 1000 infants.

**Table IV** Adjusted OR (95% CI) for the association between maternal age and adverse maternal outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Maternal age (y)</th>
<th>All adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤15 (n = 33,498)</td>
<td>16-17 (n = 119,723)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>1.08 (0.98-1.19)</td>
<td>1.04 (0.99-1.08)</td>
</tr>
<tr>
<td>Eclampsia</td>
<td>1.61 (0.86-2.42)</td>
<td>1.36 (0.89-1.85)</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>0.34 (0.29-0.40)</td>
<td>0.35 (0.31-0.40)</td>
</tr>
<tr>
<td>Urinary tract infection</td>
<td>1.03 (0.95-1.12)</td>
<td>1.01 (0.96-1.07)</td>
</tr>
<tr>
<td>Premature rupture of membranes</td>
<td>0.95 (0.90-1.01)</td>
<td>0.98 (0.95-1.02)</td>
</tr>
<tr>
<td>Third-trimester bleeding</td>
<td>0.24 (0.17-0.32)</td>
<td>0.59 (0.53-0.66)</td>
</tr>
<tr>
<td>Anemia</td>
<td>1.41 (1.33-1.50)</td>
<td>1.05 (1.00-1.10)</td>
</tr>
<tr>
<td>Cesarean delivery</td>
<td>0.87 (0.83-0.92)</td>
<td>0.80 (0.78-0.82)</td>
</tr>
<tr>
<td>Operative vaginal delivery</td>
<td>1.44 (1.32-1.57)</td>
<td>1.29 (1.21-1.38)</td>
</tr>
<tr>
<td>Episiotomy</td>
<td>2.36 (2.27-2.46)</td>
<td>1.98 (1.93-2.04)</td>
</tr>
<tr>
<td>Postpartum hemorrhage</td>
<td>1.59 (1.50-1.70)</td>
<td>1.31 (1.24-1.39)</td>
</tr>
<tr>
<td>Puerperal endometritis</td>
<td>3.81 (3.64-4.00)</td>
<td>2.08 (2.01-2.15)</td>
</tr>
<tr>
<td>Maternal death</td>
<td>4.09 (3.86-4.34)</td>
<td>0.98 (0.66-1.32)</td>
</tr>
</tbody>
</table>

* Reference group.

**Comment**

With the Perinatal Information System database we were able to establish that adolescent pregnancy in Latin
America is independently associated with increased risks of adverse pregnancy outcomes. On the basis of our findings, it appears that adolescence encompasses at least 2 maternal age groups. Although both adolescent age groups had poorer pregnancy outcomes than adult women, the youngest mothers (<16 years) had substantially higher risks for maternal and perinatal morbidity and mortality than the late adolescent age group (16-19 years). The large sample size that confers sufficient power to evaluate the relation between young maternal age and rare adverse pregnancy outcomes such as maternal, fetal, and early neonatal deaths, the possibility to control for many confounding factors, and the relatively homogeneous population of women studied support the findings of our study.

The prominent finding in this large hospital-based retrospective cross-sectional study is the risk of dying from pregnancy-related causes is 4 times higher for adolescents under 16 years than for women in their early twenties. The increased risks of anemia, postpartum hemorrhage, and puerperal endometritis may have contributed to the increased risk of maternal death among young adolescents. In general, this result agrees with previous studies from developing and developed countries.10,11 Most of these studies, however, did not adjust for confounding factors. A nested case-control study from Bangladesh12 evaluated risk factors for 390 maternal deaths and found that, compared with women aged 20 to 29 years, mothers aged 15 to 19 years had a 65% increased risk of death (OR 1.65, 95% CI 1.12-1.45) after adjustment for interpregnancy interval, area of residence, maternal education, religion, and year of birth. In contrast, a study from Ethiopia13 that controlled for the effect of antenatal care, income, occupation, marital status, and parity did not find statistically significant increase in the risk of maternal death among young adolescents compared with mothers aged 25 to 29 years (adjusted OR 2.0; 95% CI 0.4-10.3). A report evaluating the maternal deaths in the United Kingdom showed that adolescents younger than 18 years had an increased risk of maternal death.10 Nevertheless, these studies included a small number of adolescent maternal deaths. A recent national population-based report summarizing surveillance data for pregnancy-related deaths in the United States for the period 1991 through 1997 showed that, compared with women aged 20 to 24 years, the pregnancy-related mortality ratio was higher for adolescents younger than 15 years (9.4 and 15.6 maternal deaths per 100,000 live births, respectively).11

Our study found that adolescent mothers, mainly those younger than 16 years, are at increased risk of several adverse maternal outcomes. Consistent with previous reports from developed14,15 and developing countries,3,16 we observed that young adolescents had anemia more frequently than older women. Regarding to complications of labor, delivery, and the puerperium, we found that adolescent mothers are at increased risks of episiotomy, operative vaginal delivery, and puerperal endometritis in agreement with several published studies.16,17 It has been suggested that in young adolescents, the pelvic bones and the birth canal may still be in the process of growth, increasing the risk of prolonged and obstructed labor, episiotomy, use of forceps and ventouse, and puerperal endometritis.17 If this theory of biologic immaturity accounted for the greatest proportion of delivery complications, we would expect the rates of cesarean delivery to be higher in this population. However, our study found that adolescent mothers are at decreased risk of cesarean delivery corroborating the findings from previous reports.3,14,18,20 The reasons for this association are unclear. Contrary to our finding of increased risk of postpartum hemorrhage among adolescents, Jolly et al14 found the risk of postpartum hemorrhage was lower in adolescents younger than 18 years. In accordance with previous studies,14,21 we found that gestational diabetes was less common in adolescents than in adult women, which supports the recommendation that screening for

### Table V

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Maternal age (y)</th>
<th>All adolescents</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>≤15 (n = 33,498)</td>
<td>16-17 (n = 119,723)</td>
</tr>
<tr>
<td>LBW</td>
<td>1.62 (1.54-1.71)</td>
<td>1.27 (1.23-1.32)</td>
</tr>
<tr>
<td>Very LWB</td>
<td>1.25 (1.12-1.39)</td>
<td>1.24 (1.16-1.33)</td>
</tr>
<tr>
<td>Preterm delivery</td>
<td>1.66 (1.59-1.74)</td>
<td>1.25 (1.20-1.31)</td>
</tr>
<tr>
<td>Very preterm delivery</td>
<td>1.51 (1.37-1.67)</td>
<td>1.35 (1.26-1.45)</td>
</tr>
<tr>
<td>SGA</td>
<td>1.50 (1.45-1.56)</td>
<td>1.41 (1.37-1.46)</td>
</tr>
<tr>
<td>Fetal death</td>
<td>1.03 (0.92-1.15)</td>
<td>0.98 (0.91-1.06)</td>
</tr>
<tr>
<td>Early neonatal death</td>
<td>1.50 (1.33-1.70)</td>
<td>1.05 (0.95-1.16)</td>
</tr>
<tr>
<td>Low Apgar scores at 5 min</td>
<td>0.97 (0.85-1.10)</td>
<td>0.98 (0.91-1.06)</td>
</tr>
</tbody>
</table>

* Reference group.
gestational diabetes of pregnant adolescents be performed only on the basis of risk factors. 21

With regard to adverse perinatal outcomes, we found a higher risk of LBW, very LBW, preterm delivery, very preterm delivery, and SGA among infants of adolescent mothers, with the youngest age groups running the highest risks. Although there are several reports indicating that adolescent pregnancy is not associated with increased risks of adverse perinatal outcomes, 19,22 most studies from both developed and developing countries have consistently reported that pregnant adolescents are at increased risk for preterm delivery 3,14,15,23 and LBW. 15,17,18,23 The relation between adolescent pregnancy and SGA is less clear in the literature. An increased incidence of SGA infants in adolescent mothers has been reported by some authors 23 but not by others. 14,22 Previous investigations of perinatal mortality in adolescent pregnancy have yielded conflicting results. Some studies have found increased risk of neonatal mortality among infants born to adolescent mothers, 17,24 whereas others found no increase. 18,20 In our study, infants of adolescent mothers had a higher risk of early neonatal death mainly associated to problems of preterm delivery and LBW. In accordance with several studies, we confirmed that adolescents were at no greater risk of fetal death than adult women. 14,18,22,24

Controversy exists in the literature about the factors responsible for higher rates of adverse pregnancy outcomes in adolescent pregnancy. Socioeconomic factors associated with young age, such as inadequate prenatal care, poverty, unmarried status, low educational levels, psychologic stress, and illicit drug use have generally been put forward as the most important determinants of the adolescents’ increased risks of adverse pregnancy outcomes. 19,22 However, there are also studies indicating that young maternal age, independent of socioeconomic factors, increases the risks of poor pregnancy outcomes. 14,18,23,24 The biologic mechanisms underlying the association between decreasing maternal age and a greater risk of adverse pregnancy outcomes remain speculative. It has been attributed to the factor that young adolescent mothers who themselves continue to grow during pregnancy could compete with the developing fetus for nutrients. 3,23 Other biologic factors such as low prepregnancy weight and height, parity, contracted pelvis, and low pregnancy weight gain have been implicated in the poorer pregnancy outcomes in adolescents. 3

Our results could have been influenced by the fact that we were unable to evaluate the effect of some of the above mentioned socioeconomic factors known to affect the risk of adverse pregnancy outcomes because these data were not available from the database. However, because the use of alcohol and illicit drugs is lower among adolescent mothers compared with adult women 3,15 and that some authors 15 have found young maternal age and race/ethnicity do not appear to interact in a manner that produces a differential effect on pregnancy outcomes, it is unlikely these factors influenced our results. Other potential constraints of our study must be considered. The accuracy of specific diagnoses registered in this large database has not been extensively checked, but it is unlikely inaccuracies are more or less frequent in adolescents compared with other age groups. Moreover, Uruguay and Argentina contributed almost 50% of births registered at the database. Thus, our results may not be generalized to the whole of the Latin American adolescents. Finally, it should be emphasized that our study is based on a population coming from developing countries and its findings may not be generalized to other populations.

In conclusion, results of this study provide support that adolescent pregnancy, mainly in mothers younger than 16 years, increases the risk of adverse pregnancy outcomes. By reducing the number of adolescent pregnancies and by providing better prenatal and obstetric care to those adolescents who become pregnant, maternal and perinatal morbidity and mortality in the developing world could be reduced. Currently, we do not yet have a clear solution to the problem of high pregnancy rates among adolescents. A recent systematic review showed that primary prevention strategies do not reduce the rate of pregnancies in adolescent women, delay the initiation of sexual intercourse, or improve use of birth control among adolescent men and women. 25 Thus, interventions to reduce unintended pregnancies among adolescents need to be designed and evaluations of these interventions that follow the adolescents into adulthood should be performed.

References

Cervicovaginal fibronectin improves the prediction of preterm delivery based on sonographic cervical length in patients with preterm uterine contractions and intact membranes

Ricardo Gomez, MD,a Roberto Romero, MD,b Luis Medina, MD,a Jyh Kae Nien, MD,b Tinnakorn Chaiworapongs, MD,c Mario Carstens, MD,a Rogelio González, MD,a Jimmy Espinoza, MD,b Jay D. Iams, MD,d Sam Edwin, PhD,b Iván Rojas, MDa

Center for Perinatal Diagnosis and Research (CEDIP), Sótero del Río Hospital, P. Universidad Católica de Chile, Puente Alto, Chile,a Perinatology Research Branch, National Institute of Child Health and Human Development, NIH, DHHS, Bethesda, Md, and Detroit, Mich,b Department of Obstetrics and Gynecology, Wayne State University School of Medicine, Detroit, Mich,c and Department of Obstetrics and Gynecology, The Ohio State University, Columbus, Ohio d

Received for publication June 14, 2004; revised September 16, 2004; accepted September 23, 2004

Objective: The purpose of this study was to examine the diagnostic performance of ultrasonographic measurement of the cervical length and vaginal fetal fibronectin determination in the prediction of preterm delivery in patients with preterm uterine contractions and intact membranes.

Study design: Ultrasound examination of the cervical length and fetal fibronectin determination in vaginal secretions were performed in 215 patients admitted with preterm uterine contractions (22-35 weeks) and cervical dilatation of ≤3 cm. Outcome variables were the occurrence of preterm delivery within 48 hours, 7 days, and 14 days of admission, delivery ≤32 and ≤35 weeks, as well as the admission-to-delivery interval. Statistical analysis included chi-square test, receiver-operator characteristic (ROC) curve analysis, logistic regression, and survival analysis.

Results: The overall prevalence of preterm delivery ≤35 weeks was 20% (43/215). The prevalence of spontaneous preterm delivery within 48 hours, 7 days, and 14 days of admission, and delivery ≤32 and ≤35 weeks were 7.9% (17/215), 13.0% (28/215), 15.8% (34/215), 8.9% (9/101), and 15.8% (34/215), respectively. ROC curve analysis and contingency tables showed a significant relationship between the occurrence of preterm delivery and both cervical length and fetal fibronectin results (P < .01 for each). Both tests performed comparably in the prediction of spontaneous preterm delivery. However, when fetal fibronectin results were added to those of cervical length (≤30 mm), a significant improvement in the prediction of preterm delivery was achieved.
Conclusion: Fetal fibronectin adds prognostic information to that provided by sonographic measurement of the cervical length in patients with preterm uterine contractions and intact membranes.

The diagnosis of preterm labor remains a clinical challenge. Meta-analysis of clinical trials in which patients presenting with preterm labor were randomized to either placebo or beta-adrenergic agents indicates that 37% of those allocated to placebo delivered after 37 weeks. This implies that placebo has a high rate of efficacy in the treatment of preterm labor or, alternatively, that the diagnosis of preterm labor is difficult. Assessing the probability of preterm delivery is important because the standard clinical interventions, namely tocolysis, steroids administration, and transfer to a tertiary care facility, are potentially risky and expensive.

Three methods are currently available to assess the likelihood of preterm delivery in patients with premature uterine contractions: (1) digital examination of the cervix; (2) cervical sonography; and (3) fetal fibronectin. Previous studies have shown that sonographic cervical length is more effective than digital examination of the cervix. The objective of this study was to determine if the combined use of fetal fibronectin and cervical sonography can improve the prediction of spontaneous preterm delivery in patients presenting with preterm uterine contractions and intact membranes.

Patients and methods

Study design

This is a prospective cohort study of patients admitted between July 1998 and October 2002 to the Sotero del Rio Hospital, Chile, with the diagnosis of increased preterm uterine contractility and intact membranes. Criteria for entry into the study were: (1) singleton gestation; (2) uterine contractility of 3 in 30 minutes, which brought the patient into the hospital; (3) gestational age between 22 and 35 weeks; (4) cervical dilatation \( \geq 3 \) cm by digital examination; (5) intact membranes as determined by sterile speculum examination; and (6) signed informed consent, approved by the Institutional Review Boards of both the Sotero del Rio Hospital and the National Institute of Child Health and Human Development (Table I).

Definitions and study procedures

Patients were diagnosed to have increased uterine contractility in the presence of regular uterine contractions of at least 3 in 30 minutes. Digital examination of the cervix was performed, and the dilatation and effacement recorded. Tocolysis was administered to patients with persistent uterine contractility for at least 2 hours after intravenous hydration. The beta-adrenergic agent fenoterol and, occasionally, magnesium sulfate were used for tocolysis. Magnesium sulfate was used as a second-line agent after fenoterol. Steroids (betamethasone) were administered between 24 and 34 weeks. Endovaginal ultrasonography was performed shortly after admission, around the time of amniocentesis with a 5- to 7.5-MHz transvaginal probe. Patients were asked to empty their bladder before the procedure. Measurements were obtained by orienting the transducer so that the endocervical canal and the internal cervical os were visualized in the same sagittal plane, in the absence of uterine contractions. Three images were obtained, and the one showing the shortest cervical length was used to generate cervical biometric parameters. For fetal fibronectin determinations, fluid was collected from the posterior fornix of the vagina before ultrasonographic and digital examinations were performed and stored at

<table>
<thead>
<tr>
<th>Table I</th>
<th>Demographic characteristics of the study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (mean ± SD, years)</td>
<td>24.7 ± 8.2</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
</tr>
<tr>
<td>Nulliparous (%, n)</td>
<td>45% (97/215)</td>
</tr>
<tr>
<td>Multiparous (%, n)</td>
<td>55% (118/215)</td>
</tr>
<tr>
<td>Prior preterm delivery (%, n)</td>
<td>13% (28/215)</td>
</tr>
<tr>
<td>Gestational age at admission (mean ± SD, weeks)</td>
<td>31.7 ± 2.8</td>
</tr>
<tr>
<td>Gestational age at delivery (mean ± SD, weeks)</td>
<td>37.5 ± 2.8</td>
</tr>
<tr>
<td>Admission to delivery interval (mean ± SD, days)</td>
<td>41.2 ± 28.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table II</th>
<th>Frequency of outcome variables, cervical length and fetal fibronectin results</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall prevalence of preterm delivery ≤ 35 weeks</td>
<td>20% (43/215)</td>
</tr>
<tr>
<td>Spontaneous delivery within 48 hours</td>
<td>7.9% (17/215)</td>
</tr>
<tr>
<td>Spontaneous delivery within 7 days</td>
<td>13.0% (28/215)</td>
</tr>
<tr>
<td>Spontaneous delivery within 14 days</td>
<td>15.8% (34/215)</td>
</tr>
<tr>
<td>Spontaneous preterm delivery ≤ 32 weeks</td>
<td>8.9% (9/101)</td>
</tr>
<tr>
<td>Spontaneous preterm delivery ≤ 35 weeks</td>
<td>15.8% (34/215)</td>
</tr>
<tr>
<td>Cervical length (median, range, mm)</td>
<td>29 (1-58)</td>
</tr>
<tr>
<td>Cervical length &lt; 15 mm</td>
<td>14% (30/215)</td>
</tr>
<tr>
<td>Cervical length ≥ 30 mm</td>
<td>50% (107/215)</td>
</tr>
<tr>
<td>Vaginal fibronectin (+)</td>
<td>24% (52/215)</td>
</tr>
</tbody>
</table>
Figure 1  ROC curve analysis for cervical length results (mm) and vaginal fetal fibronectin determination (ng/mL) in the identification of delivery within (A) 48 hours, (B) 7 days, (C) 14 days, (D) delivery ≤ 32 weeks, and (E) ≤ 35 weeks.
-70°C until assayed with a commercially available immunoassay (Adeza Corp., Sunnyvale, Calif). The details of the assays have been previously described. The sensitivity of the assay was 16 ng/mL. A concentration of 50 ng/mL in the vaginal fluid was indicative of a positive test. However, the absolute concentration of fetal fibronectin was obtained with the immunoassay. The intra- and interassay coefficients of variation were 3.7% and 3.6%, respectively.

### Analysis

Outcome variables were the occurrence of spontaneous preterm delivery within 48 hours, 7 days and 14 days of admission, delivery ≤32 weeks and ≤35 weeks, as well as the admission-to-delivery interval. Proportions were compared with chi-square or Fisher exact tests. Diagnostic indices (sensitivity and specificity) as well as positive and negative predictive values for endocervical length and vaginal fibronectin were calculated. Logistic regression analysis was used to investigate the relationship between the occurrence of spontaneous preterm delivery and various explanatory variables, including the results of both the ultrasonographic examination of the uterine cervix and fetal fibronectin test in the vaginal posterior fornix. A Kaplan-Meier survival analysis was performed to assess the admission-to-delivery interval according to the results of the sonographic cervical length as well as those of the vaginal fetal fibronectin. Patients who delivered preterm for maternal or fetal indications were included in the analysis with a censored time equal to the examination-to-intervention interval.

### Results

#### Clinical characteristics of the study population

Two hundred fifteen patients met the entry criteria for the study. Table I describes the clinical characteristics of the patients enrolled. The mean gestational age at admission was 31.7 weeks (±2.8), whereas the mean gestational age at delivery was 37.5 weeks (±2.8). The overall prevalence of preterm delivery ≤35 weeks was 20% (43/215). The rate of spontaneous delivery within 48 hours, 7 days, and 14 days of admission, delivery ≤32 weeks and ≤35 weeks was 7.9% (17/215), 13.0% (28/215), 15.8% (34/215), 8.9% (9/101), and 15.8% (34/215), respectively.

The median cervical length was 29 mm (range 1-58 mm). The frequency of a cervical length <15 mm, <20 mm, <25 mm, and <30 mm was 14% (30/215), 22% (48/215), 34% (73/215), and 50% (108/215), respectively. Thus, 50% (107/215) of the study population had a cervical length ≥30 mm. The prevalence of a positive...
fetal fibronectin in vaginal fluid was 24% (52/215). The rate of a positive fetal fibronectin was 13% (14/107) among patients with a cervical length $\geq 30$ mm and 35% (38/108) for patients with a cervical length $< 30$ mm ($P < .01$). Moreover, the rate of a positive fetal fibronectin was 19% (36/185) among patients with a cervical length $< 15$ mm and 53% (16/30) for those with a cervical length $\geq 15$ mm ($P < .01$). Using this cutoff, the agreement between cervical length and vaginal fetal fibronectin results was 76% (kappa = 0.26, $P < .01$).

### Relationship between cervical length, vaginal fetal fibronectin, and the occurrence of preterm delivery

The rate of spontaneous preterm delivery within 48 hours, 7 days, and 14 days of admission, delivery $\leq 32$ weeks and $\leq 35$ weeks are displayed in Table II. Contingency tables and receiver-operator characteristic (ROC) curve analysis showed a significant relationship between cervical length results or fetal fibronectin concentration in the vaginal fluid and the occurrence of preterm delivery or impending preterm delivery (within 48 hours and within 7 days). See area under the curve and $P$ values for each outcome in Figure 1, A-D, as well as Tables III and IV.

Patients with a cervical length $< 15$ mm had a higher rate of delivery within 48 hours, 7 days, and 14 days of admission, delivery $\leq 32$ weeks and $\leq 35$ weeks than those with a cervical length $\geq 15$ mm (Tables III and IV). Patients with a cervical length $\geq 30$ mm had a significantly lower frequency of preterm delivery within 48 hours, 7 days, and 14 days of admission, delivery $\leq 32$ weeks and $\leq 35$ weeks than those with a cervical length $< 30$ mm (Tables III and IV). Patients with a positive vaginal fibronectin had a significantly higher frequency of preterm delivery within 48 hours, 7 days, and 14 days of admission, delivery $\leq 32$ weeks and $\leq 35$ weeks than those with a negative test (Tables III and IV).

The likelihood ratios for a positive and negative test for cervical length ($< 15$ mm or $\geq 15$ mm and $< 30$ mm or $\geq 30$ mm, respectively) and vaginal fetal fibronectin were calculated for the different endpoints of the study. The likelihood ratio for a positive test was higher for...
a cervical length < 15 mm than in the case of a cervical length of <30 mm and a positive fetal fibronectin test (for delivery ≤ 35 weeks: 9.2, 2.0, and 3.6, respectively, see Tables III and IV for other outcomes).

Logistic regression analysis showed that sonographic cervical length determinations were significantly associated with the occurrence of delivery within 48 hours, 7 days and 14 days of admission, and delivery ≤ 32 weeks and ≤ 35 weeks. Similarly, vaginal fetal fibronectin results were significantly associated with preterm delivery ≤ 35 weeks, within 7 and 14 days (Table V). Moreover, contingency tables and regression derived

Figure 2  Risk of spontaneous preterm delivery within (A) 48 hours, (B) 7 days, (C) 14 days, (D) delivery ≤ 32 weeks, and (E) ≤ 35 weeks, according to cervical length results and vaginal fetal fibronectin (fFN) determination.
probability plots showed that vaginal fetal fibronectin determination resulted in a modification of the posttest probability of all outcome endpoints when used in combination with cervical length results of $< 30$ mm (Tables VI and VII, Figures 2-4).

### Analysis of the duration of pregnancy according to cervical length and fetal fibronectin results

Survival analysis of the admission-to-delivery interval was examined. Patients with an indicated preterm delivery were included in the analysis with a censored time equal to the admission-to-intervention interval. Indications for delivery included clinical chorioamnionitis, rupture of membranes, placental abruption, fetal distress, fetal demise, and others ($n = 9$). A Kaplan-Meier survival analysis with log rank test was performed to assess the examination-to-delivery interval of the following groups: (1) cervical length $\geq 15$ mm and negative fetal fibronectin; (2) cervical length $\geq 15$ mm and positive fetal fibronectin; (3) cervical length $< 15$ mm and negative fetal fibronectin; and (4) cervical length $< 15$ mm and positive fetal fibronectin. The median survival and 95% confident interval were as follows: (1) 46 days (95% confidence interval [CI] 42-50 days); (2) 32 days (95% CI 22-43 days); (3) 15 days (95% CI 0-48 days); and (4) 2 days (95% CI 0-4 days), respectively ($P < .0001$, Figure 4).

### Discussion

#### Principal findings

The findings of this study indicate that: (1) cervical length is a strong predictor of preterm delivery; (2) a short cervix (defined as a cervical length of less than 15 mm) identifies patients at risk for impending preterm delivery (those who delivered within 48 hours or 7 days of admission); (3) a long cervix (defined as a cervical length of 30 mm or
more) identifies patients at low risk for preterm delivery and impending preterm delivery; (4) a positive vaginal fibronectin test was associated with spontaneous preterm delivery. However, the likelihood ratio of a positive test was substantially lower in a positive fetal fibronectin than that of a short cervix (eg, 3.6 vs 9.2 for delivery ≤35 weeks, see Table III); and (5) the combined use of sonographic cervical length and a vaginal fetal fibronectin test improved the prediction of preterm delivery over that provided by each test alone. This effect was observed when the cervical length was less than 30 mm.

### Cervical length and the prediction of spontaneous preterm delivery

Previous studies focusing on patients with preterm labor and intact membranes have indicated that sonographic cervical length is a powerful predictor of the likelihood of spontaneous preterm delivery. Table VIII summarizes the studies reported to date. Iams et al⁴ proposed that a long cervix (defined as 30 mm or more) will identify patients at low risk for preterm delivery (negative predictive value for delivery <36 weeks of 100%; prevalence 40%), whereas Gomez et al⁵ noted that a short cervix, defined as a cervical length of 18 mm or less, was associated with a high rate of preterm delivery (positive predictive value of 67% for preterm delivery <36 weeks; prevalence: 37%). Other studies have used different cutoff values, starting with the work of Murakawa et al,¹⁵ who first reported the use of cervical ultrasound in patients with threatened preterm labor. The results of the current study largely confirm these observations with a larger sample size. The selection of 30 mm and 15 mm was based on ROC curve analysis. The likelihood ratios for a negative test (using cervical length ≥30 mm) were 0.2, 0.1, 0.2, and 0.2 for delivery ≤35 weeks, ≤32 weeks, within 7 days, and within 48 hours, respectively. Conversely, the likelihood ratios for a positive test (using cervical length <15 mm) were 9.2, 14.3, 8.7, and 6.7, respectively, for the same outcomes (see Tables III and IV). These observations, coupled with the results of previous studies indicating that cervical length is superior to digital examination (effacement and dilatation) to predict preterm delivery,⁴,⁵,¹³ have led us to conclude that cervical sonography could be used in patients admitted with preterm uterine contractions to assess the likelihood of preterm delivery. Sonographic cervical length is more objective and reproducible than digital examination of the cervix, providing an image that allows serial observations. Previous studies indicate that cervical sonography is acceptable to patients,¹⁶ and we see no advantage in continuing to rely on digital examinations in units where ultrasound is available and individuals are trained to perform this simple test.

### Vaginal fetal fibronectin and spontaneous preterm delivery

Multiple studies have provided evidence that a positive fetal fibronectin is a predictor of preterm delivery in patients presenting with preterm uterine contractions.⁶,¹¹,¹⁷-²⁵ Lockwood et al²⁹ were the first to produce evidence in support of this. Recent reviews concluded that a negative fetal fibronectin test identifies patients at low risk for preterm delivery, although a positive test has a limited positive predictive value.³⁰,³¹ Our results are consistent with these findings as the negative predictive value was 92% for delivery ≤35 weeks, and the positive predictive value was 40% for this outcome. The likelihood ratios for the identification of impending preterm delivery are displayed in Table IV. Clearly, the performance of a positive vaginal fetal fibronectin test is limited, which contrasts with that of sonographic cervical length. Further studies are required to determine if the use of different cutoffs for vaginal fetal

---

Table VIII: Summary of studies about the role of cervical length by transvaginal ultrasound in women with symptoms of preterm labor and singleton pregnancies

<table>
<thead>
<tr>
<th>Authors</th>
<th>n</th>
<th>Gestational age (weeks)</th>
<th>Cut-off (mm)</th>
<th>Definition of PTD (weeks)</th>
<th>Prevalence of PTD (%)</th>
<th>Sensitivity (%)</th>
<th>Specificity (%)</th>
<th>PPV (%)</th>
<th>NPV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Murakawa et al (1993)</td>
<td>32</td>
<td>18-37</td>
<td>&lt;20</td>
<td>&lt;27</td>
<td>34</td>
<td>27</td>
<td>100</td>
<td>100</td>
<td>72</td>
</tr>
<tr>
<td>Iams et al (1994)</td>
<td>60</td>
<td>24-35</td>
<td>&lt;30</td>
<td>36</td>
<td>40</td>
<td>100</td>
<td>44</td>
<td>55</td>
<td>100</td>
</tr>
<tr>
<td>Rizzo et al (1996)</td>
<td>108</td>
<td>24-36</td>
<td>≤20</td>
<td>37</td>
<td>43</td>
<td>68</td>
<td>79</td>
<td>71</td>
<td>76</td>
</tr>
<tr>
<td>Rozenberg et al (1997)</td>
<td>76</td>
<td>24-34</td>
<td>≤26</td>
<td>26</td>
<td>26</td>
<td>75</td>
<td>73</td>
<td>50</td>
<td>89</td>
</tr>
<tr>
<td>Cetin et al (1997)</td>
<td>65</td>
<td>26-35</td>
<td>&lt;30</td>
<td>37</td>
<td>74</td>
<td>100</td>
<td>46</td>
<td>58</td>
<td>100</td>
</tr>
<tr>
<td>Hincz et al (2002)</td>
<td>82</td>
<td>24-34</td>
<td>≤31</td>
<td>≤28</td>
<td>17</td>
<td>100</td>
<td>47</td>
<td>28</td>
<td>89</td>
</tr>
<tr>
<td>Tsoi et al (2003)</td>
<td>216</td>
<td>24-36</td>
<td>≤15</td>
<td>Within 7 days</td>
<td>8</td>
<td>94</td>
<td>86</td>
<td>37</td>
<td>99</td>
</tr>
</tbody>
</table>

PTD, Preterm delivery; PPV, positive predictive value; NPV, negative predictive value.
fibronectin concentration may improve the clinical value of the test.

**The combined use of fetal fibronectin and cervical length**

The most important observation of this study is that the combined use of sonographic cervical length and fetal fibronectin improves the diagnostic performance of each test. Our observations are in contrast to those reported by Rozenberg et al., but are in broad agreement with those of Rizzo et al. and Hincz et al.

A limitation of the present study is that patients with persistent contractions received tocolysis, a potential confounder for the outcomes “delivery within 48 hours” and “delivery within 7 days.” However, we did not observe a significant effect of tocolysis in our logistic model to predict preterm delivery when adjusting for the effect of other explanatory variables. Moreover, the performance of both cervical length and fetal fibronectin in the prediction of “delivery within 14 days,” a variable not affected by the potential confounder effect of tocolysis, was comparable to others endpoints.

It is of interest that a positive or negative fetal fibronectin test improved the performance of sonographic cervical length only when the sonographic cervical length was less than 30 mm. The practical consequence of this fact is that patients can be screened with cervical sonography, and testing with fetal fibronectin may be restricted to those with a sonographic cervical length below 30 mm. Because approximately 50% of patients presenting with preterm contractions have a cervical length of 30 mm or more, this approach will reduce the number of fetal fibronectin tests performed. On the other hand, below 30 mm, the posttest probability of cervical length in the prediction of spontaneous preterm delivery or impending delivery (48 hours and 7 days) is affected by the result of the vaginal fetal fibronectin test. This information can be of clinical value when deciding the threshold which justifies the administration of steroids as well as patient transfer to a tertiary care facility. Further studies are required to test these recommendations in clinical practice.

**References**


Plurality-dependent risk of respiratory distress syndrome among very-low-birth-weight infants and antepartum corticosteroid treatment

Isaac Blickstein, MD,a,* Eric S. Shinwell, MD,b Ayala Lusky, MSc,c Brian Reichman, MBChB,c,d in collaboration with the Israel Neonatal Network

Departments of Obstetrics and Gynecologya and Neonatology,b Kaplan Medical Center, Rehovot and the Hadassah-Hebrew University School of Medicine, Jerusalem, Israel, Women and Children Health Research Unit, Gertner Institute, Sheba Medical Center, Tel Hashomer, Israel,c and Sackler School of Medicine, Tel Aviv University, Tel Aviv, Israeld

Received for publication August 18, 2004; revised October 6, 2004; accepted October 14, 2004

KEY WORDS
Respiratory distress syndrome
Corticosteroid
Twins
Triplets

Objective: The purpose of this study was to determine the effect of antenatal corticosteroids on the incidence of respiratory distress syndrome in singleton infants and multiple infants who weigh <1500 g and are delivered at 24 to 32 weeks of gestation.

Study design: The incidence of respiratory distress syndrome was established in 4754 singleton infants, 2460 twin infants, and 906 triplet infants.

Results: The incidence of respiratory distress syndrome ranged from 58.2% among singleton infants who received a complete course of antenatal corticosteroid to 81.5% in triplets without any treatment. Complete treatment significantly reduced the incidence of respiratory distress syndrome, compared with partial or no treatment (odds ratio, 0.2-0.6). The adjusted risk for respiratory distress syndrome among infants who received a complete course of antenatal corticosteroids compared with singleton infants increased with plurality (odds ratio, 1.4 and 1.8 for twins and triplets, respectively).

Conclusion: The effect of corticosteroids decreased with increasing plurality. Irrespective of plurality, a complete course of antenatal corticosteroids significantly reduced the incidence of respiratory distress syndrome, whereas partial treatment had the same effect as no treatment. © 2005 Elsevier Inc. All rights reserved.
corresponding figures for births at <32 completed weeks of gestation were 11.9% and 36.1%, respectively.¹

A decade ago, the consensus panel of the National Institutes of Health on the effect of antenatal corticosteroid therapy for fetal lung maturity on perinatal outcomes concluded that giving a single course of corticosteroids to pregnant women who are at risk for preterm delivery reduces the risk of death, respiratory distress syndrome (RDS), and intraventricular hemorrhage in preterm infants.² Recent collective international data continue to unequivocally support the use and efficacy of a single course of antenatal corticosteroids with the dosage and interval of administration that were specified in the 1994 Consensus statement.³,⁴

The recommended treatment was 2 12-mg doses of betamethasone given intramuscularly 24 hours apart or 4 6-mg doses of dexamethasone given orally 12 hours apart. These arbitrarily selected doses were assumed to deliver corticosteroid concentrations to the fetus that are comparable to physiologic stress levels of cortisol that occur after birth in untreated premature infants in whom RDS develops.² It has been estimated that the proposed doses will occupy 75% of the available corticosteroid receptors in a singleton fetus, which results in a near maximal induction of antenatal corticosteroid receptor-mediated response in fetal target tissues.²

In practice, the recommended dose of corticosteroids is administered irrespective of maternal or fetal mass. Consequently, the benefit of corticosteroids in twin pregnancies has been questioned,⁵ but it was unclear whether the questionable effect is related to inadequate dosing⁵,⁶ or to a lower fetal responsiveness to therapy in a multiple pregnancy.⁷

Because of the ambiguity that is related to the cause for the lack of effect of corticosteroids, we conducted an observational study of a population-based cohort of very-low-birth-weight (VLBW) preterm infants to evaluate the plurality-dependent risk of RDS as related to antepartum corticosteroid treatment.

Material and methods

The Israel National VLBW Infant Database includes >99% of VLBW liveborn infants in all of the country’s 28 neonatal units. Data are collected prospectively on a prestructured form and include parental demographic information, maternal pregnancy history and antenatal care, details on delivery, infant status at birth, diagnoses, procedures and complications during hospitalization, and outcome at discharge. Variables were defined by the scientific committee before data collection and have remained unaltered since. The definitions were based on those of the Vermont-Oxford Trials Network.⁸ The data that are collected by the local investigators is sent to the database coordinator, checked for missing data and logic errors, corrected, completed, and entered into a computerized database. Patient information is cross-checked with the national birth registry, and data from any missing infant is requested from the birth hospital.

We studied all VLBW infants (n = 10,215) who were delivered from 1995 through 2001. We excluded cases (n = 2009 [19.7%] that were delivered at <24 or >32 weeks of gestation, infants with lethal malformations, cases with unknown antenatal steroid therapy status, and infants from quadruplet or quintuplet gestations. RDS was defined by characteristic clinical and radiographic findings together with supplementary oxygen or mechanical ventilation. The severity of RDS was not quantified. Delivery room or early surfactant treatment was available during the study period for the entire population.

Small for gestational age was defined as >2 SD below the mean, based on the data of Usher and McLean⁹ for singleton births. Corticosteroid therapy comprised of 2 12-mg doses of betamethasone or 4 6-mg doses of dexamethasone. Antenatal corticosteroid treatment was defined as “partial” if delivery occurred <24 hours after the first dose or >1 week after the last dose. “Complete” treatment was defined as a delivery that occurred >24 hours and <7 days after a complete course of treatment. In the partial treatment group, the interval between the first dose and birth was not recorded. Delivery room resuscitation included endotracheal intubation or external cardiac massage.

We used SAS software (version 8.2; SAS Institute Inc, Cary, NC) to compare the incidence of RDS by the chi-squared test. We derived crude odds ratio (OR) and 95% CI for the comparison between treatment groups by plurality. We used the generalized estimating equation (GEE) approach to estimate the adjusted ORs for RDS in twins and triplets versus singleton infants by category of antenatal corticosteroid therapy.¹⁰ This method is a generalization of the generalized linear models that takes into account the “within-group” correlation and encompasses logistic and probit regressions, ordinary least-squares, ordinal outcome regression, and regression models. The GEE is used when the data are grouped such that it is expected that observations within the same group would be correlated. The following potential confounders were considered: gestational age, small for gestational age, immediate postpartum resuscitation, mode of delivery, maternal hypertensive disorders, infant gender, and being Jewish or non-Jewish. The adjusted OR for RDS was calculated with singleton infants who received a complete course of corticosteroids as the reference group. Our sample size was sensitive enough to detect an OR of 0.5 with 80% power and an error 0.05 for a reduction in the risk of RDS in preterm infants when compared with singleton infants who completed the course of antenatal corticosteroids. This calculation is based on an
estimated RDS rate of 50\% in these preterm singleton infants who weighed <1500 g and who were delivered at <32 weeks of gestation.

Results

A cohort of 8120 VLBW infants who met the inclusion criteria was included in the study. The incidence of RDS as a function of plurality and antenatal steroid therapy ranged from 58.2\% among singleton infants who received a complete course of therapy to 81.5\% in triplet infants who did not receive antenatal corticosteroids (Table I). Within each treatment group, the comparison revealed no statistically significant differences between the incidence of RDS among singleton infants, twins, and triplets. Within each plurality group, the comparison showed no significant difference between the incidences of RDS when a partial course of corticosteroids or no treatment was given. Conversely, in all plurality groups, complete corticosteroid treatment was associated with a significant reduction in the incidence of RDS compared with partial or no treatment.

The GEE model found that lower gestational age, cesarean birth, maternal hypertensive disorders, and resuscitation increased the risk of RDS (adjusted OR, 1.5, 1.4, 1.5, and 3.4, respectively; all 95\% CIs, >1), whereas being small for gestational age reduced that risk (adjusted OR, 0.8; 95\% CI, <1). After adjustment for these confounders, we found that the risk for RDS increased with plurality whether corticosteroid therapy was complete, partial, or not given at all (Table II). In comparison with the reference group, twin and triplet infants who received a complete course of treatment had a 1.4- and 1.8-fold excess risk of RDS, respectively. Furthermore, within the same plurality group, the risk for RDS increased when the course of corticosteroids was incomplete or absent when compared with a full course of treatment.

Comment

There are 3 main points that are related to antenatal corticosteroid treatment in preterm multiple births. First, in the absence of effective predictors of impending preterm delivery of twin and triplet infants, there is often inadequate time to complete a full corticosteroid course before the unexpected premature birth.\(^{11}\) Second, it has been reported previously that antenatal corticosteroid treatment did not significantly reduce the incidence of RDS in preterm twins and triplets.\(^{3,5,12}\) Finally, expecting mothers of twin and triplet infants have an expanded plasma volume, are often heavier, and carry a 2 to 3 times higher fetal mass compared with singleton gestations. Therefore, one may wonder whether the standard dose of corticosteroids produces adequate therapeutic levels when given to these women.\(^{5}\) This point of view was questioned recently by Jobe and Soll\(^{13}\) who
maintained that a lower total corticoid dose per treatment might be as effective as the current recommended treatment. Jobe’s also hypothesized that there is a fetal-state that determines and characterizes the responsiveness of the fetus to glucocorticoids. The variable response of twins to corticosteroid treatment was explained by a different cause of preterm labor in twin infants, which does not “stress” the fetuses sufficiently for the fetuses to respond to antenatal glucocorticoids.

Our study, albeit observational in nature, consists of a considerably larger sample of twins compared with previous studies and includes, to the best of our knowledge, the first series of triplets. Our data suggest 2 conclusions. First, partial corticosteroid treatment has the same effect on the incidence of RDS as no treatment at all (Tables I and II). This observation was seen irrespective of plurality. Conversely, a complete course of antenatal corticosteroids reduced the incidence of RDS by 40% to 50%, irrespective of plurality. This finding is not in agreement with previous reports. Because the incidence of RDS in our population was similar to that reported by others, our finding could not be attributed to potential differences between populations that were at risk for RDS.

The second observation that was shown in the analysis is that the effect of antenatal corticosteroids in reducing the risk of RDS was less in triplets than in twins and less in twins than in singleton infants (Table II). Because the incidence of RDS was plurality-dependent even within the complete therapy groups, the suggestion that standard treatment in multiples may be inadequate to prevent RDS to the same extent as in singleton infants cannot be rejected. However, as discussed earlier, it is unknown whether this finding is related to inadequate dosing or to a lower fetal “responsiveness” to therapy in a multiple pregnancy. If twin and singleton infants indeed have a different “responsiveness” to therapy, Jobe’s theory and our observation may similarly imply that triplets and twins also have a different “responsiveness” to antenatal steroids. The increased OR for RDS (from 1.9 in singleton infants to 2.6 in twins and 3.1 in triplets), when no treatment was given supports this “plurality-dependent responsiveness” hypothesis. Moreover, because the GEE procedure controlled for confounding variables that may have caused different “responsiveness” (such as gestational age and appropriate fetal growth), this may represent a plurality-dependent different amount of target tissue. This refinement of Jobe’s theory is supported by the definite 2- to 3-fold excess in fetal mass in preterm multiple pregnancy. For instance, the median birth weight of a singleton infant at 40 weeks of gestation is achieved as early as 31 weeks by a pair of twins and by 28 weeks in a set of triplets.

The conclusions of this population-based observational study and the conclusions of other studies on multiple gestations may be affected by the use of infants rather than the pregnancies as the subjects of the study. In such circumstances, it is more likely that members of the same pregnancy will be concordant for RDS. Assessment of infants rather than pregnancies may thus spuriously increase the incidence of RDS in twin and triplet gestations. However, by using the GEE method, we were able to decrease the likelihood of this limitation. In summary, our data show a definite increase in the incidence of RDS in VLBW twins and triplets compared with singleton infants when a complete course of corticosteroids was given. A randomized trial of plurality-adjusted dose corticosteroid therapy may be appropriate to determine the optimal therapeutic regimen in multiple pregnancies. Moreover, such a trial could determine whether antenatal corticosteroids may benefit multiple gestations by improving neonatal adaptation (blood pressure) and decreasing the frequency of intraventricular hemorrhage without changing the incidence of RDS or whether corticosteroids decrease the severity of RDS without changing its incidence.

References

CLINICAL OPINION

Estimation of iatrogenic monozygotic twinning rate following assisted reproduction: Pitfalls and caveats

Isaac Blickstein, MD

Department of Obstetrics and Gynecology, Kaplan Medical Center, Rehovot, and the Hadassah-Hebrew University School of Medicine, Jerusalem, Israel

Received for publication August 29, 2004; revised October 23, 2004; accepted November 4, 2004

The true incidence of monozygosity after assisted reproduction is unknown. Proxy estimations, such as counting the number of monochorionic twins, using Weinberg's differential rule, and counting cases where the number of fetuses exceeds the number of transferred embryos, are less accurate than zygosity assessment in all twins. These methods commonly underestimate the true frequency of zygotic splitting because they disregard like-sexed monozygotic-dichorionic twins, consider twin births rather than twin pregnancies, and do not count the number of higher-order multiples with a monozygotic pair. Because zygotic splitting following assisted reproduction is of biological interest as well as of clinical significance, efforts should be directed to increase the accuracy of zygosity determination. This will improve understanding of the zygotic splitting phenomenon and its relation to pathologic processes.

Almost 20 years ago, an increased frequency of monozygotic twinning was reported in conceptions following assisted reproduction technologies (ART) and ovulation induction. These findings are of biological significance and of clinical and public health importance. First and foremost, no other biological mechanism influencing monozygotic twining was ever identified. Second, and equally important, monozygotic twins are notoriously associated with much higher rates of perinatal morbidity and mortality compared with dizygotic twins. However, many unresolved questions still exist, despite a plethora of publications related to zygotic splitting following ovulation induction and ART.

One of the basic questions—the true incidence of monozygotic twins after ART—is still undetermined because it is difficult to establish zygosity in all multiples. As a result, proxy estimations are frequently used and, sometimes, misused. This paper describes pitfalls and caveats in the estimation of iatrogenic monozygotic twinning following ART.

Monochorionic twinning

The most frequently used estimation of monozygosity counts the number of monochorionic twins, either by antenatal sonography or by postpartum placental examination, in a population of children conceived by ovulation induction and ART. Although all monochorionic twins are monozygotic, and all unlike-sex twins are dizygotic, zygosity remains unknown by clinical means in all like-sex dichorionic twins.

Recent data from the most comprehensive registry of multiples with known zygosity and placentaion, the East Flanders Prospective Twin Study, show that in
spontaneous monozygotic twins, the diagnosis of zygosity cannot be determined without genetic evaluation in as many as 43% of the cases, and therefore, clinical estimates of monozygotic twinning based on chorionicity and fetal sex will miss as many as 1/3 of the monozygotic twins. Following similar arguments, the diagnosis of iatrogenic monozygotic twins cannot be ascertained without genetic studies in nearly half of the cases, and clinical estimates of monozygotic twinning based on chorionicity fetal sex will miss as many as 1/3 of iatrogenic monozygotic twins. Studies using this method inevitably underestimate the true incidence of monozygotic twins because the assessment does not count monozygotic-dichorionic twins. For example, a recent retrospective analysis of 731 pregnancies achieved after various ART procedures used the sonographic diagnosis of monochorionic placentation to establish the incidence of monozygotic twinning. The incidence of monozygotic (in fact monochorionic) twins after in vitro fertilization (IVF), with and without zona pellucida manipulations, was 0.72% and 0.86%, respectively, for an overall rate of 0.95%, twice the spontaneous rate.

Recently, it appears that blastocyst transfers are associated with a significantly increased monozygotic twinning rate compared with day 3 embryo transfers. However, this truism might also be questioned, because the ‘classical’ zygotic splitting model (ie, early splits result in dichorionic placentas) maintains that early splitting (ie, until day 3 post-fertilization) should result in monozygotic-dichorionic twins, whereas zygotic splitting occurring after day 3 post-fertilization (ie, at the blastocyst stage) results in any type of monochorionic twins. It follows that if the trigger for zygotic division occurs in vitro, and if, for whatever reason, blastocyst transfers were indeed associated with increased splitting rates, they are more likely to result in monochorionic twins, whereas zygotic splitting in younger embryos are more likely to result in monozygotic-dichorionic twins. Consequently, if splitting rates are determined by counting the number of monochorionic twins rather than counting the number of all monozygotic twins, blastocyst transfers are likely to erroneously show an increased frequency of zygotic splitting compared with day 3 embryos.

**Weinberg’s rule**

Another method to estimate zygotic splitting after ART is using Weinberg’s differential rule, which states that in any population, the sum total of dizygotic twins is twice the number of unlike-sexed twins. The number of monozygotic twins is then derived from subtracting this sum from the total number of twins. There are strong theoretical arguments for questioning the validity of Weinberg’s rule, and it is currently unknown whether the rule holds or not. Specifically, Weinberg’s rule was never tested in a population of ART infants, where a potential deviation of the sex ratio might exist, and indeed, very recently, a 1995 to 2000 Danish national-based cohort of twins conceived by ART demonstrated a much different proportion of same-sex pairs than naturally conceived twins. Data are currently unavailable, however, to mathematically determine whether the significant difference in sex ratio in IVF twins may significantly impact the estimates of monozygotic twinning by Weinberg’s rule. It follows that, although Weinberg’s rule may be useful as a rule-of-thumb, it cannot be assumed as a basis for establishing true monozygotic twinning rates. With this caveat in mind, results of studies that use Weinberg’s rule to assess monozygotic splitting rates should be interpreted with caution.

**Number of fetuses in excess of transferred embryos**

Zygotic splitting may be also inferred when the number of fetuses exceeds the number of transferred embryos. This methodology, only applicable to IVF procedures, may underestimate the frequency of zygotic splitting because of the above-mentioned inability to differentiate like-sex dichorionic-dizygotic from dichorionic-monozygotic twins without comprehensive zygosity assessment. Moreover, when 2 embryos are transferred and 1 is lost, while the other splits into dichorionic-monozygotic twins, the number of fetuses does not exceed the number of the transferred embryos. It follows that the rate of monozygotic twins based on this method (1.2%, a 3-fold rate compared with spontaneous cases) is likely to be erroneously underestimated.

**Single embryo transfer**

A subtype of the former method to estimate zygotic splitting assumes that if multiples occur following a single embryo transfer in an IVF procedure, they are very likely to be monozygotic. It should be acknowledged that this assumption ignores the theoretical contribution of superfecundation, ie, that 1 twin is conceived via IVF and the other by spontaneous conception occurring some time after the IVF procedure. However, given that all follicles are generally aspirated during oocyte retrieval, the likelihood of a subsequent (same cycle) spontaneous ovulation and conception should be very low. On the other hand, estimations using single embryo transfers, by definition, disregard cases of zygotic splitting occurring when more than one embryo is transferred. The magnitude of this omission can be appreciated from the East Flanders Prospective Twin Study, reporting on as many as 11.4% dizygotic iatrogenic triplets, in which 1 of the 2 zygotes underwent splitting into monozygotic twins, and survived to the stage of triplet birth. It follows that using single embryo transfers to estimate the frequency of zygotic
splitting may, in fact, underestimate this frequency because the potential contribution of superfecundation seems negligible compared with the omission of dizygotic triplets.

Our group was the first to estimate zygotic splitting by single embryo transfer in a clinic-based dataset of IVF pregnancies. The results, derived from a rather small and underpowered series, suggest that monozygotic splitting was 12 times higher (4.9%) than that observed among spontaneous pregnancies.16 Moreover, this study considered twin pregnancies rather than twin births, a difference that should always be considered and highlighted in every discussion on iatrogenic monozygotic twinning. In an attempt to diminish the potential beta-error, we evaluated a population-based dataset provided by the British Human Fertilization and Embryology Authority, and found an incidence of 2.3% monozygotic twins among IVF conceptions, a figure 6 times higher than after spontaneous pregnancies as quoted in the literature.17

It should be noted that the above-mentioned analyses16,17 do not refer to elective single embryo transfers, as the data were collected before this practice gained popularity in some countries. These single embryo transfers were probably performed in circumstances where only 1 embryo was available (or suitable) for transfer. It follows that a selection bias may exist when the best embryo among other good embryos is selected for transfer in elective procedures, and that this bias may be of a different magnitude in nonelective procedures, when only 1 embryo is considered transferable.

Complete zygosity assessment

The most accurate incidence, the so-called ‘gold standard’ of zygotic splitting after assisted reproduction, is derived from large series with complete zygosity assessment (ie, various genetic markers, including DNA “fingerprinting” analysis in all same-sex dichorionic twins).5 This is the case with the East Flanders Prospective Twin Study, in which all like-sexed dichorionic twins undergo complete zygosity assessment. Using these data,5 the overall frequency of monozygotic twinning following all assisted conceptions—4.5%—was 10 times the spontaneous rate (0.45%). Interestingly, the frequency following IVF procedures (2.6%) was 6-fold the spontaneous rate, in full accord with estimates derived from single embryo transfer pregnancies.17

Unanswered questions

Data from the East Flanders Prospective Twin Study suggest that monozygotic twins comprise about 5% to 7% of all iatrogenic twins.5 If this figure is used for extrapolation from the United States ART figures,18 at least 500 to 700 iatrogenic monozygotic multiple sets were born during 2001. Because these figures relate to multiple births, it is entirely unknown how many monozygotic twins or higher-order multiples, including monozygotic twins, were actually conceived by ART but lost throughout gestation. Considering the fact that monozygotic pregnancies are associated with increased embryonic and fetal loss rates, it is likely that the phenomenon of monozygotic twinning among ART birth is even higher than generally estimated.

The potential for an adverse outcome significantly increases from monozygotic-dichorionic to monozygotic-monochorionic, and among the monochorionic twins, from monochorionic-diamniotic to monoamniotic twins. Obviously, ART procedures that are likely to result in monochorionic twins carry a higher risk for adverse perinatal outcome. It is, therefore, essential to assign the risk for a specific chorionicity setting to a specific ART procedure.

Finally, although it is true that when more embryos are available for a given woman, and there is a greater chance that one will undergo splitting [15], the magnitude of the risk of zygotic splitting following transfer of more than one embryo is unknown. Specifically, it is unknown whether the presence of more than one developing embryo increases or decreases the survival of codeveloping monozygotic twins.

Comment

The fact that ART is associated with increased incidence of zygotic splitting is generally accepted. Consequently, the occurrence of monozygotic twins should be considered among the most serious side effects of infertility treatment. From the scientific perspective, information about the incidence of monozygotic twinning following ART is important to understand the phenomenon of zygotic splitting in vitro, as well as in vivo. In contrast, for clinical purposes, the most critical information is the incidence of subset of monochorionic twins, who carry a substantially higher risk of adverse perinatal outcome compared with dichorionic twins (irrespective of zygosity).4

Given the potential pitfalls in estimating the incidence of zygotic splitting following ART that are discussed in this paper, it is my opinion that both research directions should be pursued simultaneously. In simple terms, this would imply that chorionicity should be sonographically determined during the first trimester in all iatrogenic conceptions, and that all iatrogenic same-sex twins will undergo postpartum zygosity determination by DNA “fingerprinting.” Although the latter is mainly for scientific purposes and has almost no clinical indication, the price tag of zygosity determination (using umbilical cord blood, saliva, or buccal smears) is infinitesimally small compared with the cost of ART procedures. In addition, it has been established that the parents and the twins themselves wish to ascertain identity.19
Currently, 2 theories are proposed to explain the occurrence of monozygotic twins. According to the first and more extensively cited theory, monozygosity is a result of a hatching event, caused by a breach in the integrity of the zona pellucida, herniation of the blastomeres, and splitting of the embryo. Such a breach in the zona pellucida may be caused by ART procedures such as intracytoplasmic sperm injection and assisted hatching. An alternative hypothesis, proposed by Hall in 1996 suggests that developmental discordance between adjacent cells might cause repulsion that would lead to splitting of the early embryo. In the context of this paper, the information about zygosity would help to establish the common denominator for the numerous different ART procedures, which, regrettably, are not available even from the most elaborate data from the East Flanders Prospective Twin Study.

Undoubtedly, accurate zygosity determination may enhance our understanding of early human development. For example, data from the East Flanders Prospective Twin Study show that the frequency of monozygotic pregnancies following ovulation induction (6.4%) was 14-fold the spontaneous rate, and more than twice higher than the frequency of monozygotic pregnancies following IVF procedures (2.6%). Moreover, the fact that both ovulation induction and IVF procedures are associated with zygotic splitting may lead to the logical conclusion that the common denominator for zygotic splitting resides in vivo rather than in vitro, and cast serious doubt about the blastomere herniation theory as an explanation for zygotic splitting. The doubt about the ‘hatching’ theory is increased by recent observations, suggesting that the frequency of monozygotic twinning among IFV patients is not different between zygotes conceived with or without zona manipulation.

Recent vital statistics from several countries demonstrate a continuing steady increase of twin birth rate since the early 1980s. In the US, the annual twin birth rate rose 3% in 2002 to 31.1 per 1000 total live births, representing an increased twinning rate of 38% since 1990, and 65% since 1980 (18.9 per 1000 total live births).

Because this increase is primarily due to infertility treatments, it is expected that the incidence of iatrogenic monozygotic twins will increase as well. This fact alone reemphasizes the need to join forces to create an active national or international research team that may provide the clue for the enigmatic phenomenon of monozygotic twinning.

References
Asthma controller therapy during pregnancy

Joan C. Gluck, MD,a Paul A. Gluck, MDb,*

Florida Center for Allergy and Asthma Care,a and University of Miami School of Medicine,b Miami, Fla

Received for publication April 26, 2004; revised July 15, 2004; accepted July 27, 2004

KEY WORDS
Pregnancy
Asthma
Controller therapy
Maternal and fetal outcomes

Objective: This study was undertaken to educate physicians on the safety of asthma controller use during pregnancy.

Study design: A comprehensive literature search using MEDLINE, the Cochrane Controlled Trials Register and Database of Systematic Reviews, EMBASE, and selected bibliographies identified human gestational studies of asthma controller medications from which maternal and fetal outcomes were obtained. The US Food and Drug Administration (FDA) pregnancy category ratings were identified from product package inserts.

Results: Human gestational studies were identified for the inhaled corticosteroids (ICSs) beclomethasone, budesonide, and triamcinolone and for cromolyn sodium, theophylline, and salmeterol. Human pregnancy data support an FDA Pregnancy Category B rating for budesonide. Pregnancy Category B ratings for cromolyn, nedocromil, montelukast, and zafirlukast are based primarily on safety in animal reproduction studies. ICSs other than budesonide, theophylline, zileuton, and long-acting β2-adrenergic agonists are Pregnancy Category C.

Conclusion: Human pregnancy data for many asthma controllers are lacking; nonetheless, data support a range of choices among medications rated Pregnancy Category B.

© 2005 Elsevier Inc. All rights reserved.

Up to 7% of pregnant women may be affected by asthma, making asthma one of the most common medical conditions complicating pregnancy.1 During pregnancy, approximately one third of asthmatic women experience an exacerbation.2,3 Women who have more severe disease before pregnancy appear more likely to experience worsening asthma during pregnancy.4,5

Prospective and retrospective studies have confirmed that severe or uncontrolled asthma during pregnancy may result in adverse maternal and fetal outcomes. Increased maternal complications, including preeclampsia,6-8 gestational diabetes,6,9 preterm labor,9 vaginal hemorrhage,10 placenta previa,7 and cesarean delivery,7,9 have been described. Adverse fetal outcomes include increased risk of perinatal mortality,11 intrauterine growth restriction,12 preterm birth, and low birth weight.6,9

In contrast to perinatal outcomes associated with uncontrolled asthma, adequately controlled asthma is not associated with adverse maternal and fetal outcomes.3,12-14 One prospective controlled study compared 486 actively managed pregnant asthmatic women with...
486 pregnant nonasthmatic controls. With the exception of chronic hypertension, no significant differences in maternal or fetal outcomes were demonstrated between the asthmatic and nonasthmatic women. Similarly, a longitudinal study that followed 182 pregnancies complicated by asthma over a 10-year period demonstrated no significant differences in antenatal course, labor, delivery, or perinatal outcomes in women with well-controlled asthma compared with a control group of 364 pregnant nonasthmatic controls.

Asthma is a disease of chronic airway inflammation and remodeling, with acute episodes of bronchospasm. Asthma severity is classified according to clinical features before treatment (Table I). Treatment of asthma involves the use of controller medication to minimize the inflammatory airway response and rescue medication to reverse bronchospasm.

Recognizing the need to control asthma during pregnancy, the National Asthma Education Program (NAEP) Working Group on Asthma and Pregnancy issued consensus recommendations in 1993 that stressed aggressive asthma treatment in pregnant women and advocated daily controller medication for those with persistent asthma. The findings that 49% of pregnancies occurring in the United States in 1994 were unplanned and 28% of women 15 to 44 years had had at least 1 unplanned birth in their lives warrant similar aggressive asthma management for all women of childbearing age. Overall, the NAEP Working Group has concluded that medications used to control asthma during pregnancy are generally safer for both the mother and the fetus compared to the risks of uncontrolled asthma.

In 2000, the American College of Obstetricians and Gynecologists (ACOG) and the American College of Allergy, Asthma and Immunology (ACAAI) Position Statement incorporated recommendations of the 1993 Working Group on Asthma and Pregnancy with updated information on newer asthma medications and the 1997 National Asthma Education and Prevention Program’s (NAEPP) new framework for asthma severity. The joint ACOG-ACAAI recommendations for the use of asthma medications during pregnancy are shown in Table II.

In addition to consensus recommendations, the US Food and Drug Administration (FDA) pregnancy category ratings for asthma controller medications are available to aid physicians in prescribing medications safely during pregnancy. The current system comprises 5 pregnancy categories (A, B, C, D, X) defined by the degree to which available clinical and preclinical data rule out risk for the fetus (Table III). The categories are based on the availability of human versus animal data, whether human studies are adequate and well controlled, and the observed risk to the fetus. All asthma controller medications fall within pregnancy categories B and C. To obtain a Pregnancy Category B rating, medications demonstrating fetal risk in animal studies must have sufficient data in pregnant women (by adequate or well-controlled human studies) to suggest that potential for harm to the human fetus is remote. Medications with demonstrated risk in animal reproduction studies (or a lack of animal studies) and no adequate and well-controlled human pregnancy studies are rated Pregnancy Category C, meaning that they should be used during pregnancy only if the potential benefit justifies the potential risk to the fetus.

The prevalence of asthma is increasing in women of childbearing age. Although maternal and fetal risks associated with uncontrolled asthma during pregnancy underscore the need for guideline-recommended controller therapy for pregnant women and those likely to become pregnant, safety concerns over the use of these medications may limit their use during pregnancy. In an effort to guide physicians on the safety of asthma controller medications, available pregnancy outcomes data for individual medications are provided, with a discussion of how these data support FDA pregnancy category ratings and consensus treatment guidelines.

Material and methods

Published English-language studies of asthma controller medications in pregnant patients were identified through searches of MEDLINE (1966-February 2003), the Cochrane Controlled Trials Register and Database of Systematic Reviews (1988-February 2003), and EMBASE (1988-February 2003). An updated search of MEDLINE through June 2004 was also conducted. Classes of asthma controller medications (ie, inhaled corticosteroids [ICS], leukotriene modifiers, and long-acting β2-adrenergic agonists) and individual medications (ie, budesonide, beclomethasone, betamethasone, fluticasone, flunisolide, triamcinolone, montelukast, zafirlukast, zileuton, formoterol, salmeterol, cromolyn, nedocromil, and theophylline) were used as search terms in combination with the terms asthma and pregnancy. No restrictions were placed on search fields, which included title, abstract, subject headings, key words, and MeSH terms. Bibliographies of all located articles were searched for additional pertinent studies, and product package inserts were reviewed for pregnancy-related data.

All published studies that reported perinatal outcomes with specified controller medications were included. No specific outcome (maternal or fetal) was targeted, rather any reported outcome was considered, and the time of exposure to controller medication(s) during pregnancy has been provided when available. To present a complete review of the literature, published studies were not excluded on the basis of study design, patient number, or any other measure of study quality.
Included studies have been rated using criteria set forth by the Oxford Centre for Evidence-Based Medicine. According to these criteria, the highest level of evidence, which is consistent with a grade A recommendation, is reserved for systematic reviews (with homogeneity) of randomized controlled trials, individual randomized controlled trials with narrow confidence intervals, and all-or-none studies. Grade B evidence includes systematic reviews (with homogeneity) of cohort studies, individual cohort studies (including low-quality randomized controlled trials), outcomes research, and extrapolation of data from grade A studies. Given that prospective, randomized, controlled trials designed to evaluate safety of medications in pregnancy cannot ethically be conducted, the studies included in this review are largely cohort or observational studies and are therefore assigned a lower level of evidence. Importantly, a lower Oxford rating does not preclude a study from being considered adequate and well controlled by the FDA.

**Results**

**Inhaled corticosteroids (ICS)**

According to the NAEPP, ICS, because of their anti-inflammatory action, are the most effective class of asthma controller medications and the preferred long-term controllers for persistent asthma of all severities in adults and children. Moreover, studies have shown that ICS significantly reduce the risk of an acute asthma exacerbation when used throughout pregnancy and significantly reduce the rate of asthma-related readmissions for pregnant women when used after discharge from the hospital.

Eight prospective studies, 2 retrospective studies, and 1 study that included both prospectively and retrospectively obtained data on specific ICS use in pregnant women with asthma were retrieved from the literature (Table IV). Three studies involved the use of budesonide and 5 studies involved the use of beclomethasone. One study assessed perinatal outcomes in pregnant patients receiving beclomethasone or triamcinolone acetonide. Two additional studies assessed outcomes with various ICS. Individual ICS were not specified in 1 of these studies, and 20% of patients used more than 1 ICS during pregnancy in the other.

The most extensive studies of ICS use in pregnancy exist for inhaled budesonide. In 1 large prospective population-based study reported by Källén et al., data from the Swedish Medical Birth Registry were used to determine the incidence of congenital malformations in 2014 infants born in 1995 through 1997 to mothers taking inhaled budesonide for asthma in early pregnancy. The observed rate of congenital malformations in infants exposed to budesonide (3.8%, 95% CI, 2.9-4.6) was similar to that in the overall population (3.6%) during the same period. Cases of orofacial cleft and congenital cardiac defect, the most commonly reported major structural anomalies in infants exposed to budesonide (4 and 18 cases, respectively), were similar to the expected numbers (3.3 and 17-18 cases, respectively).

A recent large study of the Swedish registry data assessed the effects of budesonide use on additional fetal outcomes, including gestational age, birth weight, and birth length. In these analyses, identical mean gestational ages were observed between 1,409 female infants exposed to budesonide during early pregnancy and all female infants born in Sweden between 1995 and 1998 (n = 143,017). For 1,559 male infants with early budesonide exposure, mean gestational age was on average 1...
day shorter than that of all males in the study population (n = 150,931). After adjusting for differences in gestational age and mother’s height, mean observed birth weights were marginally but not statistically significantly lower for female and male infants of budesonide users compared with all female and male infants (40 and 20 g lower, respectively), but there was no difference in mean birth length for infants exposed to budesonide compared with those in the study population. Mean gestational age, birth weight, and birth length of infant females (n = 101) and males (n = 106) whose mothers used budesonide throughout pregnancy were also similar to those of all infants in the study population. Moreover, stillbirth and multiple birth rates were similar in all groups.

In summary, these 3 large population-based studies do not demonstrate an increased risk of congenital abnormalities in infants who were exposed to inhaled budesonide during early pregnancy. In addition, in utero exposure to budesonide was not shown to have any clinically relevant effect on other fetal outcomes, including gestational age, birth weight or length, and rate of stillbirth.  

Five studies describing perinatal outcome in 810 women who used inhaled beclomethasone during pregnancy have been published (Table IV). One report summarizing more than 5 years’ experience with inhaled beclomethasone in 600 patients with asthma demonstrated an absence of malformations and abortions in 20 women who became pregnant while receiving an unspecified dose of beclomethasone. More recently, Greenberger and Patterson described the perinatal outcomes of 45 pregnancies in 40 women who used inhaled beclomethasone (mean daily dose, 336 µg) for severe asthma. Thirty-three pregnancies were evaluated prospectively and 12 were evaluated retrospectively. Five episodes of status asthmaticus were reported in the 45 pregnancies, but no maternal or fetal deaths occurred. Preeclampsia occurred in 1 woman and hypertension in 3 women. Compared with the general population, the rate of prematurity delivery was lower for women receiving beclomethasone (9% vs 7.1%, respectively), but the mean birth weight was higher (3233 vs 3131 g, respectively). The prevalence of congenital malformations among infants exposed to beclomethasone (1/43 live births) was within the normal range.  

Supporting these findings are results of the Kaiser-Permanente prospective study of asthma during pregnancy, in which Schatz et al found no significant relationship between major congenital malformations and corticosteroid use in women with asthma who received inhaled (beclomethasone, n = 137; other, n = 12), intranasal (beclomethasone, n = 157; other, n = 21), or oral corticosteroids at any time during pregnancy. In 64 women exposed only to ICS (predominantly beclomethasone), rates of premature birth (7.8%), low birth weight (4.7%), and preeclampsia
(10.9%) were not significantly different from those in unexposed cohorts of more than 1400 women (4.1%, 3.8%, and 7.8%, respectively).

An additional 395 pregnancies with first-trimester beclomethasone exposure were evaluated from the Michigan Medicaid database, which collected data on 229,101 pregnancies with deliveries between 1985 and 1992.\textsuperscript{32} Analysis of Medicaid claims data did not reveal any increase in the risk of total congenital malformations in 395 infants with first-trimester exposure to beclomethasone.

Table IV Human data for ICS use during pregnancy

<table>
<thead>
<tr>
<th>Study</th>
<th>ICS</th>
<th>Number exposed</th>
<th>Outcomes</th>
<th>Grade of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Källen et al\textsuperscript{26,*}</td>
<td>BUD</td>
<td>2014</td>
<td>3.8% congenital malformation rate in exposed infants vs 3.5% general population rate.</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Similar numbers of orofacial cleft, congenital cardiac defect.</td>
<td></td>
</tr>
<tr>
<td>Ericson and Källen\textsuperscript{27,*}</td>
<td>BUD</td>
<td>2534</td>
<td>Identical congenital malformation rates for exposed infants and population at large (3.6%).</td>
<td>B</td>
</tr>
<tr>
<td>Norjavaara and Gerhardsson de Verdier\textsuperscript{28,*}</td>
<td>BUD</td>
<td>2968</td>
<td>Mean gestational age identical for female infants exposed to BUD/1 d shorter for male infants. No increased rates of prematurity, stillbirth, or decreased birth weight or length vs general population.</td>
<td>B</td>
</tr>
<tr>
<td>Brown et al\textsuperscript{29}</td>
<td>BDP</td>
<td>20</td>
<td>No abnormalities or abortions (spontaneous or therapeutic).</td>
<td>B</td>
</tr>
<tr>
<td>Greenberger and Patterson\textsuperscript{30}</td>
<td>BDP</td>
<td>45</td>
<td>Prevalence of congenital malformation within normal range. Higher mean birth weight, lower rate of prematurity with BDP vs general population.</td>
<td>B</td>
</tr>
<tr>
<td>Schatz et al\textsuperscript{31,}</td>
<td>BDP</td>
<td>137</td>
<td>No significant relationship between major congenital malformations and ICS exposure.</td>
<td>B</td>
</tr>
<tr>
<td>Briggs et al\textsuperscript{32,}</td>
<td>BDP</td>
<td>395</td>
<td>RR 1.0 for congenital malformation vs general population.</td>
<td>B</td>
</tr>
<tr>
<td>Dombrowski et al\textsuperscript{33,34}</td>
<td>BDP</td>
<td>14</td>
<td>No clinically important differences in Apgar scores or gestational age between BDP- and TAA-exposed infants. Trend for lower birth weights with BDP vs TAA.</td>
<td>B</td>
</tr>
<tr>
<td>Dombrowski et al\textsuperscript{35}</td>
<td>BDP</td>
<td>199</td>
<td>No difference in perinatal outcomes compared with theophylline-treated patients.</td>
<td>A</td>
</tr>
<tr>
<td>Namazy et al\textsuperscript{36,}</td>
<td>BUD</td>
<td>43</td>
<td>Distribution of birth weight similar to the general population.</td>
<td>B</td>
</tr>
<tr>
<td>Bracken et al\textsuperscript{37,}</td>
<td>Various (unspecified)</td>
<td>176</td>
<td>Incidence of congenital anomalies, low birth weight, and preterm birth similar to the general population.</td>
<td>B</td>
</tr>
</tbody>
</table>

\textit{BUD}, Budesonide; \textit{BDP}, beclomethasone; \textit{FLU}, flunisolide; \textit{FP}, fluticasone propionate; \textit{RR}, relative risk; \textit{TAA}, triamcinolone acetonide.

\textsuperscript{*} Studies from the Swedish Medical Birth Registry.
\textsuperscript{1} Kaiser-Permanente Study.
\textsuperscript{2} Michigan Medicaid Study.
\textsuperscript{3} 20% of patients used >1 ICS.
\textsuperscript{4} Patients not included in totals for specific ICS use.
Sixteen major birth defects (4.1%) were observed compared with 16 expected (relative risk, 1.0).

In a small cohort study, approximately 1.5 years of prospectively collected data were analyzed to assess medication use, asthma control, and pregnancy outcomes in women who used beclomethasone (with or without theophylline; n = 14), triamcinolone acetonide (with or without theophylline; n = 16), or theophylline without beclomethasone or triamcinolone (n = 25), at any time during pregnancy.33,34 Overall, the study reported no anomalies and no clinically important differences in 5-minute Apgar scores or gestational age at delivery for infants of women who used beclomethasone or triamcinolone. Although the mean birth weight of infants exposed to beclomethasone was about 500 g lower than that of those exposed to triamcinolone, the difference was not significant.

A final study was conducted recently by the Maternal-Fetal Medicine Units Network of the National Institute of Child Health and Human Development to compare the effectiveness of oral theophylline (n = 199) and inhaled beclomethasone (n = 199) in the prevention of asthma exacerbations in pregnant women.35 No significant between-group differences were noted for asthma exacerbations or perinatal outcomes (e.g., pre-eclampsia, preterm delivery, hemorrhage, and cesarean delivery). However, more patients receiving theophylline had an FEV1 less than 80% predicted and side effects causing discontinuation of study medication.

Two studies assessing perinatal outcomes with general ICS use during pregnancy have recently been reported.36,37 In a study of 474 pregnant women with asthma receiving various ICS (20% of patients used more than 1 ICS), incidences of low birth weight, preterm birth, congenital anomalies, and infants small-for-gestational age were similar to the general population.36 In a recent prospective study by Bracken et al37 that enrolled 2379 pregnant women in Connecticut and Massachusetts, increasing use of theophylline and systemic steroids increased the likelihood of preterm delivery, but ICS use (individual ICSs not specified) was not associated with increased risk.

The most extensive available human safety data for specific ICS use during pregnancy comprise outcomes with inhaled budesonide. Congenital malformation rates have been assessed in more than 2500 infants exposed to budesonide, and other perinatal outcomes have been assessed in nearly 3000 infants whose mothers used budesonide during pregnancy. Pregnancy safety data for inhaled beclomethasone and triamcinolone, although reassuring, are limited to the outcomes of 810 and 16 exposed pregnancies, respectively. Published human pregnancy data for inhaled fluticasone and flunisolide also are limited (Table IV).

Risk in animal gestational studies has been demonstrated for all available ICS.38-44 However, on the basis of the extensive human data from the Swedish Medical Birth Registry, inhaled budesonide has been changed from Pregnancy Category C to Category B.42,44 All other ICSs carry a Pregnancy Category C rating, based on the lack of adequate and well-controlled human pregnancy studies and observed risk in animal studies.

Cromolyn sodium and nedocromil sodium

These drugs exert their anti-inflammatory effects by stabilizing the mast cell. Three published studies evaluated outcomes in infants of mothers treated with inhaled cromolyn (Table V). An early investigation reported a 1.35% incidence of congenital malformations in 296 term infants of mothers exposed to cromolyn throughout pregnancy (expected rate is 2%-3%).32,45 The Michigan Medicaid study reported 7 major birth defects (3.7%) in infants of 191 mothers treated with cromolyn during the first trimester of pregnancy compared with the expected number of 8.32 Finally, the Kaiser-Permanente study31 detected no significant relationship between cromolyn exposure at any time during pregnancy and the incidence of major congenital malformations in 243 patients exposed to cromolyn. The incidence was 6.2% with cromolyn exposure versus 4.9% without. In the study by Bracken et al,37 there was no increased risk of preterm delivery among 22 women who received a cromone during pregnancy.

Unlike cromolyn, there are no published human gestational studies of inhaled nedocromil, but animal data are reassuring. Animal reproductive studies have shown no evidence of teratogenicity or harm to the fetus with nedocromil.46 Adverse fetal effects with cromolyn (increased resorptions and decreased fetal weight) have occurred only at very high parenteral, not inhaled, doses that produced maternal toxicity.47 Both cromolyn and nedocromil have been rated Pregnancy Category B based primarily on safety in animal reproduction studies.

Leukotriene modifiers

Three leukotriene modifiers, including the receptor antagonists montelukast and zafirlukast and the leukotriene synthesis inhibitor zileuton, are currently approved in the United States for the treatment of asthma. These anti-inflammatory agents protect against bronchoconstriction, reduce asthma symptoms and exacerbations, and improve pulmonary function in patients with mild to moderate persistent disease.48 At present, human pregnancy data for leukotriene modifiers are limited to their reported use by 9 pregnant women in the study by Bracken et al.37 No increased risk of preterm delivery caused by leukotriene modifiers was reported in these women.37

Animal studies show no teratogenicity with montelukast or zafirlukast at doses much higher than the
<table>
<thead>
<tr>
<th>Study</th>
<th>Medication</th>
<th>Number exposed</th>
<th>Outcomes</th>
<th>Grade of recommendation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Wilson</td>
<td>Cromolyn</td>
<td>296</td>
<td>No relationship between cromolyn exposure and congenital malformations.</td>
<td>B</td>
</tr>
<tr>
<td>Briggs et al</td>
<td>Cromolyn</td>
<td>191</td>
<td>RR 0.9 for congenital malformation with cromolyn exposure vs general population.</td>
<td>B</td>
</tr>
<tr>
<td>Schatz et al</td>
<td>Cromolyn</td>
<td>243</td>
<td>No significant relationship between major congenital malformations, preeclampsia, prematurity, or low birth weight and exposure to cromolyn.</td>
<td>B</td>
</tr>
<tr>
<td></td>
<td>Theophylline</td>
<td>429</td>
<td>Theophylline exposure associated with increased rate of prematurity ( P = .034 ). No relationship between theophylline exposure and preeclampsia or stillbirth.</td>
<td>B</td>
</tr>
<tr>
<td>Briggs et al</td>
<td>Theophylline</td>
<td>1240</td>
<td>Possible association with cardiovascular defects, oral cleft, and spina bifida.</td>
<td>B</td>
</tr>
<tr>
<td>Heinonen et al</td>
<td>Theophylline</td>
<td>117</td>
<td>RR 1.3 for congenital malformation with theophylline exposure vs general population.</td>
<td>B</td>
</tr>
<tr>
<td>Stenius-Aarniala et al</td>
<td>Theophylline</td>
<td>212</td>
<td>No relationship between theophylline exposure and length of gestation, birth weight, Apgar score, or perinatal death. Theophylline exposure associated with a higher rate of preeclampsia ( P &lt; .03 ) and jaundice in the newborn infant ( P &lt; .05 )</td>
<td>B</td>
</tr>
<tr>
<td>Park et al</td>
<td>Theophylline</td>
<td>3</td>
<td>Evidence links theophylline exposure with congenital cardiovascular anomalies.</td>
<td>C</td>
</tr>
<tr>
<td>Dombrowski et al</td>
<td>Theophylline</td>
<td>85</td>
<td>Theophylline associated with a lower rate of preeclampsia.</td>
<td>B</td>
</tr>
<tr>
<td>Neff and Leviton</td>
<td>Theophylline</td>
<td>51,830</td>
<td>Theophylline exposure not associated with stillbirth.</td>
<td>B</td>
</tr>
<tr>
<td>Jones et al</td>
<td>Salmeterol</td>
<td>126</td>
<td>Rate of congenital malformations similar to that of the general population. Salmeterol use not associated with spontaneous abortion, premature delivery, or preeclampsia.</td>
<td>B</td>
</tr>
<tr>
<td>Schatz et al</td>
<td>Systemic corticosteroids</td>
<td>130</td>
<td>No relationship between corticosteroid exposure and congenital malformations. Exposure significantly associated with preeclampsia ( P = .022 ) and low birth weight ( P = .005 ).</td>
<td>B</td>
</tr>
<tr>
<td>Czeizel et al</td>
<td>Systemic corticosteroids</td>
<td>1008</td>
<td>Increased incidence of cleft lip/palate and congenital malformations of the ear with corticosteroid exposure.</td>
<td>B</td>
</tr>
<tr>
<td>Rodríguez-Pinilla and Martínz-Frías</td>
<td>Systemic corticosteroids</td>
<td>1184</td>
<td>Increased risk of cleft lip with corticosteroid exposure.</td>
<td>B</td>
</tr>
<tr>
<td>Bracken et al</td>
<td>Long-acting ( \beta_2 )-adrennergic agonists</td>
<td>64</td>
<td>Increased risk for preterm delivery with theophylline or systemic corticosteroid exposure.</td>
<td>B</td>
</tr>
</tbody>
</table>

* Michigan Medicaid Study.
1 Kaiser-Permanente Study.
1 Collaborative Perinatal Project.
1 Hungarian Congenital Abnormality Registry.
1 Spanish Collaborative Study of Congenital Malformations.
maximum recommended human daily doses.\textsuperscript{39,50} In contrast, zileuton has demonstrated adverse effects in fetal rats and a 2.5% incidence of cleft palate in rabbits at a dose equal to the maximum recommended human daily dose on a milligram per meters squared basis.\textsuperscript{51} The leukotriene modifiers zafirlukast and montelukast are rated Pregnancy Category B, based on safety in animal reproduction studies.\textsuperscript{49,50} Zileuton carries a Pregnancy Category C rating that is based on demonstrated risk in animal studies.\textsuperscript{51}

**Theophylline**

Although pharmacokinetic studies have demonstrated compromised clearance of theophylline during pregnancy,\textsuperscript{52,53} a review of data demonstrated no significantly increased risk of total congenital malformation in 4 studies involving 1861 pregnancies with first-trimester theophylline exposure.\textsuperscript{1} Relative risk of total congenital malformations in 3 large prospective studies ranged from 0.9 in the Kaiser-Permanente study\textsuperscript{31} to 1.3 in both the Michigan Medicaid\textsuperscript{32} and Collaborative Perinatal Project\textsuperscript{54} studies; the relative risk was higher (4.8) in a fourth, smaller case-control study (Table V).\textsuperscript{1,55} Analysis of data available for specific malformations in the Michigan Medicaid study suggested an association between theophylline use and cardiovascular defects, oral cleft, and spina bifida.\textsuperscript{32} Isolated cases of congenital cardiovascular defects also have been reported in infants of mothers who used theophylline throughout pregnancy.\textsuperscript{56}

Evaluations of other maternal and fetal outcomes after theophylline exposure have yielded conflicting results. The study by Bracken et al\textsuperscript{57} and the Kaiser-Permanente study\textsuperscript{31} showed increased risk of preterm birth with theophylline exposure. In the Kaiser-Permanente study, the rate of preterm births was 6.0% in exposed versus 3.6% in nonexposed pregnancies ($P = .034$). In the small case-control study, no correlation was demonstrated between theophylline dose and length of gestation.\textsuperscript{55} Similarly, the relationship between theophylline use and preeclampsia is unclear, with 1 case-control study showing a significantly increased risk of preeclampsia with theophylline exposure\textsuperscript{55} and another study showing a decreased risk.\textsuperscript{57} In the Kaiser-Permanente study, theophylline exposure was not associated with an increase or decrease in preeclampsia among 429 women with asthma after adjusting for confounders.\textsuperscript{31} Other adverse perinatal outcomes, including stillbirth, have not been associated with theophylline exposure during pregnancy.\textsuperscript{31,55,58}

Animal reproduction studies show embryotoxicity of theophylline in rats at a dose of 220 mg/kg in the absence of maternal toxicity, but teratogenicity has not been shown in mice or rats at oral doses approximately 2- and 3-fold, respectively, of the recommended human dose.\textsuperscript{59} Theophylline has been rated Pregnancy Category C because of demonstrated risk in animal reproductive studies and a lack of adequate and well-controlled studies in pregnant women.\textsuperscript{59}

**Long-acting $\beta_2$-adrenergic agonists**

Current recommendations for the use of long-acting $\beta_2$-adrenergic agonists as an adjunct to ICS therapy\textsuperscript{15} in patients with moderate and severe persistent asthma are based on the demonstration of greater improvement in some outcomes compared with ICS monotherapy and a desire to use lower doses of ICS.

Although the short-term use of oral or intravenous terbutaline is frequently used for the treatment of preterm labor, a paucity of human data exists on the safety of inhaled long-acting $\beta_2$-adrenergic agonist use during pregnancy. No human gestational studies of formoterol have been published, and only 1 recent clinical investigation of salmeterol has been published in abstract form (Table V).\textsuperscript{60} The latter was part of an ongoing prospective cohort study comparing pregnancy outcomes of 126 women with asthma who used salmeterol during the first trimester or throughout pregnancy with those of 91 women who used short-acting $\beta_2$-agonist alone and 115 nonasthmatic controls. The study reported congenital malformation rates of 4.7%, 3.9%, and 1.9%, respectively, among the groups. No significant differences in birth weight and length, premature delivery, or preeclampsia were reported. About 75% of women in the salmeterol group also used ICS, but controlling for ICS use had no significant effect on reported outcomes. Moreover, a recent study demonstrated no increased risk of preterm delivery among women ($n = 64$) who used long-acting $\beta_2$-adrenergic agonists during pregnancy.\textsuperscript{37}

Both salmeterol and formoterol demonstrate fetal risk in animal models, with delayed fetal ossification and other adverse outcomes at high doses.\textsuperscript{61,62} These agents are rated Pregnancy Category C, based on demonstrated risk in animal models.\textsuperscript{61,62}

**Systemic corticosteroids**

A low daily dose or alternate-day regimen with oral corticosteroids may be required during pregnancy for asthma not controlled by other medications. However, concerns have been raised about the link between oral corticosteroids and preeclampsia, premature birth, and low birth weight.\textsuperscript{8,31,37,63} For example, the Kaiser-Permanente study found that oral corticosteroids were independently associated with an increased risk of preeclampsia (OR, 2.0; 95% CI, 1.11-3.61; $P = .027$) after controlling for confounding variables (Table V).\textsuperscript{31}

Whether systemic corticosteroid use during pregnancy increases the risk for congenital malformations
also is unresolved. A case-control study (n = 56,557) that was based on the Hungarian Congenital Abnormality Registry demonstrated that the use of either systemic or topical corticosteroids during pregnancy conferred little, if any, teratogenic risk to the fetus (Table V). Maternal exposure to oral corticosteroids was similar among healthy controls and children with congenital abnormalities (1.41% vs 1.55%, \( P = .2 \)). Similarly, the previously described Kaiser-Permanente study indicated that the use of oral corticosteroids did not independently increase the risk of congenital malformations. With specific malformations, however, there was an increased risk of cleft lip/palate (OR, 1.27; 95% CI, 0.82-1.96) and congenital abnormalities of the ear (OR, 3.07; 95% CI, 0.82-1.96) in the Hungarian study. The labeling of systemic corticosteroid formulations is consistent with a Pregnancy Category C rating.

**Comment**

Pregnancy category ratings for asthma controller medications are shown in Table VI. Medications rated Pregnancy Category B include nedocromil, cromolyn, and the leukotriene modifiers, montelukast and zafirlukast. Among ICS, only inhaled budesonide is rated Pregnancy Category B. Other ICS, along with theophylline, zileuton, and long-acting \( \beta_2 \)-adrenergic agonists, are rated Pregnancy Category C.

In a joint position statement published in May 2000, the ACOG and ACAAI recommended ICS as first-line therapy for pregnant women with moderate- or severe-persistent asthma. Criteria for classifying asthma severity are shown in Table I. Budesonide and beclomethasone were the preferred ICS for women who were pregnant or likely to become pregnant and were beginning ICS therapy, and budesonide was favored for patients requiring a high-dose ICS. For mild persistent asthma during pregnancy, the ACOG and ACAAI recommended inhaled cromolyn (or nedocromil in patients with a good response before pregnancy) as first-line therapy, substituting an ICS only if treatment with cromolyn or nedocromil proved inadequate (Table II).

The more recent NAEPP guidelines, which were updated in 2002, state that ICS are more effective than cromolyn sodium and are the preferred long-term controller therapy for all severities of persistent asthma. The guidelines consider cromolyn and nedocromil alternative therapies for mild persistent disease. Moreover, as of 2004, the NAEPP specifically recommends ICS as first-choice controller therapy for the treatment of mild-persistent asthma in pregnant women. In their report on the management of asthma during pregnancy, the NAEPP noted the presence of more data for budesonide compared with other ICS. The joint ACOG-ACAAI recommendations for the preferred use of cromolyn in pregnant women with mild persistent asthma are thus not in line with current evidence-based asthma treatment guidelines. The ACOG-ACAAI recommendations also predate and therefore do not reflect the most recent safety data regarding ICS use in pregnancy—specifically, the upgrading of inhaled budesonide to Pregnancy Category B.

In light of the superior efficacy of ICS versus cromolyn and other asthma controller medications, ICSs should be considered the controller medication of choice for all severities of persistent asthma in pregnant women and women likely to become pregnant. Demonstrated safety of inhaled budesonide supports ACOG-ACAAI recommendations for the preferred use of this ICS during pregnancy.

In terms of add-on asthma controller therapies, the ACOG-ACAAI position statement recommends consideration of the leukotriene receptor antagonists zafirlukast and montelukast only in pregnant women with asthma resistant to other treatment who have shown a uniquely favorable response before pregnancy. Adverse effects in animal reproduction studies preclude the use of zileuton during pregnancy. Salmeterol therapy is recommended for consideration in pregnant women with moderate or severe asthma who have responded well to this agent before pregnancy or whose disease is

---

### Table VI: FDA pregnancy categories of asthma controller medications

<table>
<thead>
<tr>
<th>FDA category</th>
<th>Asthma controller medication</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy Category B</td>
<td>Budesonide, Cromolyn, Nedocromil, Montelukast, Zafirlukast</td>
</tr>
<tr>
<td>ICS</td>
<td>Budesonide, Budesonide, Cromolyn, Nedocromil, Montelukast, Zafirlukast</td>
</tr>
<tr>
<td>Leukotriene modifier</td>
<td>Beclomethasone, Flunisolide, Fluticasone, Triamcinolone</td>
</tr>
<tr>
<td>Long-acting ( \beta_2 )-adrenergic agonist</td>
<td>Zileuton, Theophylline, Formoterol, Salmeterol</td>
</tr>
</tbody>
</table>
not adequately controlled with a medium-dose ICS (Table II). Because of its potentially greater effectiveness and tolerability, salmeterol is recommended instead of or in addition to theophylline.

Thus, according to ACOG-ACAAI recommendations, long-acting β₂-adrenergic agonists are the preferred adjunct to ICS therapy in the pregnant patient with asthma. These recommendations concur with those of the NAEPP for the adult asthmatic population on the whole. The updated NAEPP guidelines recommend a low- or medium-dose ICS plus long-acting β₂-adrenergic agonist or medium-dose ICS alone for the treatment of moderate persistent asthma, and high-dose ICS plus long-acting β₂-adrenergic agonist for severe persistent disease. Leukotriene modifiers, along with theophylline, are considered alternative (not preferred) add-on therapies for the treatment of moderate persistent disease.

Similar to consensus guidelines for the asthmatic population on the whole, ACOG-ACAAI joint recommendations for the use of systemic corticosteroids in pregnant patients are limited to regular use in those with severe-persistent disease, if needed, and bursts for active symptoms (Table II). Considering the potential for adverse outcomes associated with uncontrolled severe asthma during pregnancy, the benefits of using systemic corticosteroids, if needed, outweigh the risk.

Conclusions

Uncontrolled asthma in pregnant women can result in perinatal complications and exacerbations, which can be life-threatening for the mother and fetus. Because these risks are greater than those of adverse effects because of controller medication use, women with asthma should receive controller therapy during pregnancy. Although recommendations from the ACOG and ACAAI can serve as a basis for prescribing asthma medications safely during pregnancy, new safety and efficacy data must be considered when treating pregnant women with asthma. Notably, greater effectiveness with ICS compared with cromolyn sodium and status of ICS according to the NAEPP guidelines support the preferred use of ICS for all severities of persistent asthma in pregnant women and women of childbearing age. Although other ICS are probably also safe, extensive human pregnancy data and a Pregnancy Category B rating for inhaled budesonide support the use of this particular ICS during pregnancy.

Results of a recent survey presented in abstract form confirm that obstetricians and gynecologists are in fact more likely to prescribe ICS than any other class of asthma medications, including nonsteroidal anti-inflammatory agents, for their pregnant patients. Although prescribing patterns of these physicians are consistent with NAEPP guidelines, some physicians may prescribe less effective controller medications because of safety concerns. Among the physicians surveyed, 70% were unaware that an ICS was upgraded to Pregnancy Category B. Physicians who treat potentially pregnant women should consider using a Pregnancy Category B asthma controller, taking into account their current medication and its effectiveness.

We acknowledge John Kross, MSc, DMD, and Leslie Sell, PhD, for editorial assistance in the preparation of this manuscript. Paul A. Gluck, MD, is on the Speakers Bureau of Eli Lilly, Berlex, and Proctor & Gamble.

References


68. Ostrom NK, Cruz-Rivera M. Prescription patterns among obstetrics and gynecology physicians (OB/GYNs) for pregnant and non-pregnant asthmatic patients [abstract]. Ann Allergy Asthma Immunol 2003;90:144.
Objective: This study was undertaken to report the outcome of pregnancies achieved after ovarian stimulation, including the use of the aromatase inhibitor, letrozole, for ovarian stimulation.

Study design: A cohort study comparing the outcome of pregnancies achieved after letrozole and other ovarian stimulation treatments with a control group of pregnancies spontaneously conceived without ovarian stimulation.

Results: In 3 tertiary referral centers, there were 394 pregnancy cycles in 345 infertile couples (63 pregnancies with 2.5 mg of letrozole alone or with gonadotropins, 70 pregnancies with 5.0 mg of letrozole, 113 pregnancies with clomiphene alone or with gonadotropins, 110 pregnancies with gonadotropins alone, and 38 pregnancies achieved without ovarian stimulation). Pregnancies conceived after letrozole treatments were associated with similar miscarriage and ectopic pregnancy rates compared with all other groups. In addition, letrozole use was associated with a significantly lower rate of multiple gestation compared with clomiphene citrate.

Conclusion: The favorable pregnancy outcome and low multiple gestation rate of aromatase inhibitors for ovarian stimulation is encouraging for the development of these agents as first-line ovulation induction agents.
ovarian hyperstimulation.\textsuperscript{3,4} This improvement in response to gonadotropin stimulation was not associated with the antiestrogenic effects often observed when CC was used in combination with gonadotropins.\textsuperscript{5} In addition, we reported that the use of an aromatase inhibitor might benefit women with a poor response to ovarian stimulation with gonadotropins.\textsuperscript{4} During these studies, letrozole, was given once a day from day 3 to 7 of the menstrual cycle. In our recent reviews on aromatase inhibitors, we discussed the future avenues for their use for infertility management.\textsuperscript{5-7} Recently, others have reported comparable success rates using aromatase inhibitors for ovarian stimulation.\textsuperscript{8-11}

A healthy infant after an uneventful singleton pregnancy is the goal of treatment for infertile couples. In this study, we report data on the early outcome of pregnancies that were achieved with aromatase inhibitors for ovarian stimulation compared with the outcome of pregnancies achieved after other ovarian stimulation, including CC and gonadotropins, or with pregnancies conceived without ovarian stimulation.

### Material and methods

This was a cohort study that looked at early outcome of pregnancies achieved after treatment with letrozole compared with a control group that included pregnancies achieved spontaneously or after other ovarian stimulation protocols. The treatment groups and the control group were similar in age, duration of infertility, and infertility diagnosis. The study was conducted in 3 tertiary referral academic centers: the Reproductive Biology Unit of Mount Sinai Hospital, the Toronto Center for Advanced Reproductive Technology, and the Montreal Fertility Centre. The first 2 of these clinics were affiliated with the Division of Reproductive Sciences, Department of Obstetrics and Gynecology, the University of Toronto, Canada, whereas the latter clinic was associated with McGill University. Approval for the use of letrozole and for the follow-up of pregnancies achieved after such treatment was obtained from the Institutional Research Board of Mount Sinai Hospital and the Ethics Committee of Montreal Fertility Centre.

We looked at the early pregnancies rates (all positive pregnancy tests) in these infertile couples undergoing cycle monitoring with ovarian stimulation in conjunction with timed intercourse and intrauterine insemination (IUI) during the period from August 1999 until July 2001. The follow-up continued from clinical pregnancy confirmed by ultrasonography until delivery or pregnancy loss.

During the study period, 1650 infertile couples (unexplained infertility, PCOS, or mild male factor) had completed 3045 treatment cycles. The study included the outcome of 394 treatment cycles in 345 patients in which pregnancy was diagnosed by positive pregnancy test (quantitative assay of serum level of beta human chorionic gonadotropin [hCG] > 5 U/mL 2 weeks after timed intercourse or IUI). Pregnancy was achieved after ovarian stimulation in 356 cycles, whereas in 38 cycles pregnancies were achieved spontaneously without ovarian stimulation. Treatment cycles included timed intercourse or IUI while undergoing follicular monitoring with the use of serial measurements of serum estradiol and luteinizing hormone (LH) and transvaginal ultrasonography.

CC, gonadotropins injections, and letrozole were used for ovarian stimulation either alone or in combination (CC plus gonadotropins or letrozole plus gonadotropins). CC (Serophene, Serono, Oakville, Ontario, Canada) was administered orally at a dose of 50 to 100 mg/d from day 3 to day 7 of the menstrual cycle. When combined with gonadotropins, gonadotropin injections (Gonal-F, Serono or Puregon, Organon, Scarborough, Ontario, Canada) were started on the last day of CC administration. Letrozole (Femara, Novartis, East Hanover, NJ), was given orally at a dose of 2.5 mg/d in the 2 Toronto clinics, and 5.0 mg/d in the Montreal clinic, from day 3 to day 7 of the menstrual cycle. When combined with gonadotropins, gonadotropin injections were started on the last day of letrozole administration. When given alone, gonadotropin injections started on day 3 of the menstrual cycle. Both highly purified and recombinant gonadotropins were used at a dose of 50 to 300 IU/d depending on the patient’s clinical profile. HCG (Profasi, Serono, or Pregnyl, Organon) was given subcutaneously at a dose of 10,000 units to trigger ovulation in most of the cycles when an average of 1 to 2 mature follicles (mean follicular diameter > 1.8 cm) were obtained. Pregnancy was diagnosed by beta hCG levels performed 2 weeks from the insemination or timed intercourse day, and pregnancy ultrasound was performed 2 to 4 weeks after a positive pregnancy test to confirm clinical pregnancy by cardiac activity and number of gestational sacs.

### Study groups

Pregnancy cycles were grouped according to the treatment used for ovarian stimulation into: pregnancies after treatment with the aromatase inhibitor, letrozole 2.5 mg/d alone (33 pregnancies) or letrozole 2.5 mg with gonadotropins (30 pregnancies), 5.0 mg/d letrozole alone (70 pregnancies), pregnancies after CC alone (80 pregnancies) or CC with gonadotropins (33 pregnancies), pregnancies after gonadotropins alone (110 pregnancies), and pregnancies achieved without the use of ovarian stimulation (38 pregnancies).

The patients were not randomly assigned and the choice of receiving an aromatase inhibitor for ovarian
stimulation was based on discussion between the patient and the prescribing physician, often after previously failing to conceive with another stimulation protocol. At the end of the study period, analysis of the patients’ characteristics revealed no significant difference among the study groups on age, duration of infertility, or infertility diagnosis. The number of prior treatment cycles and type of insemination (timed intercourse or IUI) were also comparable (data not shown). The empiric use of stimulation treatments was based on decisions usually shared by the treating physician and patient. We did not use strict algorithms for ovarian stimulation in any of these cases and these factors explain, at least in part, the absence of significant differences in patients’ characteristics between the various groups. However, this does not correct the non-randomized design of this study, and a randomized design may result in more homogenous study and control groups. However, because pregnancy outcome and not the pregnancy rate was being examined, we believe that the results of this study provide useful clinical information.

### Statistical analysis

The various outcome measures, including positive pregnancy tests, chemical pregnancies, clinical pregnancies, multiple gestations, and ectopic pregnancies, were expressed as numbers and rates (percentage per treatment cycle). The Student t test, \( \chi^2 \) test, and Bonferroni t test as well as analysis of variance were used where appropriate to analyze the various data among the study groups. \( P \) value less than .05 was considered statistically significant. The statistical tests were performed with SigmaStat for Windows Version 1.0 software (SigmaStat Software HighEdit Professional copyright 1993, MicroHelp Inc, and HeilerSoftware GmbH, San Rafael, Calif).

### Results

The Table shows pregnancy rates per cycle and rates of pregnancy loss (chemical pregnancy, miscarriage, and total pregnancy loss) associated with the various ovarian stimulation treatments. Pregnancies achieved after letrozole use were not associated with increased risk for chemical pregnancies or miscarriage. The rates of total pregnancy loss ranged from 18.2% to 31.3% (5.0 mg/d letrozole treatment and CC treatment, respectively). The rate of total pregnancy loss in spontaneous conceptions without ovarian stimulation was 29%. There was no statistically significant difference in rate of pregnancy loss between letrozole alone (2.5 or 5 mg) or with gonadotropins and other treatment protocols. \( P \) values were .94, .52, .61, and .93 comparing letrozole 2.5 mg with CC, CC plus gonadotropins, gonadotropins, or spontaneous cycles, respectively. \( P \) values were .17, .81, .45, and .33 for letrozole 5 mg and .73, .72, .88, and .88 for letrozole plus gonadotropins for the same comparisons.

The rate of multiple gestation (all multiple gestation cases were twins) was significantly lower with 2.5 mg (\( P \) values were .03, .04, .05, and .05) or 5.0 mg/d (\( P \) values were .02, .03, .04, and .05) letrozole treatment (alone) when compared with all other ovarian stimulation treatments (CC, CC plus gonadotropins, gonadotropins alone, and letrozole plus gonadotropins respectively) as shown in the Figure. CC treatment (alone or with gonadotropins) was associated with the highest multiple gestation rate when compared with the other ovarian stimulation treatments (letrozole alone or with gonadotropins) and gonadotropins alone. There was no significant difference between the rate of multiple gestation after gonadotropin only treatment and letrozole plus gonadotropins treatment. There were no multiple pregnancies achieved without ovarian stimulation.

There were 9 cases of ectopic pregnancies (all tubal ectopic pregnancies), 1 case after CC plus gonadotropin

<table>
<thead>
<tr>
<th>Table</th>
<th>Pregnancy rates and the rates of pregnancy loss (chemical pregnancy, miscarriage, and total pregnancy loss) associated with various ovarian stimulation treatments</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>All started cycles</td>
</tr>
<tr>
<td>CC</td>
<td>994</td>
</tr>
<tr>
<td>Gonadotropins alone</td>
<td>671</td>
</tr>
<tr>
<td>Letrozole (2.5 mg/d)</td>
<td>167</td>
</tr>
<tr>
<td>Letrozole (5.0 mg/d)</td>
<td>432</td>
</tr>
<tr>
<td>CC + gonadotropins</td>
<td>205</td>
</tr>
<tr>
<td>Letrozole + gonadotropins</td>
<td>153</td>
</tr>
<tr>
<td>Spontaneous (no ovarian stimulation)</td>
<td>423</td>
</tr>
<tr>
<td>All cycles</td>
<td>3045</td>
</tr>
</tbody>
</table>
treatment, one case in the spontaneous pregnancy group (no ovarian stimulation), 2 after CC treatment, 2 after gonadotropins alone treatment, and 3 after letrozole plus gonadotropins treatment. There were no cases of ectopic pregnancy after letrozole only treatment regardless of the dose used. There was no statistically significant difference in the rates of ectopic pregnancies among the various ovarian stimulation treatments. The overall low rate of ectopic pregnancy (2.3%) in these patients may be explained by the absence of any demonstrable tubal damage as a criterion for selection for IUI. It is possible that 1 or more cases of ectopic pregnancy could have occurred in the letrozole groups but spontaneously resolved before clinical detection and were counted as cases of chemical pregnancy (total of 21 cases).

Comment

This study reports the outcome of pregnancies conceived after use of an aromatase inhibitor for ovarian stimulation. Pregnancies achieved after treatment with letrozole for ovarian stimulation were associated with comparable rates of pregnancy loss (chemical pregnancy and miscarriage) but with noticeably lower rates of multiple gestations. Interestingly, no ectopic pregnancies were recorded among 103 patients who achieved a pregnancy with either 2.5 mg or 5.0 mg/d of letrozole.

The success of aromatase inhibitors in inducing ovulation may be based on multiple mechanisms of action. We hypothesized that aromatase inhibition results in a temporary reduction of estrogen production, early in the menstrual cycle resulting in the release of the pituitary and/or the hypothalamus from estrogen negative feedback. This interruption of negative feedback results in an increase in endogenous gonadotropin production that stimulates the development of ovarian follicles. Peripherally, at the level of the ovaries, we also postulated that a possible temporary accumulation of intraovarian androgens, as a result of preventing their conversion into estrogens, would enhance follicle-stimulating hormone (FSH) receptor gene expression12,13 leading to an increase in the sensitivity of the ovarian follicles to gonadotropins stimulation.

We believe that the short half-life of the new aromatase inhibitors (2 days)14,15 as well as the reversibility of the aromatase enzyme inhibition results in production of estrogen at more physiologic concentrations during the later part of the follicular phase. This normal increase in estrogen allows healthy development of the follicles as well as the peripheral estrogen-sensitive genital tissues (the endometrium and cervix) and may avoid any undesirable antiestrogenic effects associated with CC treatment. The peripheral estrogen receptor (ER) depletion associated with CC is believed to be responsible for the decreased pregnancy rates, despite high ovulation rates with CC treatment.16,17

It was previously suggested that 5.0 mg/d of letrozole may lead to better follicular development and perhaps higher pregnancy rates.10 In the current study, we failed to observe significant differences in pregnancy rates or outcome between 2.5 mg/d and 5.0 mg/d of letrozole. This finding suggests that 2.5 mg/d of letrozole is sufficient to induce competent ovulation. A large prospective study is required to determine whether 2.5 mg/d or 5.0 mg/d is the optimal dose for ovulation induction.

The rates of pregnancy loss after ovarian stimulation in our series are in concordance with what has previously been reported for infertile patients.18-21 However, the rates of preclinical pregnancy loss (chemical pregnancy) are slightly higher in our series. We believe that this may be due to our study design in which we measured beta hCG levels very early (2 weeks after timed intercourse or IUI). Together with the higher sensitivity of the currently available bioassays for beta hCG, we would anticipate the detection of more early pregnancy losses. A significant number of early pregnancy losses may be missed when hCG is assayed later or when applying a less sensitive hormonal assay as reported in earlier studies.22,23
In this study, CC treatment with or without FSH addition was associated with the highest rate of multiple gestation. The lowest multiple gestation rate was associated with letrozole treatment and is an exciting potential advantage of aromatase inhibitor treatment. In our studies of letrozole for ovarian stimulation, we found CC treatment to be consistently associated with development of more ovarian follicles than with aromatase inhibitor treatment.\(^1,2\) We believe this observation is consistent with the long tissue half-life of CC\(^24\) resulting in long-lasting ER depletion centrally. ER depletion centrally prevents negative feedback suppression of FSH levels by rising E2 from growing follicles and results in multiple follicle development on ovulation. A major advantage of applying aromatase inhibitors for ovulation induction is mono-ovulation, particularly in patients with PCOS. This advantage likely arises from the preservation of the physiologic estrogen feedback mechanisms in the hypothalamus and pituitary caused by the absence of ER depletion with aromatase inhibitor treatment as discussed recently.\(^25\)

Clomiphene treatment was associated with the highest rate of multiple gestations, whereas gonadotropins treatment was associated with much lower multiple gestation rates than expected. We believe that in addition to the nonrandomization design of the study, 2 reasons might also explain the high multiple gestation rate associated with CC treatments: first, the rate of multiple gestation has been calculated per clinical pregnancy rather than per positive pregnancy test. This would increase the rate by decreasing the denominator. Second, multiple gestation was diagnosed on the basis of an early ultrasound (2-4 weeks after a positive pregnancy test). It is known that the rate of multiple gestation decreases with advancing gestational age as sometimes 1 or more of the embryos may vanish away as pregnancy progresses.\(^26-28\) This known phenomenon may lead to a higher rate of multiple gestation when an ultrasound is performed early in pregnancy to determine the number of gestational sacs. On the other hand, the lower than expected multiple gestation rate with gonadotropin treatment may be a function of liberal cycle conversion to in vitro fertilization when stimulation with gonadotropins resulted in a high risk for multiple pregnancy, or to cycle cancellation.

In the current trial, we demonstrate promising clinical pregnancy rates after using an aromatase inhibitor for ovarian stimulation, similar pregnancy outcome compared with other stimulation regimens, and a reduced risk of a multiple gestation. These results suggest that aromatase inhibitors are successful for ovarian stimulation and could possibly be applied in the future as a first-line treatment for World Health Organization (WHO) type 2 anovulation. Moreover, the oral administration of an aromatase inhibitor without the need for close cycle monitoring would enhance the clinical application of these agents for ovarian stimulation in the absence of sophisticated and expensive monitoring for infertility management.

### References


Estrogen therapy and risk of cognitive decline: Results from the Women’s Estrogen for Stroke Trial (WEST)

Catherine M. Viscoli, PhD,a,* Lawrence M. Brass, MD,b Walter N. Kernan, MD,a Philip M. Sarrel, MD,c Samy Suissa, PhD,d Ralph I. Horwitz, MD,e

Department of Internal Medicine, Yale University School of Medicine, a Departments of Neurology and Epidemiology and Public Health, Yale University School of Medicine, and the Veterans Administration Connecticut Healthcare System, b Departments of Gynecology and Obstetrics, Neurology and Epidemiology and Public Health, Yale University School of Medicine, New Haven, Conn; Departments of Epidemiology and Biostatistics and Medicine, McGill University, Montreal, Canada; School of Medicine, Case Western Reserve University, Cleveland, Ohio

Received for publication May 27, 2004; revised August 11, 2004; accepted August 17, 2004

KEY WORDS
Estrogen
Cognitive function
Mini-Mental State Examination
Stroke
Clinical trials
Menopause

Objective: This study was undertaken to assess whether estrogen therapy (ET) reduces the risk of cognitive decline in women with cerebrovascular disease.

Study design: We conducted a randomized, double-blind trial of estradiol 17β versus placebo for secondary stroke prevention in 664 postmenopausal women with a recent stroke or transient ischemic attack. The Mini-Mental State Examination (MMSE) and 5 domain measures were obtained at baseline and exit.

Results: Among 461 women withdrawn alive without stroke, ET did not have a significant effect on cognitive measures after an average of 3 years (relative risk of MMSE decline: 0.74, 95% CI, 0.49-1.13). In women with normal MMSE at entry, estrogen was associated with less decline (relative risk, 0.46, 95% CI, 0.24-0.87).

Conclusion: In this study, estradiol did not have significant effects on cognitive measures. However, in women with normal function at baseline, there may be a benefit for ET in reducing the risk for cognitive decline.

West was supported by a grant (1-RO1-N531251) from the National Institute of Neurological Disorders and Stroke, National Institutes of Health. Additional support and study drug was provided by Mead Johnson Laboratories. Dr Suissa was supported by a Senior Scientist award from the Medical Research Council of Canada and also received research funding from Schering and Organon. Drs Viscoli, Brass, Kernan, Suissa, and Horwitz have received research support from Novartis.

* Reprint requests to: Catherine M. Viscoli, PhD, Yale University School of Medicine, Department of Internal Medicine, 333 Cedar St, Room IE 61 SHM, PO Box 208025, New Haven, CT 06520-8025. E-mail: Catherine.Viscoli@yale.edu

Numerous studies have suggested that estrogen therapy (ET) in postmenopausal women may prevent or retard decline in cognitive function. A meta-analysis of observational studies concluded that ET was associated with a 34% decrease in the risk of dementia (odds ratio, 0.66, 95% CI: 0.53-0.82.) Results of randomized controlled trials (RCTs), however, have not shown a consistent trend for cognitive improvement with estrogen treatment. These conflicting RCT results had been attributed, in part, to small sample sizes, brief durations of subject follow-up, and variations in cognitive function.
measures, treatment regimes, and patient characteristics. Recent reports of 2 large clinical trials have failed to find a beneficial effect of daily estrogen and progestin treatment on cognitive performance or risk of dementia.2-5

The scientific rationale for the potential of estrogen to protect against cognitive decline is based on evidence for beneficial effects on vessels (eg, vasodilatation and increased nitric oxide production and release6), neuronal structures (eg, synaptic and dendritic remodeling7), and psychological processes.8 Estrogen receptors, however, are not uniformly distributed in the brain and ET may not be expected to affect all cognitive domains equally. Earlier studies have pointed out that the hippocampus and amygdala, which mediate learning and memory, are rich in estrogen receptors and may be highly responsive to the effects of ET.9 In addition, brain regions involved in the early phases of neurodegeneration, such as the limbic system, have extensive concentrations of estrogen receptors. The failure of a recent study of unopposed estrogen in women with mild to moderate Alzheimer’s disease to show a beneficial effect of treatment has led to provocative speculation that ET may be effective only when estrogen-sensitive neurons in the brain are still viable and, hence, may have a potential therapeutic role in delaying the onset of cognitive decline rather than in slowing its progression.10

Assessment of the effect of estrogen on cognitive function was a defined secondary aim of the Women’s Estrogen for Stroke Trial (WEST), a randomized, double-blind clinical trial comparing estradiol to placebo in postmenopausal women with a recent stroke or transient ischemic attack (TIA). In a prior publication, we reported that treatment was ineffective in reducing risk for the primary trial endpoints of stroke or death.11 Global and domain-specific measures of cognitive function were assessed in WEST participants and in this report we describe results that are based on the prespecified analytic plan for these data. In addition, we present an exploratory analysis of cognitive effects of estrogen in women with and without normal cognitive ability at entry that was motivated by the hypothesis that estrogen may be effective in reducing the initiation, rather than the propagation of, neurodegeneration.12,13

Material and methods

Study design and intervention

The design and methods of the WEST have been reported.14 Postmenopausal women were enrolled within 3 months of a qualifying nondisabling ischemic stroke or TIA. Women were recruited between December 1993 and May 1998 and were randomly assigned to receive either estradiol-17β (Estrace, Mead Johnson, Evansville, Ind) at the standard replacement dose of 1 mg daily or matching placebo. Approval was obtained from the institutional review board at each participating hospital and all women provided written informed consent. For women with a uterus, a progestin (5 mg medroxyprogesterone acetate) was administered for 12 days each year to screen for evidence of hyperplasia (drug was started after completion of annual cognitive tests.) Primary endpoints were nonfatal stroke and death from any cause. Subjects with recurrent stroke were withdrawn from treatment and further follow-up. All women alive without stroke were withdrawn at the trial closeout over a 5-month period.

Cognitive measures

Principal outcome — Global function

The principal cognitive measure specified by the WEST protocol was change in performance on the Mini-Mental State Examination (MMSE). The MMSE was administered by a research nurse during the initial home visit (average 19 [± 14] days before randomization) and at the end of a subject’s participation (exit).

The MMSE is a test of global cognitive function, composed of 6 subtests of orientation, repetition, attention (best score on counting backward from 100 by 7 for 5 subtractions or spelling “world” backward), recall (3 words from repetition test), language, and design copying.15 The maximum score is 30, and, by consensus, scores of 23 or less are considered suggestive of cognitive impairment, whereas scores of 28 or more define normal cognitive ability.16

Secondary outcomes — Domain measures

To examine the effects of estrogen on specific areas of cognition, 5 domain tests were administered at baseline, annually during follow-up, and at exit. These measures included: (1) Modified Boston Naming Test17 (number of 18 line-drawn figures correctly named), a test of language function and naming ability, sensitive to aphasia, and early word finding difficulties in dementia; (2) Digit Span18 (maximum correct repetition of up to 9 digits recited forward), a test of attention and short-term verbal memory and storage capacity; (3) Word List Generation19 (number of 4-legged animals named in 60 seconds), a test of verbal fluency and semantic memory, sensitive to language and frontal sequencing disorders; (4) Disk Spatial Recognition20 (number of new disks identified without error; best score from 2 series of 17 disks; series stopped after first error), a test of visual recognition memory and ability to learn new visual information; and (5) Delayed Naming21 (recall of items named in Boston Naming Test), a test of incidental memory.

Depression index

The Center for Epidemiologic Studies of Depression (CES-D) scale was administered to participants at
baseline, annually during follow-up, and at exit.22 This index measures graded responses to 20 statements regarding feelings in the prior week. Possible scores range from 0 to 60, with scores above 15 considered suggestive of depression.23

Statistical analyses

Because the primary cognitive measure was change in MMSE from entry to exit, our focus in this report was restricted to subjects withdrawn alive from the trial without stroke. Subjects who died after randomization did not have MMSE measured during follow-up. Moreover, although exit MMSE scores were obtained after nonfatal stroke endpoints, these assessments would be confounded by the effect of the recurrent stroke, making it infeasible to assess the independent effect of estrogen on cognition in these women. Therefore, because treatment assignment did not affect the overall risk of death or stroke recurrence during follow-up, we excluded subjects with these outcomes from the main analysis (29% of estradiol group and 28% of placebo group).

Refusal to perform any cognitive test was scored as an error (zero score imputed). (To assess the effect of imputed scores, analyses were also conducted with exclusion of all refused tests.) Inability to complete a test because of not understanding instructions or aphasia was scored as an error. Tests not completed because of noncognitive impairments (ie, hand weakness, impaired eyesight, or illiteracy) were considered missing. Total MMSE scores were adjusted to account for missing subscores. Subjects who refused the entire MMSE or the complete battery of cognitive tests were excluded from the analysis.

We compared baseline features for treatment groups using $\chi^2$ tests and $t$ tests for categorical and continuous measures, respectively. Baseline differences between treatment groups on each cognitive measure were assessed by the Wilcoxon rank sum test and changes in scores from baseline to exit were evaluated by analysis of variance. Decline on each cognitive measure was defined as a categorical outcome by the following procedure: Within strata defined by baseline score, we calculated the corresponding rank for each change score and defined decline as a ranked score greater than 1 SD from the mean in the direction of worsening (ie, less than $-1$). In an exploratory analysis to assess the effects of treatment in preserving cognitive function, we calculated the risk of decline on each cognitive measure and the risk of development of evidence of cognitive impairment (defined as MMSE $<24$) in subgroups of women defined by the presence or absence of normal cognition at baseline (MMSE $\geq 28$). In addition, we analyzed the response to estrogen in groups defined by the following subject characteristics at entry (yes/no): age less than 70 years, 12th grade education, depressed mood (CES-D $\geq 16$), menopausal complaints (hot flashes a "real problem" in prior month). No differential effects of estrogen were observed (data not shown). Relative risks for estradiol subjects compared with placebo subjects were estimated using generalized linear models (GENMOD procedure) with a log link and binomial distribution specified. For each analysis, any baseline feature that differed between treatment groups ($P$-value of .20 or less) was evaluated for its effect on the treatment parameter estimate. If inclusion of a term changed the unadjusted estimate by 10% or greater, it was retained in a final adjusted model. Year of follow-up (entry to exit) was included a priori in all adjusted models. All models were estimated by using SAS software (SAS 8.02 version for Windows, Cary, NC).

Results

Of the eligible women identified, 28% were randomly assigned into the trial. In a previous report, we demonstrated that the rates of the primary endpoints (stroke and death) were not different for subjects randomly assigned to estrogen or placebo.11 Among the 337 women assigned to estradiol, 48 died and 51 had a nonfatal stroke compared with 41 deaths and 52 nonfatal strokes among the 327 women randomly assigned to placebo. Of the 472 women who were alive without recurrent stroke at the end of the trial, 231 estradiol and 230 placebo subjects are included in the study cohort for the present analysis (1 estradiol subject did not have MMSE measured at entry, and 6 estradiol and 4 placebo subjects refused all cognitive testing at exit).

Overall, compared with subjects who died or experienced a stroke during follow-up, women who completed the trial alive without stroke were significantly ($P < .05$) younger and less likely to have a history of myocardial infarction, congestive heart failure, diabetes, prior stroke, stroke as index event (compared with TIA), residual neurologic deficits (National Institutes of Health severity score [NIHSS] $>1$), and impaired physical performance (physical performance test [PPT] $<17$). Baseline MMSE scores for the study cohort were significantly higher than for the excluded subjects (mean 27.0 $\pm$ 3.6; Wilcoxon $P$-value: .0002).

The mean age of women in the study cohort was 70 years (range 46-90), average education was 12 ($\pm 3$) years and 39% had CES-D scores compatible with depression at entry. Comparison of subjects in the study cohort by randomized treatment assignment revealed no important differences in baseline characteristics, except for use of antithrombotic treatment at entry (Table 1).

The median number of days from the index neurologic event to the baseline MMSE was 34 in the estradiol
subjects and 35 in the placebo subjects. The median number of months from baseline to exit MMSE was 38 in the estradiol subjects and 40 in placebo subjects. The distribution of years of follow-up in the trial was comparable by treatment group ($\chi^2 P$ value .52).

### Cognitive performance

#### Global function

Refusal rates for individual MMSE tests at baseline and exit were less than 1%, except for serial sevens (9 subjects in each treatment group) and spelling “world” backward (7 estradiol and 6 placebo subjects). Non-cognitive impairments (ie, sight, illiteracy, hand weakness) made it impossible for 15 estradiol and 14 placebo subjects to complete at least 1 language subtest, and for 7 subjects in each group to complete the design copy test. Aphasia affected only 2 subjects’ ability to complete an MMSE test item (1 in each treatment group).

MMSE scores ranged from 11 to 30 at entry, with mean baseline values of 26.9 (±3.5) and 27.1 (±3.5) for estrogen and placebo subjects, respectively (Table II). The median MMSE score was 28 in both treatment groups. On average, MMSE scores changed by less than 1 point from baseline to exit (range −21 to +9 points). Scores for women in the estrogen group fell less (experienced less cognitive decline) than for women in the placebo group, but the difference was small and not statistically significant (mean change −0.6 compared with −0.9, $P = .37$; adjusted difference in mean change: 0.18, $P = .56$). Seventy-four women were classified as having had a decline in MMSE. The median change in MMSE for women with decline was −4 compared with zero for women without decline. When we compared treatment groups, 13.4% of estrogen women and 18.7% of placebo women were classified as having a decline in MMSE (adjusted relative risk, 0.74, 95% CI, 0.49-1.13, Table III).

#### Domain measures

Treatment groups did not differ significantly on baseline scores for any of the 5 domain measures (Table II). Compared with the placebo group, average score changes from baseline to exit in the estradiol group were slightly better on Boston Naming (mean change −0.1 ± 2.4 compared with −0.3 ± 2.2, $P = .14$; adjusted difference in mean change: 0.27, $P = .20$) and disk recognition tests (mean change +0.1 ± 4.3 compared with −0.5 ± 4.5, $P = .13$; adjusted difference: 0.64, $P = .13$), were indistinguishable on word list (mean change +0.3 ± 3.6 compared with +0.3 ± 4.2, $P = .98$; adjusted difference: 0.06, $P = .87$) and digit span (mean change −0.1 ± 1.4 compared with −0.2 ± 1.3, $P = .87$; adjusted difference: 0.02, $P = .89$), and slightly worse on delayed recall (mean change +0.8 ± 2.6 compared with +1.3 ± 2.7, $P = .04$; adjusted difference: −0.47, $P = .06$). Risk of decline was lower in estradiol subjects on all domain tests, except for delayed recall, although no difference was statistically significant (Table III).
Depression
The incidence of depressed mood was similar by treatment group. Of estradiol and placebo subjects, 39% had CES-D scores above 15 at baseline. On average, CES-D scores improved slightly during follow-up and the rate of depressed mood at exit was 32% in the estradiol group compared with 28% for the placebo group ($P = .36$). Mean change in CES-D score was 1.9 (±12.7) for estradiol subjects and 2.6 (±11.1) for placebo subjects ($P = .51$). Results were essentially unchanged when subjects reporting hot flashes at baseline were excluded from the analysis of CES-D change. In addition, there was no difference between treatment groups in the use of antidepressant drugs at baseline or exit. For the estradiol group, the proportion of women who remained on, started, stopped, or remained off antidepressant drug treatment between baseline and exit was 9%, 12%, 4%, and 75%, respectively; for the placebo group, the rates were 7%, 13%, 4%, and 76%, respectively.

Effect of estrogen on cognitive function in normal subjects
Among the 268 subjects with baseline MMSE in the normal range (28-30), those assigned to the estrogen group experienced significantly less reduction in MMSE scores than did women in the placebo group (mean change $-0.6 \pm 1.6$ compared with mean change $-1.2 \pm 2.5$, $P = .04$; adjusted difference in mean change: 0.53, $P = .05$). The estrogen group also had a significantly lower rate of decline compared with the placebo group (8.8% compared with 19.6%, adjusted relative risk, 0.46, 95% CI, 0.24-0.87; Table IV). In contrast, no beneficial effect of estrogen was observed in women who scored below normal at baseline (mean change in MMSE in estradiol group, $-0.7 \pm 4.5$ compared with $-0.5 \pm 4.6$ in placebo group, $P = .85$; adjusted difference in mean change: $-0.76$, $P = .25$; rate of decline 18.9% compared with 17.2%, adjusted relative risk, 1.37, 95% CI, 0.73-2.54). For the 5 specific domain tests, similar nonsignificant changes favoring estradiol were restricted to women who scored in the normal range for MMSE at baseline (Table IV).

In the subgroup of normal women at baseline, 2 in the estradiol group (1.6%) and 9 in the placebo group (6.3%) had exit MMSE scores below 24 (adjusted relative risk, 0.25; 95% CI, 0.05-1.18). No protective effect of estrogen was observed in the 135 women who scored 24 to 27 at entry (26.9% of estradiol subjects and 21.0% of placebo subjects had MMSE $\leq 24$ at exit; adjusted relative risk, 1.08; 95% CI, 0.61-1.93).

Comment
In this randomized controlled trial of estradiol for secondary prevention of cerebrovascular disease, among women who survived without a recurrent stroke, initiation of therapy was not associated with significant effects on global or domain-specific cognitive measures over 3 years of follow-up. In this group, MMSE scores fell less and risk of decline on the MMSE was lower in the estradiol group compared with placebo, but these differences did not achieve statistical significance. In an

Table II Baseline cognitive scores for study cohort by treatment group

<table>
<thead>
<tr>
<th>Measure (maximum*)</th>
<th>Cognitive domain</th>
<th>Estradiol group (n = 231) Mean (SD)</th>
<th>Placebo group (n = 230) Mean (SD)</th>
<th>$P$ value$^{1}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE (30)</td>
<td>Global cognition</td>
<td>26.9 (3.5)</td>
<td>27.1 (3.5)</td>
<td>.32</td>
</tr>
<tr>
<td>Boston naming (18)</td>
<td>Language; executive function</td>
<td>16.8 (2.3)</td>
<td>17.1 (1.7)</td>
<td>.32</td>
</tr>
<tr>
<td>Word list (24)</td>
<td>Language; verbal fluency</td>
<td>10.5 (3.9)</td>
<td>10.6 (3.9)</td>
<td>.61</td>
</tr>
<tr>
<td>Digit span, forward (9)</td>
<td>Attention; immediate verbal memory</td>
<td>5.6 (1.3)</td>
<td>5.5 (1.3)</td>
<td>.54</td>
</tr>
<tr>
<td>Disk recognition (17)</td>
<td>Attention; immediate visual memory</td>
<td>8.2 (3.3)</td>
<td>8.6 (3.4)</td>
<td>.18</td>
</tr>
<tr>
<td>Recall (18)</td>
<td>Incidental recall, delayed memory</td>
<td>5.4 (2.4)</td>
<td>5.3 (2.3)</td>
<td>.82</td>
</tr>
</tbody>
</table>

* Maximum score possible for each test (except word list, which is maximum observed).

$^{1}$ $P$ value from Wilcoxon rank sum test.

Table III Cognitive test results by treatment group

<table>
<thead>
<tr>
<th>Cognitive test</th>
<th>Estradiol group (n = 231)</th>
<th>Placebo group (n = 230)</th>
<th>Adjusted relative risk (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMSE</td>
<td>13.4</td>
<td>18.7</td>
<td>0.74 (0.49-1.13)</td>
</tr>
<tr>
<td>Boston naming</td>
<td>14.5</td>
<td>16.5</td>
<td>0.90 (0.58-1.38)</td>
</tr>
<tr>
<td>Word list</td>
<td>16.1</td>
<td>18.8</td>
<td>0.84 (0.56-1.26)</td>
</tr>
<tr>
<td>Digit span, forward</td>
<td>17.9</td>
<td>20.1</td>
<td>0.89 (0.61-1.30)</td>
</tr>
<tr>
<td>Disk recognition</td>
<td>16.2</td>
<td>19.1</td>
<td>0.81 (0.54-1.22)</td>
</tr>
<tr>
<td>Recall</td>
<td>18.5</td>
<td>14.3</td>
<td>1.33 (0.87-2.05)</td>
</tr>
</tbody>
</table>

* Decline defined as a ranked score change (exit-baseline) greater than 1 SD from the mean in the direction of worsened function within baseline stratum.

$^{1}$ Features included in adjusted models by measure (all models include year of follow-up): Boston naming: hot flashes; Word list and Digit span: congestive heart failure; Disk recognition: congestive heart failure, antithrombotic therapy; Recall: antithrombotic therapy.
Evidence from prior research has suggested that estrogen may have protective effects on direct memory and concentration, which are primarily evaluated by tests of attention. A recently reported randomized trial of a selective estrogen-receptor modulator (raloxifene) in postmenopausal women with osteoporosis (mean age 66 years) found no significant differences in cognitive function at entry was normal (MMSE 28-30), we observed significantly less decline in MMSE over the course of the trial and beneficial trends on domain-specific cognitive measures in the estradiol group compared with the placebo group.

Of potential importance is a finding in our data that exploratory analysis among women whose cognitive function at entry was normal (MMSE 28-30), we observed significantly less decline in MMSE over the course of the trial and beneficial trends on domain-specific cognitive measures in the estradiol group compared with the placebo group.

The expectation that ET may preserve cognitive abilities is based on extensive brain effects associated with estrogen, including changes in cerebral blood flow, and modulation of neurotransmitter and amyloid precursor protein metabolism. Antioxidant, neurotrophic, and synaptogenic properties have also been reported. The finding from the WEST that estrogen prevents decline among women with normal cognitive function at baseline is consistent with these data.

The Heart and Estrogen/Progestin Replacement Study (HERS), a large, placebo-controlled trial of hormone therapy for secondary prevention of cardiac disease reported the results of an ancillary study of cognitive outcomes in 1063 participants. Among 7479 participants aged 65 to 79 years who were free of probable dementia at baseline, after an average 4 to 5 years, daily treatment with 0.625 mg of conjugated estrogen plus 2.5 mg of medroxyprogesterone acetate did not improve performance on 6 cognitive measures after 4 years. However, function was assessed only at the end of the trial and the effect of baseline cognitive impairment on response to treatment could not be evaluated in HERS.

The WEST findings do not support an overall effect for estrogen in reducing the risk for decline in cognitive function in women who completed the trial without recurrent stroke. However, the size of the WEST study was determined by the primary analysis of recurrent stroke and death, and, as such, our ability to fully address the secondary aim of cognitive effects was limited. The power to detect the difference in the observed rates of MMSE decline (13.4% vs 18.7%) was only 30% in these data. Another limitation of the WEST, as well as the other completed trials, is their lack of applicability to women who initiate estrogen therapy at younger ages closer to the onset of menopause.

Of potential importance is a finding in our data that suggests a benefit for estradiol in reducing risk of decline in women with normal cognition. A possible reason for
the observed benefit includes the prior hypothesis that estrogen may be effective in delaying initiation of decline, rather than modifying its progression. Although this finding of benefit may be due to chance as it came out of an exploratory analysis, judgments about validity involve more than statistical criteria. Other factors to consider in interpreting these results include the rigor of the study design, which included assignment of treatment within a randomized trial; validity of the analytic methods, as demonstrated by the baseline similarity of the compared groups; consistency of effect across different cognitive measures; and the clinical pertinence of the subgroups chosen for analysis.

The hypothesis that unopposed estrogen may reduce risk of decline in women with normal cognition, as well as the unaddressed question of the effect of earlier initiation of ET, needs to be tested in other studies. Although any therapeutic use of estrogen must be evaluated in the context of its known risks, a potential role for ET in terms of preservation of function in cognitively intact postmenopausal women would have substantial implications for clinical and public health policy and warrants further investigation.

References

Feasibility and clinical outcome of laparoscopic colorectal resection for endometriosis

Emile Darai, MD, PhD,a,* Isabelle Thomassin, MD,b Emmanuel Barranger, MD,a Romain Detchev, MD,a Annie Cortez, MD,c Sydney Houry, MD,d Marc Bazot, MDb

Service de Gynécologie, Obstétrique et Médecine de la Reproduction,a Service de Radiologie,b Service d’Anatomie Pathologique,c Service de Chirurgie Digestive,d Hôpital Tenon, Université Saint-Antoine Paris VI, Assistance Publique des Hôpitaux de Paris, France

Received for publication April 26, 2004; revised August 5, 2004; accepted August 23, 2004

KEY WORDS
Endometriosis
Laparoscopy
Colorectal resection
Magnetic resonance imaging
Rectal endoscopic sonography

Objective: This study was undertaken to evaluate the feasibility and complications of laparoscopic segmental colorectal resection for endometriosis and its efficacy on gynecologic and digestive symptoms.

Study design: After magnetic resonance imaging and rectal endoscopic sonographic evaluation of symptomatic colorectal endometriosis, 40 consecutive women requiring colorectal resection were included in this study. Symptom questionnaires were completed before and after the procedure. Perioperative complications and linear intensity scores for several gynecologic and digestive symptoms were recorded.

Results: Thirty-six women (90%) underwent laparoscopic segmental colorectal resection and 4 required laparoconversion. Major complications occurred in 4 cases (10%), including 3 rectovaginal fistulae and 1 pelvic abscess. Transient urinary dysfunction occurred in 7 women (17.5%). Median follow-up after colorectal resection was 15 months (3-22 months). Median overall preoperative and postoperative pain scores were 8 ± 1 (range 4-10) and 2 ± 2 (0-10), respectively (P < .0001). Nonmenstrual pelvic pain (P = .0001), dysmenorrhea (P < .0001), dyspareunia (P = .0001), and pain on defecation (P < .0005) were improved by colorectal resection. Lower back pain and asthenia were not improved.

Conclusion: Our results suggest that laparoscopic segmental colorectal resection for endometriosis is feasible but carries a risk of major postoperative complications. Colorectal resection improved gynecologic and digestive symptoms, and the overall pain score.

© 2005 Elsevier Inc. All rights reserved.

Endometriosis is a gynecologic disorder defined by the presence of endometrial gland and stroma outside the uterus. Deep infiltrating pelvic endometriosis and bowel endometriosis are less frequent than peritoneal and ovarian endometriosis but can cause severe discomfort and alter these women’s quality of life.1,2

The estimated incidence of bowel endometriosis is between 5.3% and 12%.3,4 The rectum and rectosigmoid junction together account for 70% to 93% of all

* Reprint requests: Professor Emile Darai, MD, PhD, Service de Gynécologie, Hôpital Tenon 4 rue de la Chine, 75020, Paris, France.
E-mail: emile.darai@tnn.ap-hop-paris.fr

doi:10.1016/j.ajog.2004.08.033
intestinal endometriotic lesions. Colorectal endometriosis is difficult to diagnose, because of the nonspecific nature of symptoms and the poor yield of clinical examination. Magnetic resonance imaging (MRI) and rectal endoscopic sonography (RES) have been recommended to detect colorectal endometriosis and can reveal multiple endometriotic sites that may warrant extensive surgery. Previous studies have demonstrated the safety of laparoscopic treatment of deep infiltrating endometriosis, with an improvement in symptoms and quality of life. Medical therapy is rarely successful, but surgical removal of colorectal endometriosis is controversial, because of the risk of major complications depending on the extent and depth of bowel infiltration.

Urbach et al reported an improvement in dysmenorrhea, dyspareunia, and digestive symptoms among women who had undergone laparotomic resection of colorectal endometriosis. In a preliminary study, we found that colorectal resection for endometriosis relieved some digestive and gynecologic symptoms but carried a risk of de novo urinary symptoms. Since the first report by Nezhat et al on laparoscopic colorectal resection for endometriosis, only a few large series have been reported. Therefore, the aim of this study was to evaluate the feasibility and short-term clinical outcome of laparoscopic segmental colorectal resection for endometriosis.

Patients and methods

Patients

Between March 2001 and March 2003, 46 women with colorectal endometriosis were referred to the gynecology department of Tenon Hôpital, Paris, France. Before surgery, all women underwent both MRI and RES. To avoid a possible bias linked to the type of surgery, only women with a muscularis involvement detected by MRI and RES underwent a segmental colorectal resection and were included in this study; those with no muscularis involvement underwent superficial rectal resection (6 women) and were excluded. Therefore, the study population was comprised of 40 women. The patient characteristics of the 40 women and their relevant surgical and medical histories are shown in Table I. The median age of the patients was 33 years and 75% of them were nulliparous. The median interval between symptom onset and diagnosis of colorectal endometriosis was 60 months. A previous surgery for endometriosis was recorded in 62.5% of the patients. Infertility was noted in 13 patients (32.5%). In addition to previous medical treatments, all the patients received gonadotropin-releasing hormone (GnRh) analogues for 3 months before surgery.

Table I: Demographic characteristics of the 40 women with colorectal endometriosis

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Women (N = 40)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median age (y) (range)</td>
<td>33 (21-46)</td>
</tr>
<tr>
<td>Median parity (range)</td>
<td>0.3 (0-3)</td>
</tr>
<tr>
<td>Median gestation (range)</td>
<td>0.5 (0-4)</td>
</tr>
<tr>
<td>Nulliparous (%)</td>
<td>30 (75%)</td>
</tr>
<tr>
<td>Median diagnostic delay (mo) (range)</td>
<td>60 (3-216)</td>
</tr>
<tr>
<td>Previous surgery for endometriosis (%)</td>
<td>25 (62.5%)</td>
</tr>
<tr>
<td>Previous medical treatment (%)*</td>
<td></td>
</tr>
<tr>
<td>Danazol</td>
<td>1 (2.5%)</td>
</tr>
<tr>
<td>GnRh analogues</td>
<td>27 (67.5%)</td>
</tr>
<tr>
<td>Progestins, followed by GnRh analogs</td>
<td>12 (30%)</td>
</tr>
<tr>
<td>Infertility</td>
<td>13 (32.5%)</td>
</tr>
</tbody>
</table>

* In addition to previous medical treatments, all 40 women received 3 months of GnRH analog therapy before surgery.

Methods

All patients signed an informed consent form and were informed of the possible need risk for laparosconversion.

All the women completed symptom questionnaires on gynecologic (dysmenorrhea, nonmenstrual pelvic pain, dyspareunia), digestive (diarrhea and/or constipation, pain on bowel movement, intestinal cramping, pain on defecation, cyclic rectal bleeding), and nonspecific disorders (lower back pain, asthenia). The local institutional review board approved the symptom questionnaires used before and after colorectal resection. Before surgery, the women completed an interview-based questionnaire (10-point analogue rating scale; 0 = absent, 10 = unbearable). They completed the same questionnaire after the operation and were also asked about any further surgery and changes in bowel, urinary, or sexual function.

MRI was performed on a 1.5-T system (Magnetom Vision, Siemens, Erlangen, Germany) with a phased-array body coil, after a 3-hour fast. MRI sections were acquired every 5 mm with a gap of 1 mm.

The MRI criteria used to diagnose colorectal endometriosis were signal abnormalities and morphologic criteria. Signal abnormalities included the presence of tissue regions (related to fibrosis and/or muscle hypertrophy) with a signal intensity close to that of pelvic muscle on T1- and T2-weighted images, with or without hyperintense spots on T1-weighted and/or fat-suppression T1-weighted MRI (related to hemorrhagic foci) or small hyperintense cavities on T2-weighted images.

Morphologic criteria included the size and pattern of the abnormalities, and their borders (regular or irregular stellate). The anterior wall of the rectosigmoid was examined for obliteration of the adipose tissue plane lying between the uterus and the rectosigmoid by a tissue mass; obliteration of the hypointense signal of the
anterior wall of the rectosigmoid on T2-weighted images; obtuse angles of the lesion with the wall of the rectosigmoid; the degree of extension (particularly the distance between the lower border of the fibrotic mass and the junction of the pelvic and perineal rectum); and contrast enhancement.

Associated abnormalities such as endometriomas, or affecting the uterosacral ligaments, torus uterinus (anatomically defined by the presence of a small transverse thickening binding the original insertion of uterosacral ligaments on the posterior wall of the uterus), posterior fornix of the vagina, and/or the cervix and rectovaginal septum were recorded. Adenomyosis was defined by a junctional zone of at least 12 mm or an ill-defined low-signal intensity area of myometrium or punctuate high-intensity myometrial foci.

After a rectal enema, RES was performed with an Olympus GF UM 20 echo-endoscope (SCOP Medicine Olympus, Rungis, France) with a diameter 11.4 mm and equipped with 7.5- and 12-MHz probes. Involvement of the different layers of the colorectal wall was evaluated, including the hypoechoic muscularis propria and the hyperechoic submucosa and mucosa. The largest diameter of the lesions was measured.

The laparoscopic procedure was performed in the modified dorsolithotomy position under endotracheal general anesthesia. Prophylactic anticoagulant therapy was given the evening before the operation, and prophylactic antibiotic therapy was given at the beginning of the operation. An umbilical and 3 suprapubic trocars, including a 12-mm trocar in the right iliac fossa, a 15-mm trocar in the median suprapubic area, and a 5-mm trocar in the left iliac fossa were introduced. The colorectum was examined to verify the presence of deep infiltrating endometriosis with bowel involvement. Then, the sigmoid and rectum were released and all endometriotic lesions, including those affecting the uterosacral ligaments, torus uterinus, peritoneum of the pouch of Douglas, and colorectum, were mobilized before sectioning the colorectum with an endo GIA 45 (Auto Suture, Tyco SA, Elancourt, France). After withdrawing the median suprapubic trocar, the incision was enlarged to 3 cm to allow the colorectum to be exteriorized and resected before creating a purse for the anvil by using Purstring 45 (Auto Suture, Tyco SA). The colon was placed in the pelvic cavity before closing the suprapubic abdominal incision. An end-to-end colorectal anastomosis was then created by using rectally introduced CCEA forceps (Auto Suture, Tyco SA). A drain was inserted behind colorectal anastomosis before closure of the trocar incisions.

The operating time was calculated from insertion of the Veress needle to skin suture. Perioperative and postoperative complications were recorded. Blood loss was estimated from the difference in the hemoglobin level before and 24 hours after the procedure.

Histopathologic criteria for colorectal endometriosis included the presence of ectopic endometrial and stromal tissues penetrating through the bowel wall.

Parametric and nonparametric continuous variables were compared with Student t test and the Mann-Whitney test, and categorical variables were compared with the χ2 test or Fisher exact test, as appropriate. Correlations were identified with analysis of variance and Spearman test. P values less than .05 were considered statistically significant.

Results

Preoperative MRI and RES findings

MRI showed colorectal endometriosis in all 40 women (Figure 1, A and B). The median lesion size was 2.5 cm (range: 1.5-5.5 cm). Colorectal endometriosis was associated with endometriomas in 17 women (42.5%) (bilateral in 6 cases, left-sided in 7 cases, and right-sided in 4 cases), and with uterosacral ligament involvement in 33 women (82.5%). Rectovaginal septum involvement was found in 7 women (17.5%), and uterine adenomyosis in 7 women (17.5%). One woman had cervical involvement. RES showed colorectal endometriosis in 37 women (92.5%). The median lesion size was 2.4 cm (range: 0.4-6 cm). RES showed submucosal or mucosal colorectal involvement in 2 women.

Surgical findings

Thirty-six women (90%) underwent segmental colorectal resection by laparoscopy. The other 4 women (10%) required conversion to open surgery because of difficulties of dissection. Initial laparoscopic inspection showed severe adhesions in 2 of these women: 1 woman had previously had 3 laparotomic procedures, including 2 cystectomies for endometrioma and 1 for myomectomy; and the second woman had a history of colostomy for rectal perforation related to surgical treatment of deep pelvic infiltrating endometriosis. Two laparoconversions were required, for ureteral involvement by endometriosis in 1 case (requiring segmental ureterectomy with reimplantation into the bladder) and for incomplete circular stapled anastomosis at the end of the laparoscopic procedure in the other case. During surgery, 33 of the 40 women (82.5%) were found to have complete obliteration of the pouch of Douglas. In addition to segmental colorectal resection, 17 women (42.5%) underwent ovarian cystectomy with complete removal of the cystic wall. No vaporization technique was associated. Torus resection was performed in all 40 women, together with unilateral or bilateral uterosacral ligament resection in 2 and 33
women, respectively. Thirty-five women (87.5%) had 1 rectosigmoid endometriotic nodule and 5 women (12.5%) had multiple rectosigmoid lesions. Extensive ureterolysis was required in 27 women (67.5%; bilateral, left, and right in 12, 10, and 5 cases, respectively). Partial vaginal resection was necessary in 12 women (30%) and hysterectomy in 4 women (10%).

Perioperative complications

The mean operating time was 6.3 hours (range: 4-13 hours). The woman with the longest operating time had a history of colorectal resection by both vaginal and laparotomic approaches and required laparoconversion. Median blood loss was equivalent to 2.4 g Hb/dL (range

Figure 1  MRI diagnosis of a rectosigmoid endometriosis and a macroscopic view of the same lesion after laparoscopic colorectal resection. A, Sagittal T2-weighted MR image showing a large fibrotic area protruding into the lumen related to rectal wall involvement associated with posterior uterine leiomyomas. B, Sagittal T1-weighted MR image after intravenous injection of gadolinium, showing a large fibrotic nodule in the rectosigmoid junction. C, Macroscopic view of the specimen.
Six women (15%) required blood transfusion, including 2 of the 4 women who underwent laparotomy. Hypercapnia associated with subcutaneous emphysema occurred in 6 women (15%) who did not require laparoconversion. None of the women had protective colostomy.

Three women (7.5%) had a rectovaginal fistula develop, diagnosed on postoperative day 7 or 8 on the basis of a febrile syndrome, followed by fecal expulsion through the vagina; 2 of these women underwent colostomy and the third had Hartmann surgery. All 3 women had undergone partial resection of the vagina. Three months later, these women underwent laparotomy to reestablish bowel continuity with protective colostomy. Another woman had an abscess behind colorectal anastomosis diagnosed on postoperative day 6, based on a febrile syndrome and pelvic pain. Computed tomography confirmed the diagnosis and the abscess was successfully drained during repeat laparoscopy.

Transient urinary retention or dysuria occurred in 7 women (17.5%), including the 3 women with a rectovaginal fistula. Urinary retention required bladder catheterization for a median of 2 weeks (range: 2-4 weeks).

**Histology**

Histologic examination of the surgical specimens confirmed colorectal endometriosis in all 40 women (Figure 1, C). The median length of the resected colorectal segment was 8.2 cm (range: 4-20 cm). The median largest diameter of the lesions on histologic examination was 3 cm (range: 1-7 cm). The muscularis propria was involved in 30 cases and both the submucosa and mucosa in 10 cases. The margins were in sano (without endometriosis on wedges of colorectal specimen) in all the women but 1, who had submucosal endometriotic foci. Lesion size correlated between histology and RES ($P = .03$, rho = 0.35), but not between histology and MRI ($P = .14$, rho = 0.274).

All ovarian cysts removed were endometriomas. For the 4 women who underwent a hysterectomy, adenomyosis detected by MRI was confirmed by histology.

**Table II** Changes in symptoms or signs after colorectal resection for endometriosis

<table>
<thead>
<tr>
<th>Symptoms or signs</th>
<th>Women with symptoms or signs before surgery</th>
<th>Disappearance after surgery (%)</th>
<th>Decrease after surgery (%)</th>
<th>Same after surgery (%)</th>
<th>Increase after surgery (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysmenorrhea</td>
<td>36</td>
<td>22 (61)</td>
<td>13 (36)</td>
<td>1 (3)</td>
<td>0</td>
</tr>
<tr>
<td>Nonmenstrual pain</td>
<td>16</td>
<td>9 (56)</td>
<td>7 (44)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>34</td>
<td>26 (76)</td>
<td>2 (6)</td>
<td>5 (15)</td>
<td>1 (3)</td>
</tr>
<tr>
<td>Bowel movement pain or cramping</td>
<td>13</td>
<td>9 (69)</td>
<td>4 (31)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Constipation and diarrhea</td>
<td>11</td>
<td>8 (73)</td>
<td>1 (9)</td>
<td>2 (18)</td>
<td>0</td>
</tr>
<tr>
<td>Pain on defecation</td>
<td>22</td>
<td>15 (68)</td>
<td>6 (27)</td>
<td>1 (4)</td>
<td>0</td>
</tr>
<tr>
<td>Cyclic rectal bleeding</td>
<td>11</td>
<td>11 (100)</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Lower back pain</td>
<td>11</td>
<td>8 (73)</td>
<td>3 (27)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Asthenia</td>
<td>21</td>
<td>9 (43)</td>
<td>2 (9)</td>
<td>8 (38)</td>
<td>2 (9)</td>
</tr>
</tbody>
</table>

Median follow-up after colorectal resection was 15 months (3-22 months).

Qualitative data on symptoms before and after colorectal resection are shown in Table II. Dysmenorrhea disappeared after colorectal resection in 22 of the 36 women concerned. Nonmenstrual pelvic pain disappeared in 9 of the 16 women concerned. One woman with isolated preoperative dysmenorrhea had nonmenstrual pelvic pain after surgery. Dyspareunia disappeared after surgery in 26 of the 34 women concerned, but 1 woman complained of increased dyspareunia and 1 woman complained of de novo dyspareunia. Pain on defecation disappeared in 15 of 22 women, but 4 of 22 women who were free of pain on defecation before the operation subsequently reported abdominal pain related to constipation. Pain on bowel movement and intestinal cramping diminished or disappeared in all 13 women concerned. Cyclic rectal bleeding disappeared in all 11 women concerned.

The median total preoperative and postoperative pain scores were $8 \pm 1$ (range: 4-10) and $2 \pm 2$ (0-10),
respectively \((P < .0001)\) (Figure 2). The overall pain score improved in 38 (95%) of the 40 women. The remaining 2 women had de novo dyspareunia and persistent constipation and diarrhea.

Preoperative and postoperative symptom intensity scores are compared in Table III. All gynecologic symptoms, including dysmenorrhea \((P < .0001)\), nonmenstrual pelvic pain \((P = .001)\), and dyspareunia \((P = .0001)\) were significantly improved by surgery. The intensity of pain on defecation diminished after surgery \((P < .0005)\). The intensity of pain on bowel movement diminished, without reaching significance, because of de novo constipation and diarrhea. Asthenia tended to improve, but lower back pain did not.

There were no recurrences of colorectal endometriosis during follow-up. All but 1 of the women stated that, with hindsight, they would have opted for the same treatment.

### Comment

This study demonstrates that laparoscopic segmental colorectal resection for endometriosis is feasible and significantly improves both gynecologic and digestive symptoms. Rectovaginal fistula was a significant complication, especially when partial vaginal resection was required.

The feasibility and safety of laparoscopic surgical techniques can be estimated on the basis of the laparoscopic conversion rate and perioperative complications. In contrast to previous studies,\textsuperscript{16-19} we only enrolled women who required segmental colorectal resection. Of the 40 women in this study, 4 required laparoscopic conversion. This rate of conversion (10%) is acceptable, especially as most patients had previously had at least 1 operation for endometriosis. It is also in keeping with previous reports (up to 12.5%).\textsuperscript{17,19} In our experience, median blood loss was of 2.4 g Hb/dL and 6 women (15%) required blood transfusion reflecting operative difficulties to remove colorectal endometriosis caused by involvement of adjacent anatomic structures. Little data are available on bleeding and transfusion after colorectal resection for endometriosis. Tran et al\textsuperscript{7} reported a mean blood lost of 2200 mL after laparotomic colorectal resection.

The procedures were relatively long in our study (mean 6.3 hours, including conversions), because of extensive lesions requiring, in most cases, extensive resection (ovarian cystectomy, torus uterinus, and uterosacral resection; hysterectomy and ureterolysis). This operating time is nevertheless in line with previous reports that used laparoscopy,\textsuperscript{16,17,19} but seems higher than those reported by laparotomy.\textsuperscript{5-7}

Complications requiring further surgery occurred in 10% of women, for rectovaginal fistulae in 3 cases and pelvic abscess in 1 case. Duepree et al\textsuperscript{19} have reported that the rate of postoperative complications was 11.1% among women undergoing segmental resection, compared with 3.8% among those undergoing superficial excision. In our series, the 3 rectovaginal fistulae occurred in women requiring colorectal and vaginal resection suggesting that a systematic protective colostomy should be used in this specific situation. No rectovaginal fistulae were reported by Nezhat et al\textsuperscript{16} and Redwine and Wright\textsuperscript{13} after laparoscopic segmental colorectal resection for endometriosis in 10 and 6 women, respectively, but no information on concomitant vaginal resection was given. Jerby et al\textsuperscript{17} observed 1 rectovaginal fistula among 7 women undergoing segmental colorectal resection. With laparotomy, Urbach et al\textsuperscript{14} reported a rectovaginal fistula (3.3%) with rectal endometriosis, whereas Bailey et al\textsuperscript{18} observed no rectovaginal fistula after laparotomic resection. We observed no cases of anastomotic dehiscence, whereas Possover et al\textsuperscript{18} reported a rate of 6.6%. One pelvic abscess was observed in our series and was successfully drained by laparoscopy. The occurrence of abscess and rectovaginal fistula in the current study could be partly explained by the systematic use of drainage. Indeed, a previous randomized study has demonstrated that the use of prophylactic pelvic drainage after elective rectal or anal anastomosis increases the risk of abscess and fistula.\textsuperscript{20} Coronado et al\textsuperscript{5} reported a 10.4% febrile

<table>
<thead>
<tr>
<th>Symptom or sign</th>
<th>Preoperative intensity score (median ± SD, range)</th>
<th>Postoperative intensity score (median ± SD, range)</th>
<th>(P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dysmenorrhea</td>
<td>9 ± 2 (4-10)</td>
<td>2 ± 3 (0-8)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Nonmenstrual pelvic pain</td>
<td>8 ± 2 (0-10)</td>
<td>2 ± 3 (0-8)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Dyspareunia</td>
<td>6 ± 3 (0-10)</td>
<td>2 ± 3 (0-10)</td>
<td>&lt; .0001</td>
</tr>
<tr>
<td>Digestive and rectal symptoms</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pain on defecation</td>
<td>7 ± 4 (0-10)</td>
<td>1 ± 2 (0-8)</td>
<td>.0005</td>
</tr>
<tr>
<td>Bowel movement pain</td>
<td>5 ± 4 (0-10)</td>
<td>2 ± 2 (0-10)</td>
<td>.11</td>
</tr>
<tr>
<td>Lower back pain</td>
<td>5 ± 4 (0-10)</td>
<td>3 ± 3 (0-8)</td>
<td>.17</td>
</tr>
<tr>
<td>Asthenia</td>
<td>3 ± 3 (1-6)</td>
<td>2 ± 1 (1-3)</td>
<td>.06</td>
</tr>
</tbody>
</table>
morbidity rate after laparotomic resection of colorectal endometriosis and noted that 3% of patients required a second laparotomy for pelvic collections in the first postoperative month.

The indications of colorectal resection for endometriosis are controversial, and the likely risk/benefit ratio must be discussed with each patient. Nonmenstrual pelvic pain, pain on bowel movement, cramping, and cyclic rectal bleeding improved or disappeared in all the women concerned, in keeping with previous studies of colorectal resection for endometriosis. Dysmenorrhea, dyspareunia, pain on defecation, and nonmenstrual pelvic pain improved significantly, on the basis of visual analog scores, whereas no impact was noted on pain on bowel movement, lower back pain, or asthenia. Our results confirm those of Redwine and Wright, showing that women with dysmenorrhea, dyspareunia, pain on defecation, or nonmenstrual pelvic pain associated with complete endometriotic obliteration of the sac of Douglas are the best candidates for extensive resection.

In our series, transient digestive and urinary symptoms occurred in respectively 37.5% and 17.5% of women. Transient urine retention and de novo dysuria and digestive symptoms are well-known complications after colorectal resection for intestinal malignancies, affecting 60% and 41% of patients, respectively. Despite these transient adverse urinary effects, 38 of the 40 women (95%) in this study reported a significant improvement in their symptoms, in keeping with previous reports of improved quality of life and acceptable postoperative morbidity after extensive surgery for deep infiltrating pelvic or rectovaginal endometriosis. In conclusion, our results suggest that laparoscopic segmental colorectal resection for endometriosis is feasible but carries a risk of major postoperative complications. However, despite transient urinary and digestive adverse effects, colorectal resection markedly improved gynecologic and digestive symptoms and the overall pain score.

References

Ultrasonography and color Doppler-based triage for adnexal masses to provide the most appropriate surgical approach

Stefano Guerriero, MD,* Silvia Ajossa, MD, Nicoletta Garau, MD, Bruno Piras, MD, Anna Maria Paoletti, MD, Gian Benedetto Melis, MD

Department of Obstetrics and Gynecology of the University of Cagliari, Cagliari, Italy

Received for publication June 22, 2004; revised July 22, 2004; accepted September 2, 2004

KEY WORDS
Laparoscopy
Ultrasonography
Color Doppler
Triage
Adnexal masses

Objective: This study was undertaken to identify whether ultrasonography with color Doppler can identify and triage the patients with adnexal masses to the most appropriate surgical approach.

Study design: Four hundred fifty-three pelvic masses were included in the study and underwent ultrasonography before surgical treatment for adnexal masses. Masses that showed a typical benign pattern at B-mode ultrasonography (very low risk of malignancy) were treated by conventional laparoscopy without further evaluation. Masses that extended above the umbilicus were considered at very high risk and treated by laparotomy. All other adnexal masses were evaluated with power Doppler. Masses with central vascularization (high risk of malignancy) were submitted to laparotomy or laparoscopy with additional tools, whereas masses with peripheral or absent flow (low risk of malignancy) were submitted to conventional laparoscopy.

Results: Among 284 very low-risk, 32 low-risk, 46 high-risk, 91 very high-risk masses, the rate of malignant masses were 0%, 0%, 52%, and 78%, respectively. The use of color Doppler increases the diagnostic accuracy of B-mode ultrasonography in the diagnosis of adnexal malignancies because of a significantly higher specificity (0.91 vs 0.82, \( P < .001 \)).

Conclusion: The evaluation of vessel distribution by color Doppler seems a safe diagnostic procedure, permitting to treat by laparoscopy 91% of benign masses.

© 2005 Elsevier Inc. All rights reserved.

Laparoscopic surgery has become the gold standard in the treatment of benign adnexal masses because this technique significantly reduces both the intensity of postoperative pain and the length of convalescence compared with laparotomy.\(^1\)\(^-\)\(^7\) For these reasons, preoperative evaluation should be performed to increase the number of benign cysts that are submitted to laparoscopy, also to reduce the risk of unexpected malignancy. The use of triage has been proposed in the emergency department and in other fields of gynecology for managing metrorrhagia.\(^8\)\(^,\)\(^9\) However, to our knowledge, there is only 1 study in the English literature regarding the role of ultrasonography in the selection of the most appropriate surgical approach in the treatment of...
This study uses a complex scoring system and introduces the diagnostic algorithm of the color Doppler only to obtain a quantitative parameter such as resistance index (RI). In the presence of several conflicting results, the use of this parameter has been criticized, which makes this approach impractical from a clinical point of view. On the contrary, further evidence shows that the evaluation of flow location by color Doppler (power or conventional) is a useful technique. In previous studies, we suggested that color Doppler should be used only to grade masses with central vascular flow or vascular flow within excrescences previously identified by B-mode ultrasonography as malignant or indeterminate. As a matter of fact, the main problem of gray scale morphologic ultrasonography is the high false-positive rate in the differential diagnosis of adnexal malignancies, even with several scoring systems. Nevertheless, gray scale ultrasonography is precise enough in the diagnosis of several benign adnexal conditions; therefore, the actual problem of false-positive findings is reduced for those complex or "indeterminate" masses, increasing the risk for submitting to laparotomy several benign masses presenting dubious B-mode findings.

The aim of this study was to identify whether ultrasonography coupled with color Doppler can provide the necessary information to triage the patients with adnexal masses to the most appropriate surgical approach (laparotomy or laparoscopy), decreasing the number of false-positive cases in the differential diagnosis of adnexal malignancies from benign pelvic masses, and avoiding the problem of false-negative cases.

| Table I | Histologic diagnosis and ultrasonographic risk of malignancy |
|-----------------|-----------------|-----------------|-----------------|-----------------|
| Histotype | Very low risk | Low risk | High risk | Very high risk |
| Ovarian cancer stages I-II | 9 (50.0%) | 9 (50.0%) | 2 (5.3%) | 36 (94.7%) |
| Ovarian cancer stages III-IV | 8 (40.0%) | 12 (60.0%) | 5 (26.3%) | 14 (73.7%) |
| Borderline | 2 (3.6%) | 2 (3.6%) | 1 (0.7%) | 1 (0.7%) |
| Other malignancies | 3 (7.3%) | 1 (2.5%) | 1 (0.7%) | 1 (0.7%) |
| Endometrioma | 124 (89.9%) | 8 (5.8%) | 5 (3.6%) | 14 (73.7%) |
| Dermoid cyst | 47 (85.5%) | 2 (3.6%) | 1 (0.7%) | 1 (0.7%) |
| Serous cyst | 37 (90.2%) | 3 (7.3%) | — | 1 (2.5%) |
| Serous cystadenoma | 11 (73.3%) | 3 (2.0%) | — | 1 (0.7%) |
| Mucinous cystadenoma | 15 (57.7%) | 3 (11.5%) | 2 (7.7%) | 6 (23.1%) |
| Hydrosalpinx | 13 (92.9%) | 1 (7.1%) | — | — |
| Hemorrhagic cyst | 18 (66.7%) | 2 (7.4%) | 1 (3.7%) | — |
| Paraovarian cyst | — | — | — | — |
| Ovarian fibroid | — | — | 3 (60.0%) | 2 (40.0%) |
| Miscellaneous | 17 (48.6%) | 6 (17.1%) | 8 (22.9%) | 4 (11.4%) |
| Total | 284 (62.7%) | 32 (7.1%) | 46 (10.1%) | 91 (20.1%) |

Material and methods

The study, performed at Department of Obstetrics and Gynecology of University of Cagliari, Italy, included 424 women who underwent transvaginal ultrasonography before elective surgical treatment for adnexal masses. The study was approved by our Institutional Review Board and all participating patients gave their informed consent. The average ± SD age of the study population was 39 ± 15 years (range, 14-79 years). Three hundred twenty-three patients (76%) were premenopausal, and 101 (24%) were postmenopausal. Within 2 days before surgery, all patients underwent transvaginal ultrasonography with the use of an Acuson XP/10 OB (Acuson Inc, Mountain View, Calif) or an EUB-6000 Astro MP (Hitachi Medical Corporation by Esaote, Genova, Italy) with a 6.5 to 7 MHz transvaginal probe. With the use of B-mode sonography, a mass was thought to be benign when it had a typical benign pattern that was based on the following morphologic criteria: endometrioma, characterized by circular homogeneous, hypoechoic "tissue" without papillary proliferations and a clear demarcation from the ovarian parenchyma; cystic teratoma was characterized by one of the following 3 echo patterns: (1) a densely echogenic mural tubercle with a posterior acoustic shadow associated with a cystic echo pattern; (2) echogenic, thin, band-like echoes (hyperechoic sparkling lines and dots in dark field); and (3) a dense echo pattern associated with posterior acoustic shadow with or without a cystic component. Serous cyst and serous cystadenoma were characterized by an anechoic unilocular or bilocular cystic mass with a thin regular wall without endocystic vegetation; mucinous cystadenoma, multilocular mass with a thin regular wall and septa and liquid content with variable echogenicity, without endocystic vegetation; hemorrhagic cyst, mass with hyperechoic content and thin septa; paraovarian cyst, unilocular anechoic cyst separate from the ipsilateral ovary, with thin wall; hydrosalpinx, characterized by an irregular, elongated mass filled with anechoic fluid. Each mass with these characteristics was considered at...
“very low risk” of malignancy and eligible for laparoscopic treatment without further evaluation. Masses with evidence of gross metastatic disease and/or masses that extended above the umbilicus and/or ascites and/or very large masses (>90 mm) with mostly solid aspects were considered at “very high risk” and treated by laparotomy. All other adnexal masses defined as indeterminate are evaluated for vascular flow with color Doppler (conventional or power). Malignancy was suggested when arterial flow was demonstrated in an echogenic structure or in an irregular solid portion and the mass was submitted to laparotomy or laparoscopy with intraoperative surgical evaluation, the use of a plastic bag to avoid peritoneal spillage, and the immediate frozen section. Laparoscopy was attempted when a low-grade malignancy or a borderline was believed to be due to the presence of a vascularized papillary structure on an internal wall. On the contrary, when a vascularized, irregular, mostly solid portion was present, a laparotomy was performed. A mass was thought to be benign on transvaginal color Doppler imaging when flow was detected only in the wall of the mass or when it was absent, then the mass was treated by laparoscopy.

The results of pathologic examination were obtained for each adnexal mass. Malignant ovarian tumors were surgically staged according to the criteria of the International Federation of Gynecology and Obstetrics (FIGO). The sensitivity, specificity, and positive and negative predictive values of B-mode transvaginal ultrasonography and other methods were calculated for each mass. The z statistics for the comparison of 2 proportions were used to evaluate the results. In addition, to evaluate the overall agreement between a test result and the actual outcome using the 2 different methodologies, the kappa index was calculated according to Fleiss. The use of kappa statistics allows the comparison of different diagnostic tests in different populations. Kappa values ranging between 0.40 and 0.75 were assumed to indicate a strong agreement.

Figure The proposed algorithm for the preoperative assessment of adnexal masses, based on the use of color Doppler as a secondary test after B-mode evaluation. *Converted to laparotomy for the presence of thick adherences between ovaries, uterus, and bowel.
Results

Four hundred fifty-three adnexal masses diagnosed in 424 patients were included in the study. Three hundred fifty-eight (79%) masses were benign and 95 (21%) were malignant. Thirty-four (36%) of the malignant masses were in the premenopausal group and 61 (64%) were in the postmenopausal group. Of the 95 ovarian carcinomas, 20 were low-malignancy potential tumors, 18 were stages I and II according the FIGO, and 38 were stages III and IV. Five masses were ovarian metastases of a carcinoma of the colon in 3 cases and a carcinoma of the breast in 2 cases. The remaining 14 malignant masses were 4 lymphomas, 1 Brenner tumor, and 9 recurrent ovarian cancers. Table I showed the distribution of masses in the 4 classes of risk according to the preoperative echographic evaluation. Two hundred eighty-four masses were in the very low-risk group and all submitted to laparoscopy. On the contrary, submitting to laparotomy all suspicious masses at B-mode evaluation, the rate of benign masses submitted to laparoscopy decline to 79% (P < .001).

Comment

The use of color Doppler to increase the specificity of B-mode ultrasonography in the diagnosis of ovarian cancer is a safe diagnostic procedure with a very low risk of false-negative cases. In the current study, we propose in a large study population a new algorithm for the preoperative assessment of adnexal masses that is based on the use of color Doppler as a secondary test after B-mode evaluation. This B-mode evaluation remains the key of this approach because masses with a typical benign finding were considered to be at a very low risk of malignancy without further evaluation. The study of Berlanda et al proposed a similar approach, but with the use of a B-mode scoring system. Several studies have criticized this approach. As stated by Valent in 2004, the use of gray scale ultrasound morphology to characterize a pelvic mass, also called “pattern recognition,” is superior to all other ultrasound methods such as scoring systems and mathematical models for calculating the risk of malignancy for discrimination between benign and malignant extrauterine pelvic masses, also in terms of reproducibility and simplicity. As a matter of fact, many pelvic masses have such a typical macroscopic appearance that a fairly confident diagnosis can be made on the basis of their macroscopic appearance alone, ie, on the basis of their gray scale ultrasound image. In addition with their scoring system, Berlanda et al used a complex algorithm with different parameters such as dimensions, monolaterality or bilaterality, mobility, serum CA 125 levels, and RI. In fact, these authors used the color Doppler only to perform a pulsed Doppler evaluation. This approach has been criticized because a large overlap of values has been reported by several authors and a more recent approach that is based on color Doppler should include only the evaluation of flow

Table II The diagnostic accuracy of combined methods in the diagnosis of ovarian masses

<table>
<thead>
<tr>
<th></th>
<th>B-mode findings</th>
<th>Color Doppler findings</th>
</tr>
</thead>
<tbody>
<tr>
<td>Specificity</td>
<td>82%(293/358)</td>
<td>91%(326/358)</td>
</tr>
<tr>
<td></td>
<td>95% CI, 77%-86%</td>
<td>95% CI, 87%-94%</td>
</tr>
<tr>
<td>Sensitivity</td>
<td>100%(95/95)</td>
<td>100%(95/95)</td>
</tr>
<tr>
<td></td>
<td>95% CI, 95%-100%</td>
<td>95% CI, 95%-100%</td>
</tr>
<tr>
<td>Positive predictive value</td>
<td>59%(95/160)</td>
<td>75%(95/127)</td>
</tr>
<tr>
<td>Negative predictive value</td>
<td>100%(293/293)</td>
<td>100%(326/326)</td>
</tr>
<tr>
<td>LR+</td>
<td>5.5</td>
<td>11</td>
</tr>
<tr>
<td></td>
<td>95% CI, 4.4-6.9</td>
<td>95% CI, 8.0-15.6</td>
</tr>
<tr>
<td>LR−</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Kappa value</td>
<td>0.65</td>
<td>0.81</td>
</tr>
<tr>
<td></td>
<td>95% CI, 0.60-0.66</td>
<td>95% CI, 0.75-0.82</td>
</tr>
</tbody>
</table>

LR+, Likelihood positive; LR−, likelihood negative.
location. For these reasons we proposed a simplified approach in which all adnexal masses without typical benign findings, with a mean diameter less than 90 mm, were defined as indeterminate and evaluated for vascular flow location with power Doppler. Only recently had the role of vascular distribution received increasing attention, because it seems an easy method to investigate adnexal masses. After the articles of Buy et al. and Guerriero et al. that identify the presence of solid elements and central flow as the most important features of malignancy, several logistic regressions confirmed this assumption. To further validate this approach, a collaborative work at 3 European university departments of obstetrics and gynecology was performed and a total of 826 complex pelvic masses were included in the study. An adnexal mass was first studied in gray scale sonography and a probable histologic type was predicted. Second, solid excrescences or solid portions of the tumor were evaluated for vascular flow with color Doppler sonography (conventional or power). This study confirmed that color Doppler evaluation was more accurate in the diagnosis of adnexal malignancies in comparison with gray scale sonography (kappa = 0.82 and 0.65, respectively) because of significantly higher specificity (0.94 vs 0.84; \(P < .001\)).

With this approach, a mass was considered at high risk of malignancy if flow was demonstrated within the excrescences or solid areas and submitted to laparotomy or laparoscopy with intraoperative surgical evaluation, the use of endobag, and the immediate frozen section, and at low risk of malignancy if there was peripheral or absent flow and, for these reasons, was scheduled for conventional operative laparoscopy. On the contrary, masses with evidence of gross metastatic disease and/or masses that extended above the umbilicus and/or ascites were considered at very high risk of malignancy and underwent laparotomy. The fundamental aim of a presurgical triage performed before elective surgical treatment for adnexal masses is to reduce the number of unnecessary laparotomic procedures in patients with benign adnexal masses, reducing the false-positive rate of B-mode evaluation used alone. With the use of our presurgical algorithm, this objective was fully achieved because the specificity of color Doppler evaluation was higher (91%) than that of B-mode evaluation used alone (82%). In fact, among 78 adnexal masses indeterminate at B-mode evaluation, 32 were correctly identified as low risk and submitted to laparoscopic approach.

In terms of surgical treatment, the current method of preoperative assessment could permit the approach by laparoscopy of 91% of the benign masses, whereas for B-mode used alone this rate seems to be significantly lower (79%). In addition, this approach could allow select masses with a fair risk to being malignant to be managed by an expert laparoscopist able to use additional tools such as endobag to reduce the risk of spillage and immediate frozen section. Of 46 masses with high risk, 16 were submitted to laparoscopy because of a low-grade malignancy or a borderline malignancy that was supposed to be due to the presence of a vascularized papillary structure on internal wall. The presence of a borderline lesion was confirmed in 4 cases. We suggest that to avoid the risk of failure to diagnose ovarian malignancy, tumor spillage, inability to proceed immediately with staging procedure, and delay in therapy, only those centers with extensive experience in oncology and laparoscopic surgery be able to treat by laparoscopy an adnexal mass that is suspected to be malignant. The use of this simple distinction in 4 classes introduced by our algorithm may be useful for proper counselling and for planning surgical procedures. In particular, low-risk and very low-risk masses, with a 0.316 risk of malignancy, are candidates for laparoscopic treatment; high-risk masses, with a 1.09:1 risk of malignancy (usually early stage carcinomas or borderline, Table I), are candidates for laparoscopic evaluation by an operating surgeon skilled in advanced operative laparoscopy combined with availability of immediate frozen-section diagnosis; very high-risk masses, with a 3.55:1 risk of malignancy, should be managed by a gynecologic oncologist directly with laparotomy. This approach can permit directing high-risk masses to selected departments. In these departments, it should possible, with advanced technologies and advanced skills, to remove most adnexal masses, including early-stage ovarian cancer, laparoscopically with an efficient removal of mass without causing spread or delay of additional therapy and further morbidity and mortality. In addition, this approach can avoid some laparotomies for the treatment of benign masses. In conclusion, the evaluation of vessel distribution by color Doppler seems a simple, cheap, and safe diagnostic procedure, permitting treatment by laparoscopy for more than 90% of the benign masses without mismanagement of ovarian cancer.

References
Population characteristics in cervical cancer trials: Search for external validity

Annie Yessaian, MD, Alberto A. Mendivil, MD, Wendy R. Brewster, MD, PhD*

University of California, Irvine, Irvine, Calif

Received for publication March 3, 2004; revised August 18, 2004; accepted August 23, 2004

Objective: The purpose of this study was to compare the characteristics of patients with cervical cancer who were enrolled in cooperative group trials with characteristics of the cervical cancer population of the United States and to determine the generalizability of the results of those trials to the disease population in the United States.

Study design: Phase III trials in cervical cancer that were conducted by cooperative groups in the United States from 1981 through 1997 were identified. These groups were placed into 4 categories on the basis of disease stage and primary treatment modality: Stage IB, negative pelvic nodes that were treated with radical hysterectomy (n = 277 patients); Stage IB-IIA, positive pelvic nodes that were treated with radical hysterectomy (n = 239 patients); Stage IB2, negative pelvic and para-aortic nodes that were treated with radiation therapy (n = 369 patients); and Stage IIB-IVA, negative para-aortic nodes that were treated with radiation therapy (n = 1190 patients). For each category, comparable patients from the Surveillance, Epidemiology, and End Results (SEER) database were identified. The age and ethnic distributions of each study population and the distributions of the SEER program were compared.

Results: The age distributions were equivalent, except for patients with IB2 disease that was treated with radiation therapy where cooperative group subjects were more likely to be younger than 50 years, (odds ratio, 0.17; 95% CI, 0.11-0.26). A statistically significant higher proportion of black and Hispanic women enrolled in cooperative group studies in comparison with surveillance, epidemiology, and end results.

Conclusion: Hispanic and black women were recruited proportionately to cooperative group randomized cervical cancer trials in comparison to the United States population. The age distribution of the clinical trial population is also comparable to that of the general population.

In 2004 an estimated 10,520 new diagnoses of invasive cervical cancer were predicted in the United States, and 3900 deaths were expected from this disease. The incidence and the mortality rates of invasive cervical cancer have decreased steadily over the past several decades, with an average decline in mortality of approximately –1.5% per year since 1982.1

The age-adjusted disease incidence and mortality rates of cervical cancer vary considerably among various ethnic groups in the United States. Hispanic...
women have the highest age-adjusted incidence rate (15.8/100,000 person-years), followed by black women (11.8/100,000 person-years) and Asian-Pacific Islanders (10.3/100,000 person-years), with non-Hispanic white women having the lowest incidence rate at 7.1 per 100,000 person-years. When age-adjusted mortality rates are compared, black women have the highest rates (6.0/100,000 person-years), followed by Hispanic women (3.5/100,000 person-years), Asian-Pacific Islander women (2.8/100,000 person-years), and non-Hispanic white women (2.4/100,000 person-years). The differences in the mortality rates among the different ethnic groups appear primarily to be due to differences in socioeconomic status and stage at diagnosis. Fifty-five percent of non-Hispanic white women who are diagnosed with cervical cancer will have localized disease, compared with only 45% among women of black descent.

To decrease the mortality rate of cervical cancer, investigators have directed their efforts at different levels of prevention. However, because of the imperfections of primary prevention, there is a need to improve the treatment of this disease. To achieve this goal, investigators have developed novel approaches to cervical cancer treatment and compared them with standard therapies in phase III trials. However, the results of these trials must be applicable to the general disease population if society is expected to benefit from the new cancer therapy. External validity or extrapolability refers to the extent to which the results of an experiment can be generalized or extended beyond the conditions of the experiment. Insufficient numbers of minority subjects and women participants in a clinical trial decrease the confidence with which findings can be applied to the dissimilar diseased population.

The National Institute of Health (NIH) has mandated that cooperative research groups comply with guidelines for the inclusion of minority subjects and underserved populations in NIH-funded clinical trials to ensure that the results and benefits of these trials will be applicable to women and minority populations. Minority groups are defined as “...a readily identifiable subset of the US population which is distinguished by either racial, ethnic, and/or cultural heritage.” These guidelines began with the report of the Public Health Service Task Force on Women’s Health in 1985 and later evolved into more policies that were published in the NIH Guide to Grants and Contracts in subsequent years. In 1993, Congress passed into law the NIH Revitalization Act (Public Law 103-43). In response to these guidelines, investigators have directed considerable effort to increase the participation of women and minority subjects in cancer trials and have generally achieved a sociodemographic composition that parallels that of the general population. However, even though subjects from minority groups are enrolled into clinical trials, the small numbers of these participants often preclude a rigorous statistical comparison of their sociodemographic composition and disease information with that of the target population.

To compare the most basic of demographic characteristics (age and ethnicity) of patients with cervical cancer who are enrolled in large cooperative group trials with those of the cervical cancer population in the United States, we used data from the Surveillance, Epidemiology and End Results (SEER) program (1973-1998) of the National Cancer Institute. The SEER program, administered by the National Cancer Institute, was initiated in 1973 to report population-based estimates of cancer incidence and deaths. The program includes cancer cases from geographic regions that capture approximately 14% of the US population. The regional tumor registries in the SEER program abstract demographic information and data on primary surgery, the radiotherapy that was administered, and survival.

Methods

Peer-reviewed, published, randomized phase III treatment trials in primary cervical cancer that were conducted by large cooperative groups in the United States between 1985 and 2000 were identified and reviewed. The search was performed with the use of the National Library of Medicine–PubMed web site, with the terms squamous cell carcinoma of the cervix and treatment. The search was limited to randomized controlled trials that were published in the English language between 1990 and 2001 in humans. Forty-eight articles were identified that met the search parameters. The results were further limited by the elimination of 31 studies that were conducted outside the United States, trials that were conducted by non–cooperative groups, and studies of recurrent or metastatic disease. Nonrandomized observational studies or those studies that were related to the evaluation of risk factors, quality control issues, and disease were also excluded.

Eight published trials met the criteria. Data that were collected from each of these studies included the number of evaluable patients, disease stage, lymph node status, primary treatment modality, age group distribution, and racial-ethnic composition of the study populations. To facilitate comparison with the SEER cervical cancer population, these studies were grouped into 4 main categories that were based on primary treatment modality and disease characteristics (such as stage and lymph node status). The following categories were created:

Patients with stage IB cervical cancer with negative pelvic nodes

This category included patients from Gynecologic Oncology Group Trial Number 92 (GOG-92). The subjects in this study were women with intermediate
risk factors who were assigned randomly to pelvic radiation therapy (RT) versus no further treatment after radical hysterectomy and pelvic lymphadenectomy. This study was activated in March 1988 and closed in September 1995.

Patients with stage IB-IIA cervical cancer with positive pelvic nodes that were treated with radical hysterectomy

This category was inclusive of the subjects who were enrolled in either GOG-109 or the Southwest Oncology Group Trial Number 8797. These subjects were assigned randomly to either RT with adjuvant concurrent chemotherapy or to pelvic RT alone after radical hysterectomy. The study was activated in September 1990 and closed in December 1996.

Patients with stage IB₂ cervical cancer with negative pelvic and para-aortic lymph nodes that were treated with radiation

This category included the patients who were enrolled in GOG-123 who were assigned randomly to cisplatin, RT, and adjuvant extrafascial hysterectomy versus RT and adjuvant hysterectomy. This study was activated in February 1992 and closed in April of 1997.

Patients with stage IIB-IVA cervical cancer with negative para-aortic nodes that were treated with radiotherapy

This category included patients with locally advanced disease and negative para-aortic lymph nodes who were treated with RT and adjunct chemotherapy and included GOG-56, GOG-85/Southwest Oncology Group Trial Number-8695, and GOG-120. GOG-56 was activated in June 1981 and closed December 1985. In this study, patients with locally advanced disease with negative para-aortic nodes were assigned randomly to pelvic RT with misonidazole or to pelvic RT with hydroxyurea. GOG-85 was activated in August 1986 and closed in December 1990. These patients all had locally advanced cervical cancer and negative para-aortic nodes. They were assigned randomly to receive RT with 5-FU infusion and cisplatin or with hydroxyurea. GOG-120 was activated in April 1992 and closed in April 1997. In this study, patients with locally advanced cervical cancer who had negative para-aortic nodes were assigned randomly to 1 of the 3 chemotherapy regimens in addition to RT.

The SEER public use database, 1973 through 1998, was used to identify the reference population, which consisted of patients with cervical cancer who received a diagnosis over the same calendar interval with comparable disease stage and treatment methods as the patients enrolled in the cooperative group trials.

Within each of the categories that were created, characteristics such as ethnic composition, disease stage, and age distribution were compared between the cooperative group trial population and SEER. Statistical analyses were performed with SAS software (SAS Institute Inc, Cary, NC). The statistical tests that were used were chi-squared and logistic regression. Only 2-tailed probability values <.05 were considered statistically significant.

Results

There were 277 evaluable patients in GOG-92 with stage I-B cervical cancer disease who underwent radical hysterectomy and also had negative pelvic lymph nodes. There were 556 comparable patients in the SEER database with the same disease stage who underwent radical hysterectomy with negative pelvic nodes that was diagnosed between 1988 and 1995 (Table I). There was no difference in the proportion of age groups between SEER and GOG-92 (P = .81). The proportion of black women with cervical cancer was higher for the GOG study in comparison with SEER (20% vs 8.9%; P < .05).

GOG-109 reported on 243 evaluable patients. The appropriate SEER comparison comprised 117 patients who received a diagnosis of stage IB-IIA cervical cancer with positive pelvic lymph nodes and who were treated with radical hysterectomy over the same interval.

In the GOG-109 trial, there was no report on the presence or absence of Asian subjects. Therefore, we presumed that Asian women were categorized as “Others” and included Asian women in the SEER group to facilitate a comparison (Table II). A comparison of

<table>
<thead>
<tr>
<th>Table I</th>
<th>Comparison of subjects in GOG-92 and SEER database with stage IB cervical cancer and negative pelvic nodes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variable</td>
<td>GOG-92 (n)*</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>151 (55%)</td>
</tr>
<tr>
<td>Black</td>
<td>56 (20%)</td>
</tr>
<tr>
<td>Asian</td>
<td>21 (8%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>27 (10%)</td>
</tr>
<tr>
<td>Other</td>
<td>22 (8%)</td>
</tr>
<tr>
<td>Total</td>
<td>277</td>
</tr>
<tr>
<td>Age group (y)</td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>32 (11.5%)</td>
</tr>
<tr>
<td>31-40</td>
<td>106 (38.2%)</td>
</tr>
<tr>
<td>41-50</td>
<td>75 (27%)</td>
</tr>
<tr>
<td>51-60</td>
<td>38 (13.7%)</td>
</tr>
<tr>
<td>&gt;61</td>
<td>26 (9.3%)</td>
</tr>
</tbody>
</table>


† P < .05.
SEER and GOG-109 demonstrated a significant difference in ethnic group distribution, with a larger proportional representation of Hispanic women in GOG-109 as compared with SEER (12.1% vs 5.1%; \( P < .05 \)). There was also a significant difference in disease stage distribution, which favored more patients with stage IB in GOG-109 as compared with the SEER population (stage IB, 94.2% vs 82%; \( P < .05 \)).

For the third comparison, GOG-123 had 369 evaluable patients, all of whom had negative pelvic and para-aortic lymph nodes that were found by imaging. The SEER database contained 113 comparable patients. In GOG-123, there was no identification of Asian women. Therefore, for the SEER comparison group, the Asian women were classified as “Others.” There was a significant difference in the ethnic group distribution, which favored a larger Hispanic proportion in the GOG-123 trial (12.4% vs 7.0%) and more “Others” in the SEER population (5.1% vs 13.8%; \( P < .05 \)). In comparison with SEER, subjects of the GOG-123 trial were significantly younger. The GOG population was 83% more likely to be \( < 50 \) years of age as compared with the women in SEER (odds ratio, 0.17; 95% CI, 0.11-0.26; Table III).

The clinical trials of locally advanced cervical cancer (GOG-56, -85, -120) were grouped together in a fourth category. The data on disease stage, age, and ethnic group distribution were averaged and cumulatively compared with those of the SEER population. There were 1184 evaluable patients in these 3 trials combined (GOG-56 had 296 evaluable patients; GOG-85 had 368 evaluable patients, and GOG-120 had 526 evaluable patients). For GOG-120, the total number of patients (526) was calculated by adding the number of subjects in each treatment arm. In GOG-56, 6 patients with stage III disease that was not specified otherwise to stage III-A or III-B were eliminated from the final analysis (Table IV). In the SEER database, a total of 1951 patients with locally advanced cervical cancer were identified. Of those, 607 patients (31.1%) had their lymph nodes examined. One hundred ninety-four of those 607 subjects (31.9%) had no evidence of metastatic cancer in their nodes. This group comprised 9.9% of the locally advanced disease population for the specified time interval, and only this subpopulation was analyzed.

The publications from GOG-56 and GOG-85 did not provide information on the ethnicity of the study population. Therefore, the ethnic group distribution of GOG-120 population was compared with that of the SEER population. The difference was statistically significant in favor of a larger proportional representation of black and Hispanic women in the GOG-120 trial compared with the SEER population (\( P < .05 \)). A higher percentage of “Asian” and “Other” women were also noted within the SEER population as compared with that of the GOG-120. There was no significant difference in disease stage and age group distribution between the locally advanced cervical cancer clinical trial population and the comparable SEER group (\( P = .31 \)).

Because examination of para-aortic lymph nodes is not standard in the treatment of locally advanced cervical cancer, the selected SEER group with the negative para-aortic nodes composed only 9.9% of the entire locally advanced cervical cancer population.

### Table II Comparison of subjects in GOG-109 with SEER: Stages IB-IIA cervical cancer with positive pelvic nodes

<table>
<thead>
<tr>
<th>Variable</th>
<th>GOG-109 ( n )</th>
<th>SEER ( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>158 (66.1%)</td>
<td>76 (64.9%)</td>
</tr>
<tr>
<td>Black</td>
<td>41 (17.1%)</td>
<td>14 (11.9%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>29 (12.1%)</td>
<td>6 (5.13%)</td>
</tr>
<tr>
<td>Other</td>
<td>11 (4.6%)</td>
<td>21 (17.8%)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Median</td>
<td>39.5</td>
<td>42</td>
</tr>
<tr>
<td>Range</td>
<td>20-77</td>
<td>20-77</td>
</tr>
<tr>
<td>Disease stage</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IB</td>
<td>229 (94.2%)</td>
<td>96 (82%)</td>
</tr>
<tr>
<td>IIA</td>
<td>14 (5.7%)</td>
<td>21 (17.9%)</td>
</tr>
<tr>
<td>Total</td>
<td>239</td>
<td>117</td>
</tr>
</tbody>
</table>


\( P < .05 \).

### Table III Comparison of GOG-123 subjects with SEER: Stage IB2 cervical cancer with negative pelvic and para-aortic nodes

<table>
<thead>
<tr>
<th>Variable</th>
<th>GOG-123 ( n )</th>
<th>SEER ( n )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>218 (59%)</td>
<td>69 (61%)</td>
</tr>
<tr>
<td>Black</td>
<td>86 (23.3%)</td>
<td>21 (18.5%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>46 (12.4%)</td>
<td>8 (7%)</td>
</tr>
<tr>
<td>Other</td>
<td>19 (5.1%)</td>
<td>15 (13.8%)</td>
</tr>
<tr>
<td>Total</td>
<td>369</td>
<td>113</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>38 (10.2%)</td>
<td>3 (2.6%)</td>
</tr>
<tr>
<td>31-60</td>
<td>143 (38.7%)</td>
<td>25 (22.1%)</td>
</tr>
<tr>
<td>61-70</td>
<td>125 (33.8%)</td>
<td>23 (20.3%)</td>
</tr>
<tr>
<td>71</td>
<td>35 (9.4%)</td>
<td>30 (26.5%)</td>
</tr>
<tr>
<td>Total</td>
<td>10 (2.7%)</td>
<td>19 (16.8%)</td>
</tr>
</tbody>
</table>


\( P < .05 \).
This subset of women is limited in its representation of women with locally advanced cervical cancer.

Comment

In the conduct of a clinical trial, the use of a homogenous patient population may be a preferred method to improve the quality of the research design and reduce cost. However, when minorities are excluded, when minorities are included in inadequate numbers, or when the demographics of the sample are not reported sufficiently, there is loss of generalizability of these results, particularly to “special populations” of patients.25

The external validity of a study is endangered by many factors (such as selection bias), where the initial choice of the population that was sampled is not a true cross section of the desired (reference) population.26 Awareness of this bias is important when the applicability of trial results to the general population is evaluated. However, this bias remains unmentioned and probably unexamined in most published clinical trials.

Nonparticipants in a trial may have different socio-demographic, biologic, and clinical profiles that could affect the validity of the extrapolation of the results of these trials.27 Racial differences exist in the response to medications such as beta-blockers, angiotensin-convert-ing enzyme inhibitors,28 antidepressants,29 interferon,30 and the metabolism of tamoxifen.31 In an extensive review of pharmacology literature, Wood and Zhou32 and Johnson33 documented the influence of ethnicity on pharmacokinetics of drugs and provided many examples of racial differences in bioavailability, distribution, metabolism, and renal elimination. These examples indicate the need for adequate representation of racial minorities in clinical drug trials. Unfortunately, many investigators do not seem to adequately take into account racial differences as a potential source of variability.34 This results in unexpected side effects when medications are marketed to a population that is quite different from the clinical trial population.27 However, the design of clinical trials to assess race and ethnicity interactions is complex, and there are practical limitations to the development of such studies. The subtleties that surround this issue have been debated extensively.35

Current available data do not provide age-adjusted incidence of cervical cancer of the ethnic groups at the multiple trial recruitment sites. This analysis could be performed only with the use of a comparison of

<table>
<thead>
<tr>
<th>Variable</th>
<th>SEER (n)</th>
<th>GOG-average (n)</th>
<th>GOG120 (n)*</th>
<th>GOG -85 (n)†</th>
<th>GOG -56 (n)‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>117 (60.3%)</td>
<td>—</td>
<td>303 (57.6%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Black</td>
<td>33 (17%)</td>
<td>—</td>
<td>130 (24.7%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Asian</td>
<td>9 (9.7%)</td>
<td>—</td>
<td>20 (3.8%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9 (9.2%)</td>
<td>—</td>
<td>67 (12.7%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Other</td>
<td>7 (3.6%)</td>
<td>—</td>
<td>6 (1.1%)</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;30</td>
<td>9 (4.6%)</td>
<td>62 (5.2%)</td>
<td>26 (4.9%)</td>
<td>21 (5.7%)</td>
<td>15 (5%)</td>
</tr>
<tr>
<td>31-40</td>
<td>39 (20.1%)</td>
<td>261 (21.9%)</td>
<td>129 (24.5%)</td>
<td>80 (21.7%)</td>
<td>52 (17.5%)</td>
</tr>
<tr>
<td>41-50</td>
<td>49 (25.26%)</td>
<td>346 (29%)</td>
<td>148 (28.1%)</td>
<td>122 (33.1%)</td>
<td>76 (25.6%)</td>
</tr>
<tr>
<td>51-60</td>
<td>50 (25.7%)</td>
<td>300 (25.2%)</td>
<td>129 (24.5%)</td>
<td>82 (22.2%)</td>
<td>89 (30%)</td>
</tr>
<tr>
<td>61-70</td>
<td>34 (17.5%)</td>
<td>181 (15.2%)</td>
<td>77 (14.6%)</td>
<td>52 (14.1%)</td>
<td>52 (17.5%)</td>
</tr>
<tr>
<td>&gt;71</td>
<td>13 (6.7%)</td>
<td>40 (3.36%)</td>
<td>17 (3.2%)</td>
<td>11 (2.9%)</td>
<td>12 (4%)</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>1190</td>
<td>526</td>
<td>368</td>
<td>296</td>
</tr>
<tr>
<td>Disease stage</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>II-B</td>
<td>124 (63.9%)</td>
<td>683 (57.6%)</td>
<td>275 (52.2%)</td>
<td>228 (61.9%)</td>
<td>180 (60.8%)</td>
</tr>
<tr>
<td>III-A</td>
<td>13 (6.7%)</td>
<td>26 (2.19%)</td>
<td>15 (2.85%)</td>
<td>10 (2.7%)</td>
<td>1 (0.3%)</td>
</tr>
<tr>
<td>III-B</td>
<td>44 (22.6%)</td>
<td>438 (36.9%)</td>
<td>220 (41.8%)</td>
<td>118 (32%)</td>
<td>100 (33.7%)</td>
</tr>
<tr>
<td>III</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>6 (2%)</td>
</tr>
<tr>
<td>IV-A</td>
<td>13 (6.7%)</td>
<td>37 (3.12%)</td>
<td>16 (3%)</td>
<td>12 (3.2%)</td>
<td>9 (3%)</td>
</tr>
<tr>
<td>Total</td>
<td>194</td>
<td>1184</td>
<td>526</td>
<td>368</td>
<td>296</td>
</tr>
</tbody>
</table>


\( p < .05.\)
proportions. The authors could not include the non-evaluable patients who were enrolled in the various clinical trials because these specific demographic data were not provided in the published reports, neither could we identify the subjects who participated in the clinical trials who were represented in the SEER dataset.

Although 8 published trials met our inclusion criteria,\textsuperscript{17-24} the results of Radiation Therapy Oncology Group 79-20 were not included in category III because they included other disease stages along with stage IB2 (stage IB, 17.2%; stage IIA, 9.7%; stage IIB, 72.9%). This would have confounded the comparison to the SEER group. Similarly, the results of RTOG-9001\textsuperscript{23} were eliminated from category IV because they included patients with earlier stages of disease (stage IB, 27.3%; stage IIA, 6.1%).

We compared the age and ethnic distribution of the GOG cervical cancer study population to the reference general disease population. There were no significant differences in the age group proportions between cooperative group trial populations and that of the general disease population, with the exception of the subjects in GOG-123 who were younger than the comparison population. For both populations (the cooperative group trial and SEER) differences are identified in the age distribution of different cervical cancer stages and treatment groups (ie, a younger patient population with early stage disease who underwent radical surgery and an older population with more advanced disease stage who underwent RT). These differences in the SEER population are consistent with previously published reports\textsuperscript{36} and might be attributed to underlying comorbid factors that preclude radical surgical procedures in the older population.

The similarity with regard to age and race between the SEER population and the cervical cancer trials populations does not eliminate the selection-bias that is associated with these clinical trials. The representative nature of these basic demographic factors does not confirm that the findings of the GOG cervical cancer trials are generalizable to the cervical cancer population. It is typical for only a subset of patients from the target population to access a clinical trial. Furthermore, entry into the trials that are described in this article generally imposed limits on the subjects’ pretreatment laboratory values and functional status, whereas the performance status for patients within the SEER comparison groups is not recorded. Finally, many of the subjects who are enrolled in the GOG trials are recruited from cancer centers or university-based facilities that are not selected randomly, and have differences in recruitment success. However, because patient recruitment sites are not mentioned in the published reports of clinical trials, our ability to assess selection bias remains limited.

In summary, for each of the examined categories that were defined by clinically significant trials, there was no significant difference in age distribution between the patient population that was enrolled in cooperative group trials and that of the general cervical cancer population that was described by SEER. Women of black and Hispanic groups are recruited proportionately to cooperative group cervical cancer trials. Cooperative group trials provide a unique opportunity to analyze outcomes by race in an equal-care setting. This argues for diligent collection and reporting of demographic data for participating and nonparticipating patients at individual recruitment sites.

References


Objective: The purpose of this study was to determine cervical cancer screening practices of obstetrician/gynecologists in the US after recent revised guidelines.

Study design: Questionnaires were mailed to 355 randomly selected US obstetrician/gynecologists. Questions were structured as clinical vignettes.

Results: Questionnaires were returned by 60% (213/355) of recipients; 185 were eligible. Seventy-four percent would begin screening virginal girls at age 18. Sixty percent would continue annual screening in a 35-year-old woman with 3 or more normal tests. Frequent screening is common in women after total hysterectomy for symptomatic fibroids and no history of dysplasia, and in 70-year-old women with a 30-year history of previous normal tests. Most (82%) use liquid-based cytology; 78% of female respondents would prefer it for themselves. Most (64%) would not adopt triennial Pap/HPV DNA screening, although 58% of women would choose it for themselves.

Conclusion: Most US obstetrician/gynecologists screen low-risk women often and indefinitely, despite national guidelines designed to minimize screening harms resulting from overtesting.

In the US, an estimated 10,520 new cases of invasive cervical cancer, and 3900 cervical cancer deaths, will occur in the year 2004. Widespread use of Pap tests for cervical cancer screening has accompanied a dramatic decline in cervical cancer mortality over the last several decades. The American Cancer Society (ACS) has recently revised screening recommendations, suggesting that screening start within 3 years of sexual activity or age 21, and be less frequent than annual in women over age 30 with 3 or more previous normal tests. They also endorse cotesting with human papillomavirus (HPV) and discontinuing screening in certain women. The American College of Obstetricians and Gynecologists (ACOG) has recently made similar recommendations. Despite revised national screening guidelines, little is known about how obstetricians/gynecologists have modified their screening behavior in this important area.
of women’s health. A previous recent study examined obstetrician/gynecologists, subspecialists, and internal medicine physicians’ screening practices in 2001, before the major screening guideline changes.5

The purpose of our study was to determine the present cervical cancer screening practices of obstetrician/gynecologists in the US subsequent to publication of the ACS guidelines.

**Material and methods**

We used the American Medical Association (AMA) master list database to select a random population of 355 of the approximate total 40,000 US practicing obstetrician/gynecologists. The AMA master list was used because it has one of the largest US physician databases, and is not limited to AMA members. Stratified simple random sampling was used to generate a sample in proportion to the number from each state in the database. The sample size of 355 was selected based on the assumption of a 50% response rate and was calculated to assure a ±10% sampling error for the 95% CI for each question. Before sending out the questionnaire to subjects, the study tool was pilot tested for clarity by obstetrician/gynecologist physicians at one of our institutions. The study was approved by

**Table 1** Respondents’ baseline characteristics (n = 185)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>N</th>
<th>(%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Average Pap tests per week</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-15</td>
<td>22</td>
<td>(11.9)</td>
</tr>
<tr>
<td>16-30</td>
<td>54</td>
<td>(29.2)</td>
</tr>
<tr>
<td>More than 30</td>
<td>96</td>
<td>(51.9)</td>
</tr>
<tr>
<td>No response</td>
<td>13</td>
<td>(7.0)</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤40</td>
<td>51</td>
<td>(27.6)</td>
</tr>
<tr>
<td>41-50</td>
<td>55</td>
<td>(29.7)</td>
</tr>
<tr>
<td>51-60</td>
<td>45</td>
<td>(24.3)</td>
</tr>
<tr>
<td>≥61</td>
<td>34</td>
<td>(18.4)</td>
</tr>
<tr>
<td>Practice setting</td>
<td></td>
<td></td>
</tr>
<tr>
<td>University</td>
<td>11</td>
<td>(5.9)</td>
</tr>
<tr>
<td>Private/managed care</td>
<td>150</td>
<td>(81.1)</td>
</tr>
<tr>
<td>Health maintenance organization</td>
<td>9</td>
<td>(4.9)</td>
</tr>
<tr>
<td>County, state, military, or public health</td>
<td>6</td>
<td>(3.2)</td>
</tr>
<tr>
<td>Other</td>
<td>5</td>
<td>(2.7)</td>
</tr>
<tr>
<td>No response</td>
<td>4</td>
<td>(2.2)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>118</td>
<td>(63.8)</td>
</tr>
<tr>
<td>Female</td>
<td>67</td>
<td>(36.2)</td>
</tr>
<tr>
<td>Geographic region</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Northeast</td>
<td>31</td>
<td>(16.8)</td>
</tr>
<tr>
<td>Midwest</td>
<td>42</td>
<td>(22.7)</td>
</tr>
<tr>
<td>South</td>
<td>66</td>
<td>(35.7)</td>
</tr>
<tr>
<td>West</td>
<td>46</td>
<td>(24.9)</td>
</tr>
</tbody>
</table>
the Institutional Review Board of the University of California, San Francisco.

In May and June, 2003, letters were sent describing the study, and informing potential subjects of its upcoming arrival in the mail. One week later, the questionnaire, consent form, and $5 cash were mailed to each subject. Ten days later a postcard was sent requesting completion of the questionnaire if not yet done. A duplicate questionnaire was sent 1 week later to subjects who had not yet responded. All questionnaires and envelopes had anonymous sequential identification numbers that were kept separate from subjects’ names during data entry and analysis.

The questionnaire contained 21 categorical questions for all participants, and 8 additional questions for female participants. The questions were structured as clinical vignettes on several subjects, including age to begin and end screening, screening frequency, screening post-hysterectomy, teen pregnancy screening, techniques being used to screen, and coscreening with HPV DNA. In addition, female physicians were asked questions regarding personal frequency and preferences for cervical cancer screening.

We excluded respondents who indicated that they did not perform Pap tests (n = 4), those who were retired (n = 13), and those who were not direct patient care physicians in obstetrics/gynecology, gynecology, or obstetrics (n = 2). Subspecialists, including reproductive endocrinologists, gynecologist oncologists, and maternal-fetal medicine specialists (n = 8), were also excluded. Before randomly selecting the population from the AMA database we excluded any non–US-based resident, research, or administration-only physicians. Analysis consisted of descriptive statistics to include mean and standard deviations for continuous variables, and frequencies and crosstabs for categorical data. A chi-square test was used to compare proportions responding between the 4 geographic regions.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Age to begin screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>Yes n (%)</td>
</tr>
<tr>
<td>An 18-year-old woman who is not sexually active presents for her first gynecologic visit. Do you recommend a Pap smear?</td>
<td>136 (73.5)</td>
</tr>
<tr>
<td>An 18-year-old woman who became sexually active for the first time one month ago presents for her first gynecologic visit. Do you recommend a Pap smear?</td>
<td>175 (94.6)</td>
</tr>
<tr>
<td>A 35-year-old woman who has never had sexual intercourse presents for an annual visit. Do you recommend a Pap smear?</td>
<td>172 (93.0)</td>
</tr>
<tr>
<td>An 18-year-old woman who is 12 weeks pregnant comes to your office for her first prenatal visit. Do you recommend a Pap smear?</td>
<td>178 (96.2)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table III</th>
<th>Screening frequencies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>Pap every 6 months</td>
</tr>
<tr>
<td>A 35-year-old woman who has had 3 documented consecutive negative Pap smears performed by you and who has no new sexual partners within the last 5 years wants to know how often she needs a Pap test. You would recommend:</td>
<td>N/A</td>
</tr>
<tr>
<td>She is very anxious about cervical cancer having had a friend recently diagnosed and is insisting upon wanting Pap smear screening every 6 months. After counseling her about her risk and your recommendations for screening, you would perform:</td>
<td>46 (24.9)</td>
</tr>
</tbody>
</table>
Results

The Figure summarizes outcomes of all questionnaires mailed. The response rate was 60% of all questionnaires sent (213/355). Excluding questionnaires returned unopened (n = 4), the response rate was 60.7% (213/351). One hundred and eighty-five (86.7%) respondents were eligible; 118 (63.8%) were male, and 67 (36.2%) were female.

The majority of respondents performed more than 30 Pap tests per week, were less than 60 years of age and male, and worked in a private practice/managed care setting (Table I). Table II summarizes the age that respondents begin screening. The majority of partici-
pants (75.3%) would screen an 18-year-old patient who is not sexually active, and 96.2% of participants would screen an 18-year-old patient at her first prenatal visit. Table III summarizes the participants’ preferences for screening frequency. Most participants screen all patients annually, and 25% would screen more often than annually if a woman requested it.

Table IV shows the respondents’ answers on when to end screening. Most screen regularly in a 70-year-old patient with a previous negative 30-year history of Pap tests. If this patient had difficulty with the examination and inquires about stopping screening, 60% would continue screening, and 47% would continue screening if her life expectancy was less than 5 years. After total hysterectomy in a 55-year-old woman for symptomatic fibroids and no history of dysplasia, 79% would continue screening.

Table V displays the screening methods preferred by study participants. The majority of participants (82%) use liquid-based cytology, and of those who use conventional cytology, the majority use combination spatula/brush. If the option became available to combine screen with a Pap and HPV DNA every 3 years, 33% would adopt this strategy.

There were no major differences in screening behavior that could be detected in this population with regard to geographic region, practice setting, gender, age, or history of dysplasia, although power was limited to detect these differences.

Comment

Most obstetrician/gynecologists we surveyed begin screening at age 18, and screen annually despite ACS and ACOG recommendations allowing for less frequent screening. Most are reluctant to discontinue screening or screen less often than annually despite recommendations to do so by the ACS and the US Preventive Services Task Force (USPSTF). Scant evidence exists concerning which guidelines are most often followed by obstetrician/gynecologists. Of note, in 2001, state-mandated screening programs were more likely to follow ACS guidelines rather than those of the USPSTF.

Until recently, little was known about screening practices among US physicians. Our findings are similar to those from a recent survey, but differ in important ways. Our questionnaire was distinct because it consisted predominately of clinical vignettes, which have been shown to be a valid and comprehensive tool for measuring actual clinical behavior, and are a useful way to measure physician practices in an outpatient setting.

In contrast to the previous survey, we did not include any select groups of physicians who voluntarily participate in questionnaire studies in order to try to draw conclusions of the actual practices of a variety of physicians.

<table>
<thead>
<tr>
<th>Table V</th>
<th>Methods used for cytology screening</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>n (%)</td>
</tr>
<tr>
<td>Which cytology method do you use most often for cervical cancer screening?</td>
<td></td>
</tr>
<tr>
<td>Liquid-based cytology</td>
<td>151 (81.6)</td>
</tr>
<tr>
<td>Conventional cytology</td>
<td>31 (16.8)</td>
</tr>
<tr>
<td>No response</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>If you use conventional smears at all, which of the following collection instruments do you use most?</td>
<td></td>
</tr>
<tr>
<td>Spatula only</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Brush only</td>
<td>9 (4.9)</td>
</tr>
<tr>
<td>Combination spatula/brush</td>
<td>100 (54.0)</td>
</tr>
<tr>
<td>Combination spatula/swab</td>
<td>2 (1.1)</td>
</tr>
<tr>
<td>Combination brush/swab</td>
<td>3 (1.6)</td>
</tr>
<tr>
<td>Not applicable, do not use conventional smears at all</td>
<td>65 (35.1)</td>
</tr>
<tr>
<td>No response</td>
<td>4 (2.2)</td>
</tr>
</tbody>
</table>

The American Cancer Society (ACS) has recommended that women over age 30 years may be primarily screened with cytology plus a cervical test for types of human papillomavirus (HPV) associated with cervical cancer. The ACS recommended that if both tests are negative, screening should not be performed again for 3 years. This strategy is an alternative to more frequent screening with cytology alone. If this strategy were made available to you, would you adopt this type of screening in your practice?

| Yes | 61 (33.0) |
| No | 118 (63.8) |
| No response | 6 (3.2) |
different obstetrician/gynecologists across the US. We surveyed a randomly selected population of currently practicing obstetrician/gynecologists—those who are doing the majority of the screening, and who are most likely to be updated on the most recent guidelines compared with primary care physicians. Our study also examined obstetrician/gynecologists’ views on cervical cancer screening preferences, and patterns for themselves. This has not been the focus of much previous literature, and provides interesting insight into physicians’ personal screening practices.

One of the major changes in guidelines has been a recommendation by the ACS that screening be delayed for up to 3 years after the onset of sexual intercourse or age 21. One rationale behind this delay is the concern that screening before the 3-year period may result in an overdiagnosis of lesions that will regress spontaneously, leading to inappropriate interventions that may be more harmful than beneficial. Our survey suggests that screening within even a month of debut of sexual intercourse is nearly universal; so many young women may be put at undue harm. Clinicians should be aware that loop excision of cervical intraepithelial neoplasia has recently been associated with adverse pregnancy outcomes.9

Of interest, the single response for which there was 100% agreement among respondents was the decision to screen pregnant 18-year-olds. Although no guideline specifically addresses this issue, a cogent case can be made for this practice being controversial. The goal of cytology screening in pregnancy is to identify invasive cervical disease that will affect delivery plans. This is in sharp contrast to the goal of screening in nonpregnant women, where preinvasive lesions are sought and either destroyed or excised. Treatment of preinvasive disease is contraindicated in pregnancy. Invasive cervical cancer is extremely rare in women under age 20 years, 10 but cytologic abnormalities are not uncommon in adolescents having unprotected intercourse. This common screening practice requires a more thoughtful analysis as to its true benefits and harms.

Most respondents screen annually and use liquid-based cytology. Of note, the ACS states that clinicians using liquid-based cytology should screen every 2 years, and not annually, to avoid what they describe as “increases in the detection of atypical squamous cells of undetermined significance and low-grade abnormalities with subsequent increases in referral to colposcopy unnecessarily, risking the potential for over treatment and increased health care costs.”3 Of interest, we observed that 1 in 4 physicians would screen every 6 months in a patient who was anxious and requested more frequent screening. Women should be reassured about their low risk of cervical cancer within short periods of time following normal cytology,11 but also be aware of the likelihood of false-positive testing and subsequent invasive interventions inherent in screening low-risk women.12

Presently, several major guidelines suggest discontinuing screening in patients age 65 or 70 without a history of dysplasia with adequate recent screening. In our

<table>
<thead>
<tr>
<th>Table VI</th>
<th>Female physicians’ personal screening preferences (n = 67)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Question</td>
<td>n (%)</td>
</tr>
<tr>
<td>Has your cervix been removed? (ie, have you had a total hysterectomy?)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>No</td>
<td>59 (88.1)</td>
</tr>
<tr>
<td>When was your last Pap smear?*</td>
<td></td>
</tr>
<tr>
<td>Within the last year</td>
<td>45 (67.1)</td>
</tr>
<tr>
<td>About 2 years ago</td>
<td>8 (11.9)</td>
</tr>
<tr>
<td>About 3 years ago</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Over 3 years ago</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>No response</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>On average, you have a Pap smear performed:*</td>
<td></td>
</tr>
<tr>
<td>Annually</td>
<td>41 (61.2)</td>
</tr>
<tr>
<td>Every 2 years</td>
<td>9 (13.4)</td>
</tr>
<tr>
<td>Every 3 years</td>
<td>5 (7.4)</td>
</tr>
<tr>
<td>Less often than every 3 years</td>
<td>2 (3.0)</td>
</tr>
<tr>
<td>I do not have Pap smears performed</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>No response</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Which type of cytology screening method would you prefer your provider uses when performing your screening?</td>
<td></td>
</tr>
<tr>
<td>Conventional cytology</td>
<td>4 (6.0)</td>
</tr>
<tr>
<td>Liquid-based cytology</td>
<td>52 (77.6)</td>
</tr>
<tr>
<td>No preference</td>
<td>10 (14.9)</td>
</tr>
<tr>
<td>No response</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>If you are being screened for cervical cancer currently, would you want to be screened primarily with both a cytology test and human papillomavirus (HPV) test?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>39 (58.2)</td>
</tr>
<tr>
<td>Would not adopt this method for their patients</td>
<td>26 (66.7)</td>
</tr>
<tr>
<td>Would adopt this method for their patients</td>
<td>13 (33.3)</td>
</tr>
<tr>
<td>No</td>
<td>22 (32.8)</td>
</tr>
<tr>
<td>Would not adopt this method for their patients</td>
<td>16 (72.7)</td>
</tr>
<tr>
<td>Would adopt this method for their patients</td>
<td>6 (27.3)</td>
</tr>
<tr>
<td>No response</td>
<td>6 (8.9)</td>
</tr>
<tr>
<td>Have you ever had an abnormal Pap smear?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>15 (22.4)</td>
</tr>
<tr>
<td>No</td>
<td>51 (76.1)</td>
</tr>
<tr>
<td>No response</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Have you ever had colposcopy?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>11 (16.4)</td>
</tr>
<tr>
<td>No</td>
<td>55 (82.1)</td>
</tr>
<tr>
<td>No response</td>
<td>1 (1.5)</td>
</tr>
<tr>
<td>Have you ever had treatment for dysplasia (eg, cryotherapy, LEEP, cone biopsy)?</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>5 (7.5)</td>
</tr>
<tr>
<td>No</td>
<td>60 (89.5)</td>
</tr>
<tr>
<td>No response</td>
<td>2 (3.0)</td>
</tr>
</tbody>
</table>

* Excludes women who stated they had a total hysterectomy.
study, few respondents were comfortable discontinuing screening, but were willing to do so if the patient had difficulty undergoing the examination, or had a shortened life expectancy. However, most physicians screened older patients regularly despite many negative Pap tests, patients who had had a hysterectomy for symptomatic fibroids and no history of dysplasia. Clinicians should be aware of the poor positive predictive value of cytology in well-screened older women, and women without a cervix, and other downsides of too-frequent screening in this population. Moreover, the ACS, ACOG, and USPSTF all recommend discontinuation of screening after removal of the cervix in women without previous cervical disease.

Most physicians preferred liquid-based cytology both for use on their patients and themselves, despite lack of clear benefits over conventional cytology. Of note, the USPSTF stated after an extensive review that “the quality of the literature is poor for choosing between screening systems in US populations.” Most physicians did not elect to adopt combined Pap/HPV DNA coscreening for their patients, although the majority of female physicians expressed interest in having this type of screening done on themselves. This discrepancy possibly reflects concern that patients may receive scant medical care in the interim years between screenings because for many women, Pap tests are the main reason to seek medical care. Alternatively, clinicians in fee-for-service settings may be disinclined to adopt guidelines that involve less-than-annual visits.

Our study population appears to be demographically representative of the US population of practicing obstetrician/gynecologists and, thus, likely reflects many of the present national practices. Our study, however, has a number of limitations. First, our sample was mainly composed of private practice/managed care physicians; our results may not be applicable to other practice settings. Second, as with all questionnaires, those returning our survey may have been a select group of clinicians whose views may have not been representative of the larger sample to which we sent surveys. The demographics of nonrespondents were largely unknown, although we were able to determine if differences in rates varied by geographic regions. All regions had an over 60% response rate except the Northeast, which had a 46% response rate ($P = .02$). Our results, therefore, may not be representative of screening behaviors in that area of the country. Our overall response rate of 60% was relatively high compared with the mean response rate of 54% of published physician surveys.

Future research should focus on ascertaining the reasons why clinicians do not adhere to guidelines and devising educational interventions that will modify screening behaviors. Pap test screening is one of the greatest success stories in modern medicine, and it is commendable that physicians take this seriously and advocate regular screening for all patients. At the same time, the resources spent towards overzealous screening in low-risk populations, and the harms incurred by false-positive tests deserve focused attention. Clinicians should be better informed of the scientific evidence that underpins the rationale behind national guidelines from professional groups because they are crafted to maximize screening benefits and minimize screening harms. Concurrently, attention should be given to the best methods of educating women about optimal screening strategies to facilitate informed decision making.

Acknowledgments

We would like to thank Abigail Brekenridge, Alexis Martinez, Tracy Weitz, and Felicia Stewart in the Advancing New Standards in Reproductive Health (ANSIRH) program at the UCSF Center for Reproductive Health Research & Policy for their invaluable help.

References


A randomized trial that compared povidone iodine and chlorhexidine as antiseptics for vaginal hysterectomy

Patrick J. Culligan, MD,a,* Kari Kubik, MD,a Miles Murphy, MD, MSPH,a Linda Blackwell, RN, BSN,a James Snyder, PhDb

Department of Obstetrics, Gynecology, and Women’s Health, Division of Urogynecology and Pelvic Reconstructive Surgery,a Department of Pathology, Division of Laboratory Medicine,b University of Louisville Health Sciences Center, Louisville, Ky

Received for publication June 10, 2004; revised August 5, 2004; accepted August 5, 2004

Objective: The purpose of this study was to compare the efficacy of chlorhexidine and povidone iodine for cleansing the operative field for vaginal surgery.

Study design: This was a randomized controlled trial that compared 10% povidone iodine and 4% chlorhexidine gluconate as surgical scrubs. Our primary end point was the proportion of contaminated specimens (defined as total bacterial colony counts of ≥5000 colony-forming units) per group found throughout the surgical procedures. All patients received standard infection prophylaxis that included preoperative intravenous antibiotics. Immediately before antibiotic administration and baseline aerobic and anaerobic cultures of the vaginal flora were obtained, which were followed by cultures at 30 minutes after the surgical scrub and hourly thereafter throughout each patient’s surgery.

Results: A total of 50 patients were enrolled between October 2002 and September 2003. There were no differences between the povidone iodine (n = 27) and chlorhexidine (n = 23) groups with respect to age, race, exogenous hormone use, body mass index, gravity, parity, preoperative mean colony counts, or operative time. Among the first set of intraoperative specimens (which were obtained 30 minutes after the surgical scrub), 63% of the cultures (17/27) from the povidone iodine group and 22% of the cultures (5/23) from the chlorhexidine group were classified as contaminated (P = .003; relative risk, 6.12; 95% CI, 1.7, 21.6). Subsequent cultures failed to demonstrate significant differences.

Conclusion: Chlorhexidine gluconate was more effective than povidone iodine in decreasing the bacterial colony counts that were found in the operative field for vaginal hysterectomy.

© 2005 Elsevier Inc. All rights reserved.

Before the widespread use of aseptic techniques and prophylactic antibiotics, the rate of wound infection after vaginal hysterectomy was an unacceptably high 30% to 40%. Eventually, evidence from dozens of randomized controlled trials prompted the American College of Obstetricians and Gynecologists...
to recommend antimicrobial prophylaxis for all vaginal hysterectomies.\textsuperscript{2}

In addition to antibiotic prophylaxis, preparation of the surgical field with povidone iodine has been recommended widely.\textsuperscript{3,4} Although the exact mechanism by which iodine destroys bacteria is unknown, it has been postulated that iodine reacts with bacterial amino acids and fatty acids resulting in the destruction of their cellular structures and enzymes.\textsuperscript{5}

Another surgical antiseptic, chlorhexidine gluconate, causes the destruction of bacterial cell membranes leading to leakage of cellular constituents and coagulation of cell contents.\textsuperscript{3} Both agents have been shown to decrease cutaneous and mucosal bacterial counts in the vagina.\textsuperscript{6-8} However, there have been no prospective, randomized trials that have compared these 2 agents’ efficacy for vaginal surgery.

Therefore, our objective was to compare the efficacy of chlorhexidine and povidone iodine for cleansing the operative field for vaginal surgery.

\section*{Material and methods}

This was a randomized controlled trial that was approved by the University of Louisville Health Sciences Center Human Studies Committee that compared povidone iodine and chlorhexidine gluconate to prepare the surgical field before vaginal hysterectomy with or without reconstructive pelvic surgery. We had no outside funding source for this study; therefore, it was paid for by the University of Louisville Urogynecology Divisional account.

In preparation for this randomized trial, our group first carried out a pilot study that described the bacterial colony counts that were found throughout the course of vaginal surgery.\textsuperscript{9} In that study, we obtained serial vaginal cultures before and during 31 vaginal hysterectomies. Each of those patients received standard infection prophylaxis that included preoperative intravenous antibiotics and a standardized surgical scrub with povidone iodine. For the purposes of that study, our definition of contaminated included any specimen culture that yielded \textgeq 5000 colony-forming units per milliliter. Pilot study results were as follows:\textsuperscript{9}: The first set of intraoperative cultures were obtained 30 minutes after the completion of the scrub; 52\% of the cultures (16/31) were contaminated. In the next set of cultures (which were obtained 90 minutes after the initial scrub), 41\% of the cultures (12/29) were contaminated. The remaining sets of cultures showed progressively fewer contaminated specimens, which prompted us to conclude that future investigations regarding bacterial colony counts during vaginal surgery should focus on the initial 30 to 90 minutes of the procedure.

Using that pilot data, we performed a sample size estimate for our randomized trial. In doing so, we decided that a reduction in the contaminated specimens from 52\% to 10\% may be clinically significant. Therefore, we needed 22 patients in each arm of the study to have an 80\% power to detect that difference (\(\alpha = .05\)). All patients who underwent vaginal hysterectomy through our institution between October 2002 and September 2003 were offered enrollment. Patients were assigned randomly to receive a standardized preoperative scrub with either 10\% povidone iodine (Medline Prep Solution; Medline Industries, Mundelein, Ill) or 4\% chlorhexidine gluconate (Dynahep 4; Xtritium Laboratories Inc, Chicago, Ill). Randomization was performed immediately after enrollment on the day of surgery. A blocked random assignment technique was used to determine the allocation sequence, and opaque sealed envelopes were used to conceal the group assignments.

Our primary end point was the proportion of contaminated specimens per group that were found throughout the surgical procedures.

All patients received standard infection prophylaxis that included preoperative intravenous antibiotics within 30 minutes of the surgical start time. Cefazolin (1 g) was used, unless a patient reported an allergy to this medication. In those cases, clindamycin (900 mg) and gentamicin (120 mg) were used. Our only exclusion criterion was patient-reported allergy to iodine.

Immediately before the administration of the preoperative antibiotics, baseline aerobic and anaerobic cultures of the vaginal flora were obtained with a combined aerobic/anaerobic collection and transport system (CultureSwab Plus; Becton Dickinson, Franklin Lakes, NJ). A standard technique was used to obtain all cultures:\textsuperscript{9}: With the patient in the dorsal lithotomy position, a swab was placed in the posterior fornix and agitated throughout the length and circumference of the vagina for 1 minute. Care was taken to include the entire surface area of the vagina, but the cervix was avoided.

The same technique was used to obtain cultures of the vaginal field 30 minutes after the completion of the surgical scrub and hourly thereafter throughout each surgery. Exact time intervals between cultures were determined with a stopwatch.

Immediately after each operation, the culture transport tubes were taken to the University of Louisville Hospital Microbiology Laboratory for processing, as previously described.\textsuperscript{9} The laboratory personnel were blinded to the patient group assignments. A sterile, calibrated (0.01 mL) loop was used to inoculate the specimen onto 5\% sheep blood agar and chocolate agar plates that were incubated at 35°C in 5\% to 10\% carbon dioxide (aerobic cultures). Cultures for anaerobic micro-organisms were inoculated quantitatively on \textit{Brucella} blood agar, phenylethyl alcohol, kanamycin vancomycin agar, and \textit{Bacteroides} bile esculin agar. Manual colony counts were reported for all positive
cultures; the identification was performed according to standard biochemical methods. The approach to quantifying microbial flora involved a 0.01-mL calibrated loop. Using this technique, 1 colony is equivalent to 100 colony-forming units per milliliter of specimen. For each specimen, the "total colony count" was determined by adding all colony counts (regardless of bacteria type). For any specimen, a total colony count of \( \geq 5000 \) colony-forming units per milliliter was classified as contaminated.

To detect any clinical infections, routine postoperative office visits that included a pelvic examination were carried out between 2 and 6 weeks after surgery.

Statistical analyses were performed with SPSS software (version 11.0; SPSS Inc, Chicago, Ill). Demographic characteristics of the 2 groups were compared with the Pearson chi-squared test (for proportions) or the independent samples \( t \)-test (for continuous variables). At each time interval, the proportions of contaminated specimens were compared between groups with the Pearson chi-squared test.

### Results

Fifty patients were enrolled between October 2002 and September 2003. Only 1 eligible patient refused enrollment, citing no specific reason. The entire study protocol was completed for all 50 patients. No protocol deviations occurred. As expected in a randomized trial, there were no differences between the povidone iodine (n = 27 patients) and chlorhexidine (n = 23 patients) groups with respect to age, race, exogenous hormone use, body mass index, gravity, parity, or preoperative mean colony counts (Table).

All but 4 patients (2 in each group) received cefazolin; the remaining patients received clindamycin and gentamicin. There was no difference between the 2 groups with respect to mean surgical duration. The mean operative times for the chlorhexidine and iodine groups were 95 ± 74 minutes and 74 ± 65 minutes, respectively (\( P = .3 \)). All patients returned for at least 1 visit between 2 and 6 weeks after the operation; no patient in either group had evidence of an operative site infection.

At the first postoperative interval (30 minutes after the surgical scrub), 63% of the cultures (17/27) from the povidone iodine group and 22% of the cultures (5/23) from the chlorhexidine group were classified as contaminated (\( P = .003 \); relative risk, 6.12; 95% CI, 1.7, 21.6). The mean colony counts at 30 minutes were 10,743 ± 28,906 for the povidone iodine group and 15,730 ± 28,559 for the chlorhexidine group (\( P = .54 \)).

At the second postoperative interval (90 minutes after the surgical scrub), 36% of the povidone iodine cultures (4/11) and 14% of the chlorhexidine (2/14 cultures) groups were classified as contaminated (\( P = .12 \); relative risk, 3.4; 95% CI, 0.5, 23.8). The mean colony counts at 90 minutes were 20,472 ± 40,058 for the povidone iodine group and 1221 ± 2857 for the chlorhexidine group (\( P = .001 \)).

At the third postoperative interval (150 minutes after the surgical scrub), 50% of the povidone iodine group (3/6 cultures) and 14% of the chlorhexidine group (1/7 cultures) were classified as contaminated (\( P = .17 \); relative risk, 6.0; 95% CI, 0.4, 85.3).

### Comment

Despite the widespread use of aseptic techniques and prophylactic antibiotics, infection remains the most common complication that is associated with vaginal hysterectomy, occurring between 2.1% and 9.5% of cases. These infections arise primarily from the ascending spread of micro-organisms from the upper vagina. The obvious ideal strategy for lowering these infection rates would incorporate only randomized controlled trials, with operative site infections as their primary outcome measure. However, such studies would require large numbers of patients to detect a clinically significant difference in postoperative infections. For example, a randomized controlled trial with 80% power to detect a reduction in infection rates from 6% to 3% would require 814 patients in each arm. Given the

### Table: Demographics and baseline colony counts

<table>
<thead>
<tr>
<th>Variable</th>
<th>Povidone iodine group (n = 27)</th>
<th>Chlorhexidine group (n = 23)</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean preoperative colony count (n)</td>
<td>172,296 ± 74,866</td>
<td>211,956 ± 94,204</td>
<td>.10</td>
</tr>
<tr>
<td>Mean age (y)*</td>
<td>42.6 ± 7.8</td>
<td>45.0 ± 11.5</td>
<td>.67</td>
</tr>
<tr>
<td>Mean body mass index (kg/m²)*</td>
<td>30.4 ± 6.4</td>
<td>29.9 ± 7.8</td>
<td>.82</td>
</tr>
<tr>
<td>Mean gravidity (n)*</td>
<td>3.0 ± 1.7</td>
<td>3.1 ± 1.2</td>
<td>.77</td>
</tr>
<tr>
<td>Mean parity (n)*</td>
<td>2.6 ± 1.5</td>
<td>2.5 ± 1.0</td>
<td>.84</td>
</tr>
<tr>
<td>Hormone replacement therapy (%)†</td>
<td>0 (0/27)</td>
<td>13 (3/23)</td>
<td>.053</td>
</tr>
<tr>
<td>White (%)†</td>
<td>82 (22/27)</td>
<td>78 (18/23)</td>
<td>.78</td>
</tr>
</tbody>
</table>

* Data are given as means ± SD, compared with the use of independent samples \( t \)-test; proportions compared with the use of Pearson chi-squared test.
† Data in parentheses represent number/total.
difficulty and expense of such a study, we chose to carry out this trial using a surrogate end point for infection. While planning the protocol for this study, we encountered several operating room nurses who had been taught not to use chlorhexidine as a vaginal preparation. We performed a literature search on this subject through MEDLINE using the key words vagina and chlorhexidine. We found no evidence to support the idea that chlorhexidine is unsafe as a vaginal preparation. On the contrary, before enrolling any patients, we found 3 randomized trials in which a chlorhexidine vaginal preparation was performed on nearly 4500 patients (among the 3 studies) with no adverse events.\textsuperscript{11-14} Although a recent case report (published after completion of our study) suggests that some women can have a desquamating reaction after vaginal scrub with chlorhexidine,\textsuperscript{15} we believe the incidence of that problem must be extremely small.

Our study was the first randomized trial to compare povidone iodine and chlorhexidine in the preparation of the vaginal field for hysterectomy. Based on cultures that were obtained 30 minutes after the surgical scrubs, chlorhexidine was clearly superior. In fact, cultures from the povidone iodine group at 30 minutes were \textgreek{o}6 times as likely to be contaminated as those from the chlorhexidine group. Although a similar trend existed in the culture sets that were obtained at 90 minutes, no statistical significance was found at that time interval.

Although the exact clinical significance of this information is unclear, our findings suggest that the widespread use of chlorhexidine rather than povidone iodine might reduce the risk of operative site infections after vaginal hysterectomy.

Clearly, the purpose of a surgical scrub is to reduce the number of pathogens that are present in the operative field. Assuming that there are equivalent costs and effort associated with the use of the 2 antiseptics, our study raises 1 simple question: Why not use the antiseptic that seems to make the operative field cleaner?

**Acknowledgments**

We thank the operating room staff members at the Norton Hospital Pavilion and the University of Louisville Hospital for their cooperation and the staff members of the University of Louisville Hospital for their technical support.

**References**

The expression and function of the endothelin system in contractile properties of vaginal myofibroblasts of women with uterovaginal prolapse

Sébastien Poncet, MSc,a Sylvain Meyer, MD,b Christelle Richard, BSc,a John-David Aubert, MD,c Lucienne Juillerat-Jeanneret, PhDa,*

University Institute of Pathology,a Department of Gynecology and Obstetrics, Urogynecology Unit,b Division of Pneumology,c CHUV, Lausanne, Switzerland

Received for publication May 25, 2004; revised August 15, 2004; accepted September 15, 2004

KEY WORDS
Endothelin
Vaginal myofibroblast
Contraction
Prolapse

Objective: The endothelin-1 system regulates (myo)fibroblast contraction in wound healing. Our aim was to determine endothelin-1 system expression and function in contractile properties of vaginal myofibroblasts of women with uterovaginal prolapse.

Study design: Cultures of α-smooth muscle actin-positive myofibroblasts that were established at the time of repair surgery for prolapse (n = 30; mean age, 56 ± 14 years) were analyzed and compared for their expression of the endothelin-1 system and contractile properties to myofibroblasts from primiparous women.

Results: Myofibroblasts expressed the complete endothelin system but did not secrete endothelin-1. Endothelin-1 binding was mediated exclusively by the endothelin B-receptor. In 3-dimensional collagen gels, spontaneous contraction of myofibroblasts from estrogen-treated women with prolapse was statistically significantly lower than from young primiparous women. Exogenous addition of endothelin-1 decreased the spontaneous contraction of myofibroblasts.

Conclusion: Genital myofibroblasts of women with uterovaginal prolapse are poorly contractile, and endothelin-1 further decreases vaginal myofibroblast contraction, which is opposite to observations in skin myofibroblasts.

Supported by grants from the Swiss National Science Foundation for Scientific Research (grants 20-52490.97, 3152-059219.99 and 3200.64907.01), the Swiss League and Research against Cancer (grants KFS 947-09-1999 and SKL 107-09.00), the Novartis Foundation for Scientific Research, and a Leenaards Foundation Award (Encouragement de la Recherche Scientifique).

* Reprint requests: Lucienne Juillerat-Jeanneret, PhD, PD, MER, University Institute of Pathology, Bugnon 25, CH-1011 Lausanne, Switzerland.
E-mail: Lucienne.Juillerat@chuv.hospvd.ch

Urinary incontinence and other pelvic floor disorders (such as fecal incontinence, modifications of sexual response, uterovaginal prolapse) represent an important health problem in women. The main cause of these problems is the “birth trauma” of vaginal delivery and may affect as many as 30% of women after delivery.1,2

Endothelin-1 (ET-1) is a 21 amino-acid peptide3 that is produced from an inactive precursor (ppET-1) by the endothelin-converting enzymes-1 (ECE-1a-d).4 The biologic functions of ET-1 on mammalian cells are
mediated by 2 subtypes of G protein-coupled receptors, the endothelin A (ET\textsubscript{A}) and ET\textsubscript{B} receptors\textsuperscript{5,6}, which are expressed in various tissues and mediate a variety of physiologic functions, including constriction in smooth muscle cells, skin fibroblasts, and internal organ fibroblasts\textsuperscript{7-9} through the ET\textsubscript{A} receptor and vasorelaxation through the ET\textsubscript{B} receptor, which we have previously shown to be increased in tumor-associated myofibroblasts in human cancers.\textsuperscript{10} In models of wound healing, skin myofibroblasts that express an important contractile apparatus that is comparable to the contractile apparatus of smooth muscle cells, in particular \(\alpha\)-smooth muscle actin (\(\alpha\)-SMA), control the contractile and strength of the tissue.\textsuperscript{11-13}

Therefore, on the assumption that the main cause for uterovaginal prolapse development is vaginal delivery in women, we evaluated the expression and contractile functions of the ET-1 system in myofibroblasts that were obtained from surgical samples of estrogen-treated women who underwent surgical repair for severe uterovaginal prolapse. Myofibroblasts that were obtained from posterior vaginal wall biopsy samples that were obtained during episiotomy repair after delivery of younger primiparous women who did not have prolapse were used as a model of nondiseased uterovaginal myofibroblasts.

### Material and methods

**Human tissues**

Human vaginal samples were obtained from surgical biopsy samples of posterior/anterior vaginal wall that were obtained during uterovaginal prolapse elective surgical repair of women with severe (ie, grade 3) uterovaginal prolapse (n = 30 women; 56 ± 14 years old; parity, 2 ± 1; Table I) or during episiotomy repair of primiparous women after normal delivery (n = 21 women; 29 ± 4 years old), according to a protocol that had been approved by the Ethics Committee of the Hospital in Lausanne and with patients’ approval. Before surgery, all the women were treated by the intravaginal administration of estriol (Ortho-Gynest-D, 3 mg) 1 to 2 times weekly. Tissues were either freshly used to prepare cultures of myofibroblasts or frozen in liquid nitrogen and stored at \(-80^\circ\text{C}\) for reverse transcriptase–polymerase chain reaction (RT-PCR) experiments.

**Cell cultures**

 Cultures of human fibroblasts were obtained from surgical biopsy specimens by the explant technique. Cells were grown as monolayers to confluence in DMEM supplemented with 4.5 g/L glucose, 10 % fetal calf serum and penicillin/streptomycin (Gibco-BRL, Basel, Switzerland) at 37°C and 6% carbon dioxide. After 2 to 3 weeks of primary culture, cells were

### Table I Clinical and surgical data of women (n = 30) with uterovaginal prolapse disorders

<table>
<thead>
<tr>
<th>Variable</th>
<th>Site of defect</th>
<th>Percentage of women (%)</th>
<th>Type of surgical repair</th>
<th>Percentage of operations (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cystocele grade 3/2</td>
<td>27/17</td>
<td>Anterior repair/anti-incontinence simultaneous correction (TVT)</td>
<td>37/13</td>
</tr>
<tr>
<td></td>
<td>Uterine, vaginal vault prolapse grade 3/2</td>
<td>23/37</td>
<td>Sacrospinal vault fixation (bilateral and unilateral)/vaginal hysterectomy</td>
<td>43/23</td>
</tr>
<tr>
<td></td>
<td>Rectocele grade 3/2</td>
<td>33/20</td>
<td>Posterior repair (with and without durotomy repair)</td>
<td>57</td>
</tr>
</tbody>
</table>

### Table II Sequences of the primers used to amplify human ET-1, ET\textsubscript{A}, ET\textsubscript{B}, ECE-1, ECE-1\textsubscript{a}, ECE-1\textsubscript{b}, ECE-1\textsubscript{c}, ECE-1\textsubscript{d}, and GAPDH mRNA

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence for RT-PCR</th>
<th>Size of amplified fragment (base pair)</th>
</tr>
</thead>
<tbody>
<tr>
<td>ET-1</td>
<td>Sense 5’ CTGTTTGTCTTAGTGTTCCTC 3’</td>
<td>304</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ GTCAACACTCCCCGGACAGT 3’</td>
<td>299</td>
</tr>
<tr>
<td>ET\textsubscript{A}</td>
<td>Sense 5’ TTTGATGTTGACATTAGCATAAG 3’</td>
<td>428</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ CTTTTTACACAAAGCCTT 3’</td>
<td>353</td>
</tr>
<tr>
<td>ET\textsubscript{B}</td>
<td>Sense 5’ CTGACATCCACCTTCTCTCA 3’</td>
<td>437</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ ACTGGCCATTTGGAGCAGAT 3’</td>
<td>347</td>
</tr>
<tr>
<td>ECE-1</td>
<td>Sense 5’ CGCTGGAAGTCTGCTGAGG 3’</td>
<td>348</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ CTCTACCAGCTGGCTGTGAG 3’</td>
<td>369</td>
</tr>
<tr>
<td>ECE-1\textsubscript{a}</td>
<td>Sense 5’ CACCCTAGTGGCTCTCC 3’</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ GCTGAAGAAGTCTGGAGG 3’</td>
<td>353</td>
</tr>
<tr>
<td>ECE-1\textsubscript{b}</td>
<td>Sense 5’ CTTCCCTTGCTGGCTGGG 3’</td>
<td>347</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ GCTGAAGAAGTCTGGAGG 3’</td>
<td>347</td>
</tr>
<tr>
<td>ECE-1\textsubscript{c}</td>
<td>Sense 5’ CGGACCAGCAGAGTCTGAT 3’</td>
<td>369</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ GCTGAAGAAGTCTGGAGG 3’</td>
<td>348</td>
</tr>
<tr>
<td>ECE-1\textsubscript{d}</td>
<td>Sense 5’ ATGGAGGGCTGAGGGAT 3’</td>
<td>403</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ GCTGAAGAAGTCTGGAGG 3’</td>
<td>347</td>
</tr>
<tr>
<td>GAPDH</td>
<td>Sense 5’ AAATTTGTCATGATGACCCGTGTC 3’</td>
<td>347</td>
</tr>
<tr>
<td></td>
<td>Antisense 5’ TGACCCCTCCATGGACCT 3’</td>
<td>347</td>
</tr>
</tbody>
</table>

Poncet et al 427
passaged by trypsin-versene detachment, diluted 1:5, grown to confluence for reselection by detachment and regrowth, and frozen in liquid nitrogen. Cells were used after 2 to 3 passages.

**Immunocytochemistry**

Cells were grown on glass slides in complete DMEM, fixed in 4% paraformaldehyde in phosphate-buffered saline solution for 90 minutes at 4°C, washed, and incubated with mouse monoclonal anti-α-SMA (dilution, 1:1000; Sigma Chemical Company, St. Louis, Mo), anti-desmin (dilution 1:500; Dakopatts, Glostrup, Denmark), anti-vimentin (dilution 1:250; Dakopatts), or anti-caldesmon (dilution 1:20; NeoMarkers, Fremont, Calif) primary antibodies then with biotinylated secondary antibodies (1:300 dilution; Dakopatts) and exposed to avidin-biotin complex (Dakopatts), according to the manufacturer’s instructions. Peroxidase activity was visualized with 0.035% diaminobenzidine (Fluka, Buchs, Switzerland) as a chromogen, and the slides were counterstained with hematoxylin. Reactions performed without the primary antibody or with irrelevant antibody were used as control for nonspecific reactions.

**RT-PCR experiments**

RT-PCR determination of the components of the ET-1 system (ppET-1, ETA, ETB, ECE-1, and its isoforms by RT-PCR in uterovaginal myofibroblasts in culture. RT-PCR analysis with specific primers (described in Table II) for ppET-1, ETA, ETB, ECE-1, ECE-1α, and GAPDH mRNAs was performed with total RNA that was extracted from 2 representative cultures of a total of 24 of uterovaginal myofibroblasts. Amplified transcripts were obtained for each gene at the expected size (ppET-1, 304 base pair; ETA, 675 base pair; ETB, 400 base pair; ECE-1, 622 base pair; and GAPDH, 403 base pair).

**Western blotting experiments**

Proteins were extracted from confluent layers of myofibroblast cell cultures in 40 mmol/L HEPES pH 7.5 that contained 100 mmol/L NaCl, 0.1 mmol/L ethylenediaminetetraacetic acid, 100 mmol/L phenylmethylsulfonyl fluoride, 10% glycerol, and 0.5% NP40, and the extracts were submitted to electrophoresis (sodium dodecylsulfate–polyacrylamide gel electrophoresis). After transfer, the membranes were probed with the monoclonal anti-human α-SMA or the polyclonal antihuman cytoskeletal actin (both from Sigma Chemical Company) antibodies and revealed with the ECL detection kit (Amersham International PLC, Buckinghamshire, UK).
ET-1 measurements

The quantification of the ET-1 levels in myofibroblast culture supernatants was performed with an enzyme-linked immunosorbent assay kit (Biomedica, Vienna, Austria), essentially as previously described.14

$[^{125}I]$-ET-1 binding

Confluent cultures of myofibroblasts in 48-well plates were washed, incubated with 50 pmol/L (0.15 nCi) $[^{125}I]$-ET-1 (2.13 Ci/μmol; Anawa, Wangen, Switzerland) at the concentrations indicated in 250 μL complete DMEM for 60 minutes at 22°C. After incubation, the media was aspirated; the cells were washed and solubilized in 1% SDS-0.1 mol/L NaOH, and the radioactivity was counted in an γ-counter (Cobra, Packard, Perkin-Elmer, Foster City, Calif). Binding assays were performed in duplicate. Saturation binding experiments were performed with increasing concentrations of $[^{125}I]$ET-1 (40-800 pmol/L) in the absence (total binding) and the presence (non-specific binding) of 1 μmol/L unlabeled ET-1 (Bachem, Bubendorf, Switzerland), or 4 to 80 μmol/L bosentan (dual ET$_{A,B}$-receptor antagonist, a gift from M. Clozel, Actelion, Allschwil, Switzerland) or $10^{-15}$ to $10^{-6}$ mol/L BQ123 (ET$_A$-selective receptor antagonist) or BQ788 (ET$_B$-selective receptor antagonist both from Alexis Biochemicals, Läufelingen, Switzerland) to characterize the receptor that was involved in binding.

Collagen gel contraction assay

Fifty thousand cells were added to 500 μL of a 1:1 neutralized solution of rat tail collagen (BD Biosciences, San Jose, Calif) in culture medium that contained 4.5 g/L glucose, 10% fetal calf serum, and 6% methylcellulose (Sigma Chemical Company), and the effectors were added at the concentration that is indicated in 24-well plates. ET-1 (final concentration, $10^{-8}$ and $10^{-10}$ mol/L), BQ123, BQ788 (final concentration, 10 μmol/L) or bosentan (final concentration, 1 μmol/L) were used to investigate the role of the endothelin system in myofibroblasts contraction. Culture plates were scanned, and the contraction factor of the myofibroblast-collagen gel was quantified by measurement of the mean diameter of the contracted plug, as previously described,15 after 24 hours or 48 hours of incubation. The contraction factor was calculated as the ratio of the diameter of the contracted plug to the initial diameter of the well.

Statistics

Means and standard deviation were calculated. Statistical significance was assessed with the use of an unpaired 2-tailed Student $t$ test.

Results

Cells in culture expressed the expected differentiation markers of myofibroblasts by immunocytochemistry (Figure 1, A). Most cells, in all cultures that were examined, expressed α-SMA, including the presence of stress fibers, a reliable marker of differentiated myofibroblasts (data not shown), and vimentin, a marker of fibroblast-like cells. Desmin, a marker of smooth muscle cells and endothelial cells, or caldesmon, a marker of myofibroblasts, were expressed only by very few scattered cells in some cultures at the second passage (data not shown). A quantification of the expression of α-SMA compared with cytoskeleton actin was performed in extracts of myofibroblast cultures of women with uterovaginal prolapse or for comparison of women after normal delivery (Figure 1, B). There was no difference between the disease status of the women and the expression of α-SMA by genital myofibroblasts in culture.

All cultures of genital myofibroblasts from women with prolapse (n = 13 women) or from women after vaginal delivery (n = 11 women) expressed the messenger RNA (mRNA) for ppET-1, ECE-1, ET$_A$ and ET$_B$ (Figure 2); however, no secretion of ET-1 could be detected (results not shown), whatever the disease status. Three of the 4 mRNA isoforms of ECE-1 (ECE-1$_{a}$, ECE-1$_{c}$, and ECE-1$_{d}$) were expressed ubiquitously by all samples; ECE-1$_{b}$ was expressed only by a subpopulation of cell cultures, with no obvious relationship to the disease status of the women. The mRNAs for the 2 subtypes of endothelin receptors (ET$_B$ and ET$_A$) were detected in genital myofibroblasts in culture, with a higher expression of ET$_B$ than of ET$_A$. No differences in the transcripts were found that were related to the disease status of the women. Radioactive ET-1 binding studies were used to characterize the ET-receptor that was expressed by genital myofibroblasts (Figure 3). $[^{125}I]$ET-1 that was bound to all cultures was examined, and this binding was dose-dependently displaced by unlabeled ET-1 (10 nmol/L to 1 μ mol/L; Figure 3, A), the dual ET$_{A,B}$ antagonist bosentan (4-80 μmol/L; data not shown), the ET$_B$-selective antagonist BQ788 (Figure 3, B; $10^{-15}$ to $10^{-6}$ mol/L), but not the selective ET$_A$-antagonist BQ123 ($10^{-15}$ to $10^{-6}$ mol/L; data not shown). These effects were not dependent on the disease status of the women.

The role of the endothelin system in genital myofibroblast contraction was examined with a 3-dimensional collagen gel assay, 24 hours and 48 hours after the initiation of the 3-dimensional cultures (Table III). When myofibroblasts were seeded in the collagen matrix, we first observed a reorganization of the myofibroblasts, then the contraction of the collagen matrix. The spontaneous contraction of cells (Figure 4) was statistically significantly higher already after 24 hours (Table III) in genital myofibroblasts that were obtained from young
women after normal delivery who did not develop prolapse than in genital myofibroblasts that were obtained from older estrogen-treated women with prolapse. Bosentan, BQ123, or BQ788 did not exert a statistically significant inhibitory effect on the contraction of myofibroblasts. The effect of ET-1 was related to the concentration of the peptide. At the “high” concentration of 10 nmol/L, ET-1 statistically significantly inhibited contraction (Table III; Figure 4), although at the “low” concentration of 0.1 nmol/L, ET-1 was without effect. After > 2 days, the collagen matrix reached a maximal contraction and detached from the plastic. We never observed relaxation of the floating collagen gels.

Comments

Parity is the strongest risk factor for the development of pelvic organ prolapse, with an adjusted relative risk of 10.85 (95% CI, 4.65-33.81).\(^1,2,16,17\) The cause of these problems is poorly understood; however, it can be hypothesized that they are related to abnormal repair of the injured tissue after the stress of delivery.\(^18\) Several studies have underlined the role of a deficit in the strength of urogenital tissues, which is attributed to decreased collagen amount, altered collagen metabolism with increased collagen breakdown\(^19-21\) or genetic deficiencies in extracellular matrix remodeling or in expression of structural proteins, which includes actin, myosin, or extracellular matrix proteins.\(^22\) However, up to now, no study has assessed the functions of supporting and contractile cells (ie, fibroblasts/myofibroblasts) or has evaluated the functions and the role of the endothelin system in uterovaginal prolapse.

The expression of α-SMA is considered to be the most reliable marker of myofibroblasts,\(^13\) together with the expression of vimentin and the absence of expression of desmin. Thus, the pattern of expression of the cell cultures that we obtained from the surgical samples corresponded to the expected phenotype. Myofibroblasts from different organs and origins express different

<table>
<thead>
<tr>
<th>Status</th>
<th>N</th>
<th>24 h</th>
<th>48 h</th>
<th>24 h</th>
<th>48 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery</td>
<td>14</td>
<td>2.33 ± 0.27</td>
<td>3.64 ± 0.36</td>
<td>1.92 ± 0.22</td>
<td>2.40 ± 0.36</td>
</tr>
<tr>
<td>P value (treatment vs control)</td>
<td></td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Prolapse</td>
<td>14</td>
<td>1.58 ± 0.18</td>
<td>1.80 ± 0.19</td>
<td>1.53 ± 0.13</td>
<td>1.70 ± 0.22</td>
</tr>
<tr>
<td>P value (treatment vs control)</td>
<td></td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>P value (delivery vs prolapse)</td>
<td></td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Cells were grown in a collagen gel with the effectors indicated. At 24 and 48 hours of culture, the diameter of the collagen plug was measured. The contraction factor was calculated as the ratio of the measured plug diameters to the initial diameter. Means ± SD were calculated, and statistical significance was assessed by a 2-tailed unpaired t-test.

Figure 3  Binding characteristics of [\(^{125}\)I]-ET-1 to uterovaginal myofibroblasts. Cells were grown to confluence then incubated with [\(^{125}\)I]-ET-1 in the absence or presence of increasing concentrations of either unlabeled ET-1 (A), or the selective ET\(_B\) antagonist BQ 788 (B); and cell-bound radioactivity was measured. A, Displacement of [\(^{125}\)I]-ET-1 by 10 nmol/L to 1 μmol/L unlabeled ET-1, in 1 representative uterovaginal myofibroblast culture of the 11 that were tested. B, Inhibition of [\(^{125}\)I]-ET-1 binding by 10^-13 to 10^-6 mol/L BQ788, a selective ET\(_B\) receptor antagonist, in 1 representative myofibroblast cell culture of 11 women with prolapse (BT31) and 1 representative myofibroblast cell culture of a woman who did not experience prolapse after vaginal delivery (ML73).
properties, the percentage of α-SMA–expressing cells depends on the origin of the fibroblast population.11,23,24 The components of the endothelin system can regulate multiple functions of fibroblasts/myofibroblasts. In wound healing, skin myofibroblasts have a predominant role in tissue reconstruction, in establishing tension during wound healing in the synthesis, reorganization, and contraction of extracellular matrix through ETA.8,11,12 ET-1 mediates vasorelaxation through the ETB receptor in other cells.5,6 Thus, a defect in the ET-1 system may be responsible of the dysfunction of the (myo)-fibroblast network in women with uterovaginal prolapse.

Uterovaginal myofibroblasts expressed the mRNAs for all the components of the endothelin system, which include ppET-1, ECE-1 (a subpopulation of these cells did not express the ECE-1isoform), ETA, and ETB, but did not secrete ET-1 (thus, myofibroblasts are dependent on an exocrine source of the peptide as ligand for the membrane receptor, as already suggested for skin myofibroblasts5); only a functional ETB receptor was detected. These profiles were unrelated to disease, because we did not observe a different expression of the endothelin system and of α-SMA in women with prolapse and in younger women who did not have prolapse after delivery, which suggests that the primary cause of uterovaginal prolapse is not a deficit in the expression of the endothelin system and of α-SMA in myofibroblasts. However, although we found that myofibroblasts that were obtained from young women after normal delivery contracted the collagen matrix more rapidly and more strongly than myofibroblasts that were obtained from older women with uterovaginal prolapse under local estrogen treatment, we observed that myofibroblasts from both women populations responded comparably to ET-1. Therefore, this peptide system was not a contraction-inducing factor in these cells. These observations and the pattern of regulation by modulators of the ET-1 system suggest that the ETB receptor was involved in these mechanisms. Our observations are compatible with a relaxant functions of ET-1 through the ETB-receptor, which attenuates myofibroblast contraction and tissue strength and suggests that the selective blockade of ETB-receptor in these cells may improve the condition.

Acknowledgments
We thank B. Carnal and C. Chapuis Bernasconi for experimental help and the teams of the Gynecology Units of the Hospitals of Lausanne and Morges for their participation in the surgical samples collection.

References
The combined maternal administration of magnesium sulfate and aminophylline reduces intraventricular hemorrhage in very preterm neonates

Gian Carlo Di Renzo, MD, PhD,a,* Marcella Mignosa, MD,a Sandro Gerli, MD,a Liliana Burnelli, MD,a Giuseppe Luzi, MD,a Graziano Clerici, MD,a Fabiana Taddei, MD,a Doretta Marinelli, MD,b Patrizia Bragetti, MD,b Daniele Mezzetti, MD,b Benedetta Della Torre, MD,b Alessandra Fantauzzi, MD,b Maria Serena Lungarotti, MD, PhDb

Centre of Perinatal and Reproductive Medicinea and the Neonatal Intensive Care Unit,b University of Perugia, Perugia, Italy

Received for publication April 22, 2004; revised July 29, 2004; accepted July 30, 2004

Objective: To determine whether the adjunctive administration of aminophylline and magnesium sulfate to mothers at risk for preterm birth can reduce the rate of intraventricular hemorrhage in neonates born at less than 30 weeks of gestation.

Study design: A prospective study was conducted to determine whether the rate of intraventricular hemorrhage was different in patients at risk for preterm delivery treated with ritodrine, magnesium sulfate, aminophylline, and corticosteroids (group A) versus patients treated with ritodrine and corticosteroids (group B). During the study period (January 1996 to December 2001), 125 patients enrolled in the study. Treatment was assigned by alternative allocation, and the study was designed to compare the rate of intraventricular hemorrhage in neonates born before the 30th week of gestation (primary outcome), 78 newborns in group A and 68 in group B. The proportion of neonates with intraventricular hemorrhage was calculated, and data were analyzed with Student t test, χ², and logistic regression analysis.

Results: The frequency of severe respiratory distress syndrome needing surfactant replacement and high-pressure positive ventilation, patent ductus arteriosus, and retinopathy of prematurity was not different between the 2 groups. However, the rate of intraventricular hemorrhage was lower in neonates born before 30 weeks whose mothers received adjunctive aminophylline and magnesium sulphate (group A) than in the group that did not receive these 2 agents (group B). The overall frequency of intraventricular hemorrhage was 5.1% (4/78) versus 20.6% (14/68) (P < .001), and the frequency of intraventricular hemorrhage grade 3-4 was 1.3% (1/78) versus 10.3 % (7/68; P < .001), respectively.

* Reprint requests: Gian Carlo Di Renzo, MD, PhD, Center of Perinatal and Reproductive Medicine, Department of Gynecologic, Obstetric and Pediatric Sciences, University of Perugia, Policlinico Monteluce, 06122 Perugia, Italy.

E-mail: direnzo@unipg.it
The likelihood of survival of very preterm neonates has improved dramatically over the last 20 years. However, long-term neurologic handicap continues to be a very substantial problem. Several strategies have been proposed to decrease the risk of brain injury associated with preterm birth and neonatal intensive care. For example, corticosteroids administration reduces the rate of intraventricular hemorrhage (IVH), whereas acute treatment with magnesium sulfate appears to reduce the frequency of neurologic injury that predisposes to cerebral palsy. This hypothesis was tested after uncontrolled trials suggesting that antenatal magnesium sulfate administration may be protective against cerebral palsy, although other studies found magnesium to be only weakly or not at all associated with decreased risk of cerebral palsy.

Animal experimentation indicates that the administration of a phosphodiesterase inhibitor, aminophylline, may improve lung adaptation at birth and that the operative mechanism may be similar to that of β-adrenergic agents or glucocorticoids. Moreover, aminophylline has a stimulatory effect on the central nervous system, including the units involved in the regulation of breathing.

This study was conducted to explore whether maternal administration of aminophylline and magnesium sulfate could reduce the rate of IVH in very early preterm neonates.

**Material and methods**

A prospective study was conducted to determine whether the administration of aminophylline and magnesium sulfate reduces the rate of IVH in preterm neonates born at less than 30 weeks of gestation. The study period was January 1, 1996, to December 31, 2001. The study was approved by the Institutional Review Board of the Centre of Perinatal and Reproductive Medicine of the University Hospital in Perugia (Project 27/95) with appropriate informed consent. Patients at risk for preterm delivery admitted to the Centre of Perinatal and Reproductive Medicine were considered for inclusion into the study. Only patients with a gestational age of less than 30 weeks were eligible to participate. Dating of pregnancy was based on a midtrimester ultrasound, which was routinely performed between 20 and 22 weeks. Pregnant patients at risk for preterm delivery because of spontaneous preterm delivery, spontaneous preterm premature rupture of membranes, and complications of pregnancy that could lead to an indicated preterm delivery were invited to participate. Patients admitted to the hospital during the study period were invited to participate. Informed consent was obtained. Allocation of the patients to either treatment was accomplished by alternative allocation to either group A or B. This was not done by random allocation.

Ritodrine was administered at a rate of 0.10-0.30 mg/minute by increments of 0.05 mg/minute every 10 minutes above the initial infusion rate as recommended by the manufacturer or until contractions ceased. In the case of preterm premature rupture of membranes, ritodrine infusion was used at 0.10 mg/minute for at least 48 hours, even in patients without evidence of increased uterine contractility. At the same time that ritodrine was administered, another intravenous line was established to administer 4 g of magnesium sulfate and 240 mg of aminophylline at a rate that would have maintained the infusion for 12 hours (5.5 and 0.33 mg/minute, respectively), continuing the same infusion every 12 hours, for a minimum of 48 hours or until delivery. Corticosteroids were administered as recommended by the National Institutes of Health Consensus Conference (betamethasone 12 mg intramuscularly 24 hours apart). This group was considered group A. Patients allocated to group B received ritodrine and corticosteroids only, according to the same regimen described in the previous paragraph. Patients with preterm premature rupture of membranes received antibiotics administration (ceftriaxone 1 g intramuscularly every 12 hours) until delivery or for a maximum of a week. The study was designed to evaluate the frequency of IVH, and our pre hoc hypothesis was that this benefit would accrue in neonates born below 30 weeks of gestation that had received a full course of steroids. Therefore, infants born after 30 weeks or before the completion of the course of steroids were excluded. If a neonate was diagnosed to have a congenital anomaly not diagnosed prenatally or if there was a fetal death, the case was also excluded from analysis. Analysis was performed using Student *t* test, *χ*² test, and multiple logistic analyses as appropriate.

**Results**

During the study period, there were 78 newborns in group A and 68 in group B who met the inclusion criteria. Maternal and neonatal characteristics and route of delivery, spontaneous preterm premature rupture of membranes, and complications of pregnancy that could lead to an indicated preterm delivery were invited to participate. Patients admitted to the hospital during the study period were invited to participate. Informed consent was obtained. Allocation of the patients to either treatment was accomplished by alternative allocation to either group A or B. This was not done by random allocation.

Ritodrine was administered at a rate of 0.10-0.30 mg/minute by increments of 0.05 mg/minute every 10 minutes above the initial infusion rate as recommended by the manufacturer or until contractions ceased. In the case of preterm premature rupture of membranes, ritodrine infusion was used at 0.10 mg/minute for at least 48 hours, even in patients without evidence of increased uterine contractility. At the same time that ritodrine was administered, another intravenous line was established to administer 4 g of magnesium sulfate and 240 mg of aminophylline at a rate that would have maintained the infusion for 12 hours (5.5 and 0.33 mg/minute, respectively), continuing the same infusion every 12 hours, for a minimum of 48 hours or until delivery. Corticosteroids were administered as recommended by the National Institutes of Health Consensus Conference (betamethasone 12 mg intramuscularly 24 hours apart). This group was considered group A. Patients allocated to group B received ritodrine and corticosteroids only, according to the same regimen described in the previous paragraph. Patients with preterm premature rupture of membranes received antibiotics administration (ceftriaxone 1 g intramuscularly every 12 hours) until delivery or for a maximum of a week. The study was designed to evaluate the frequency of IVH, and our pre hoc hypothesis was that this benefit would accrue in neonates born below 30 weeks of gestation that had received a full course of steroids. Therefore, infants born after 30 weeks or before the completion of the course of steroids were excluded. If a neonate was diagnosed to have a congenital anomaly not diagnosed prenatally or if there was a fetal death, the case was also excluded from analysis. Analysis was performed using Student *t* test, *χ*² test, and multiple logistic analyses as appropriate.

**Conclusion**

Adjunctive maternal administration of aminophylline and magnesium sulfate was associated with a significant reduction in the rate of intraventricular hemorrhage in neonates born before 30 completed weeks.

© 2005 Elsevier Inc. All rights reserved.
of delivery are summarized in Table I. All newborns were inborn and immediately transferred to the neonatal intensive care unit after stabilization in the delivery room.

The incidence of respiratory distress syndrome (RDS) (severe enough to require surfactant replacement and high-positive pressure ventilation),15 IVH (total and grade 3-4),16 patent ductus arteriosus (PDA), and retinopathy of prematurity (ROP) in the 2 groups is reported in Table II. A highly significant decrease in the incidence of IVH was observed in group A (either total number or 3-4 degree), compared with group B. The frequency of neonatal death within 28 days from delivery was not significantly different between the 2 groups.

Possible confounding factors of the observed decreased association between treatment A and IVH was evaluated by multiple logistic regression, entering variables singly and in combination. The following variables were included into the models: gestational age (as a continuous variable or dichotomized); birth weight (continuous or dichotomized to those <750 g versus 750-1000 g versus >1000 g), small for gestational age (SGA; defined as those born with a birth weight less than 3 centiles for that gestational age) versus non-SGA, year of birth, gender, clinically diagnosed chorioamnionitis, mode of delivery (cesarean section versus vaginal), presence of labor, and neonatal surfactant treatment. None of these factors significantly changed the association between the rate of IVH and treatment with magnesium sulfate and aminophylline.

**Comment**

**Principal findings of our study**

The key observation of this study is that the combined used of aminophylline and magnesium sulphate was associated with a reduction in the rate of IVH (total frequency as well as the rate of grade 3 and 4) in very preterm infants (those born before the 30th week of gestation). Adjunctive treatment with this combination did not change the rate of other complications such as RDS, PDA, and ROP. We think that our observation justifies further studies of the potential value of magnesium sulphate and aminophylline in the prevention of neurologic injury.

**IVH and its clinical significance**

Preterm neonates are at increased risk for IVH, and the risk is higher the lower the gestational age at birth. Hemorrhage often originates in the subependymal germinal matrix, but it often ruptures through the

---

**Table I** Perinatal characteristics of study population

<table>
<thead>
<tr>
<th>Maternal characteristics*</th>
<th>Group A (65 pregnancies, 78 newborns)</th>
<th>Group B (60 pregnancies, 68 newborns)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multiple pregnancy (twin-triplets)</td>
<td>13</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>PIH-Pre-eclampsia-HELLP</td>
<td>14</td>
<td>10</td>
<td>NS</td>
</tr>
<tr>
<td>Severe IUGR (&lt;3 centiles)</td>
<td>10</td>
<td>9</td>
<td>NS</td>
</tr>
<tr>
<td>PPROM</td>
<td>32</td>
<td>27</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Route of delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>27 (34.6%)</td>
<td>21 (35.3%)</td>
<td>NS</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>51 (65.4%)</td>
<td>44 (64.7%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean Apgar</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min (range)</td>
<td>1-9</td>
<td>1-9</td>
<td>NS</td>
</tr>
<tr>
<td>5 min (range)</td>
<td>6-10</td>
<td>5-10</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Birth weight (g)</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range</td>
<td>565-1220</td>
<td>600-1260</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>757 ± 215</td>
<td>821 ± 275</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Timing of delivery (from admission) and length of therapy</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (d)</td>
<td>2-18</td>
<td>2-16</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>7.2 ± 5.4</td>
<td>8.1 ± 4.8</td>
<td>NS</td>
</tr>
<tr>
<td><strong>Mean gestational age at delivery</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Range (wks)</td>
<td>23-30</td>
<td>24-30</td>
<td>NS</td>
</tr>
<tr>
<td>Mean ± SD</td>
<td>26.8 ± 2.7</td>
<td>27.2 ± 2.5</td>
<td>NS</td>
</tr>
<tr>
<td>Male/female ratio</td>
<td>34/44</td>
<td>31/37</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, Nonsignificant; PIH, pregnancy-induced hypertension; HELLP, hemolysis, elevated liver enzymes, low platelets; IUGR, intrauterine growth restriction; PPROM, preterm premature rupture of membranes.

* More than one may have been present in the same pregnancy.
ependyma into the ventricular cavity. The germinal matrix undergoes dramatic changes during the late midtrimester including proliferation of glial cells and oligodendrocytes. These changes are thought to require a rich vascular supply. The germinal matrix disappears as term approaches, and it is believed that the vascular network that supplies it is not bestowed with an adequate extracellular matrix envelope and smooth muscle that would confer stability to the blood vessels. Other factors implicated in the etiology are a delay in endothelial cell tight junction formation and pathophysiologic conditions such as fluctuations in cerebral blood flow (from hypercarbia and hypotension from systemic neonatal hypotension). The bleeding can be aggravated by the suboptimal hemostasis (platelets and coagulations factors) reported in some preterm neonates. Bleeding may lead to periventricular infarction (white matter damage), which has been associated with the subsequent development of cerebral palsy. No effective preventive and/or therapeutic strategies have yet been developed other than the administration of steroids.

### Magnesium and aminophylline decrease IVH in preterm neonates born before the 30th week of gestation

The combined use of magnesium and aminophylline was associated with a decrease frequency of IVH in very preterm babies (less than 30 weeks of gestational age) but not of severe RDS, PDA, ROP, or neonatal death. The potential effect of several confounding factors was evaluated with logistic regression, but none was found to change the results. This study represent the first clinical trial in humans and is considered as preliminary evidence because it was an observational, unblinded study in which assignment was made by alternative allocation. We have no evidence that alternative allocation introduced biases, but we considered that a randomized controlled trial (RCT) is optimal and the next step in testing this hypothesis. Before the conduction of the study, we planned to restrict the analysis to neonates born before the 30th week of gestation because after that gestational age, there is a dramatic reduction in the frequency of IVH. The recent RCT of magnesium sulfate for prevention of cerebral palsy was also targeted to neonates born before the 30th week.

### Magnesium sulphate as a neuroprotective agent

Magnesium sulfate is widely used in obstetrics for seizure prophylaxis in pre-eclampsia or for tocolysis. Evidence from randomized clinical trials supports its use in pre-eclampsia, whereas the effectiveness of this agent for tocolysis is less strong. Recently there has been considerable interest in the utilization of magnesium sulphate as a neuroprotective agent. Although the evidence from nonrandomized clinical trials is contradictory, the only RCT reported to date suggests that magnesium sulphate administration to the mother for 24 hours before birth reduced significantly substantial gross motor dysfunction (6.6% in the control group and 3.4% in the treatment group; relative risk, 0.51, 95% confidence interval, 0.29-0.91) in babies born before the 30th week of gestation. Mechanisms by which magnesium sulfate might be neuroprotective include stabilization of both myocardium and blood flow in placental and fetal cerebral circulation and a reduction in the area of ischemia-reperfusion injury. Benefits might be also derived from blockage of the excitatory neurotransmitter receptors, an antioxidant effect of magnesium and a reduction in platelet adhesiveness.

### Antepartum use of aminophylline as a neuroprotective agent

Antenatal administration of aminophylline has been used for the treatment of acute asthma, tocolysis, and the treatment of fetal distress associated with uterine hyperdynamic state. Xanthines are widely used in neonatology for the prevention and/or treatment of neonatal apnea because they increase the respiratory drive. Aminophylline crosses the placenta readily, and serum theophylline concentrations in maternal venous and mixed cord blood at delivery are almost identical after intravenous bolus administration of aminophylline. There is also no evidence of neonatal

### Table II Neonatal mortality and morbidity according to different antenatal treatments

<table>
<thead>
<tr>
<th></th>
<th>Group A (78 newborns)</th>
<th>Group B (68 newborns)</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td>IVH (3-4 degree)</td>
<td>1 (1.3%)</td>
<td>7 (10.3%)</td>
<td><em>P &lt; .001</em></td>
</tr>
<tr>
<td>Total</td>
<td>4 (5.1%)</td>
<td>14 (20.6%)</td>
<td><em>P &lt; .001</em></td>
</tr>
<tr>
<td>RDS*</td>
<td>28 (35.9%)</td>
<td>26 (38.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>PDA</td>
<td>7 (9.0%)</td>
<td>5 (7.5%)</td>
<td>NS</td>
</tr>
<tr>
<td>ROP</td>
<td>2 (2.6%)</td>
<td>4 (5.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Neonatal death†</td>
<td>8 (10.2%)</td>
<td>7 (10.3%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

* Severe degree needing surfactant replacement and high-pressure positive ventilation (HPPV).
† Within 28 days from delivery.
toxicity when aminophylline is administered in the third trimester of pregnancy. Thus, maternal aminophylline administration may be a method to deliver this agent to the fetus. Previous studies reported by our group documented that short-term maternal administration of aminophylline to pregnant rabbits at 26 days improved the survival rate and the respiratory performance of the pups, which were delivered by hysterotomy far from term (mean gestational duration in rabbits is 31 days). These observations were interpreted as indicating that aminophylline administration induces maturation, which is similar to that induced by steroids. Because the mechanisms of action of steroids and aminophylline are different, we proposed that a synergistic effect could occur and hence the use of aminophylline in this study. Other investigators have also demonstrated that short treatment with aminophylline of preterm neonates is associated with a significant increase in oxyhemoglobin.

In conclusion, we present the first evidence that the combined administration of magnesium sulfate and aminophylline can reduce the rate of IVH in very preterm neonates. This benefit was observed in neonates who had received steroids (both arms of the study), and therefore the combination of magnesium sulfate and aminophylline appears to offer additional advantages over the current standard approach, which is limited to steroid administration. The pharmacologic approach reported herein is relatively simple and inexpensive, and the doses utilized have a large margin of maternal safety (8 g per day of magnesium sulfate and 480 mg/day of aminophylline). Further studies are required to dissect the precise contribution of magnesium sulfate or aminophylline to improve neonatal outcome as well as to confirm our findings. This can be accomplished only through a large multi-institutional, randomized clinical trial.

References

28. Liu DTY, Blackwell RJ. The value of a scoring system in predicting outcome to preterm labour and comparing the efficacy
Delayed interval delivery in twin pregnancies in the United States: Impact on perinatal mortality and morbidity

Yinka Oyelese, MD,a,* Cande V. Ananth, PhD, MPH,b John C. Smulian, MD, MPH,a Anthony M. Vintzileos, MDa

Division of Maternal-Fetal Medicine,a Section of Epidemiology and Biostatistics, Department of Obstetrics, Gynecology, and Reproductive Sciences,b University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School/Robert Wood Johnson University Hospital, New Brunswick, NJ

Received for publication March 22, 2004; revised June 19, 2004; accepted July 27, 2004

Objective: To estimate the incidence of delayed interval delivery in twin pregnancies in the United States and evaluate the impact of delayed delivery on perinatal outcomes.

Study design: A population-based retrospective cohort study was performed using the U.S. “matched multiple birth” file (1995 to 1998), restricting our analysis to twin sets in which the first twin was delivered vaginally at 22 to 28 weeks (n = 4257). Outcomes examined included perinatal and infant mortality and small-for-gestational-age births. Outcomes of second twins in pregnancies that underwent delayed interval delivery of 1, 2, 3, and ≥4 weeks were compared with those in which both twins were delivered contemporaneously.

Results: In this cohort, 6.1% (n = 258) of twins had delayed delivery (≥1 week) of the second twin. Decreases in perinatal and infant mortality were observed only when the first twin was delivered at 22 to 23 weeks and when the delivery interval was ≤3 weeks. However, for intervals ≥4 weeks or when the first twin was delivered at 24 to 28 weeks (regardless of delivery interval), there was no benefit in perinatal or infant mortality. Delayed delivery of ≥4 weeks was associated with increased risk of small-for-gestational-age birth in the second twin, regardless of gestational age at delivery of the first.

Conclusion: When a first twin was delivered at 22 to 23 weeks, delayed delivery of the second twin was associated with reduced perinatal and infant mortality of the second twin if the interval was less than 3 weeks. Delayed delivery of the second twin when the first was delivered at ≥24 weeks had no benefit on mortality.
managed by delivery of both fetuses, either vaginally or by cesarean. However, an increasing number of case reports have described delaying delivery of the second twin for days and even weeks after delivery of the first. \(^3\)\(^-\)\(^11\) Gestational age is the most important predictor of neonatal survival in infants delivered at <25 weeks gestation. \(^12\) In twins, survival to discharge following delivery at 22, 23, 24, and 25 weeks are 11%, 11%, 23%, and 51% respectively. \(^12\) Similarly, at these extremely premature gestational ages, even small increases in fetal weight have tremendous impact on neonatal survival. \(^12\) Thus, in pregnancies presenting at the lower limit of viability, significant prolongation of gestation and increase in fetal weight would be expected to improve fetal outcome.

Information on delayed interval delivery has been available only from sporadic case reports, most of which shows an improved outcome for the second fetus. \(^3\)\(^-\)\(^11\) However, these data may be affected by significant selection bias because unsuccessful cases are unlikely to be reported. Consequently, the true incidence of delayed interval delivery, and its impact on perinatal outcomes, is unknown. The potential benefit of delaying delivery of second twins would be expected in very preterm gestations. Therefore, we performed a population-based, retrospective cohort study to determine the incidence of delayed interval delivery in extremely premature twin pregnancies in the United States and evaluated the impact of this management on perinatal and infant survival and morbidity.

**Material and methods**

Data from the “matched multiple birth” file of the Centers for Disease Control and Prevention’s National Center for Health Statistics (NCHS) were used. \(^13\) These data comprise all twin live births, stillbirths and infant deaths in the United States during the period 1995 to 1998. The data were abstracted from live birth, fetal, and infant death certificates. The two fetuses from a twin pregnancy were linked based on a 3-stage matching algorithm developed by the NCHS. \(^14\) The algorithm has been validated and found to be 99% accurate in being able to link the 2 fetuses to a twin pregnancy. The details of the algorithm have been described elsewhere. \(^14\)

Our study population consisted of all twin pregnancies in which a first twin was delivered vaginally between 22 and 28 weeks with birth weights corresponding to these gestational ages. The birth weight cut-off ranges were based on a twin birth weight-for-gestational age nomogram corresponding to the 5th (350 g) and 95th (1459 g) centiles for birth weights at 22 and 28 weeks, respectively. \(^15\) These gestational ages and birth weight ranges were chosen because neonatal survival after 28 weeks’ gestation is generally favorable.

Gestational age in these data files was predominantly based on the last menstrual period. In a small fraction of births (<5%), it was based on a clinical estimate. The clinical estimate was used when the last menstrual period-based estimate was implausible for a given birth weight. When both the clinical estimate and the menstrual estimate of gestational age were incorrect or missing, it was imputed by the NCHS prior to release of the data. \(^16\)\(^,\)\(^17\)

Delayed interval delivery between the first and second twin was expressed in completed weeks and was defined as 1 week or greater between the gestational age at delivery of the first and second twins. Risks of perinatal and infant mortality were the primary outcomes evaluated. Perinatal mortality was defined as the number of stillbirths (at 22 to 28 weeks) plus the number of neonatal deaths within the first 28 days and was expressed per 1000 total twin births. Infant mortality was defined as the number of infant deaths within the first year and was expressed per 1000 total twin live births. Secondary outcomes that were evaluated included stillbirths (defined as a fetus born without signs of life), neonatal deaths (defined as deaths within the first 28 days of life), mean birth weight, rates of small-for-gestational age (SGA) births (defined as birth weight <10th centile for gestational age), and respiratory distress syndrome with or without the need for assisted ventilation (for at least 30 minutes).

Perinatal and infant mortality rates in first and second twins were derived in relation to delayed interval delivery. In addition, these mortality rates were calculated in second twins in relation to delayed interval delivery categories separately for each week of gestation between 22 and 28 weeks. Relative risk (RR) with 95% confidence interval (CI) was derived as the measure of effect. Two sets of RRs were derived: (1) for perinatal mortality in second twins, compared with first twins, at each delivery interval strata (1, 2, 3, and ≥4 weeks), using 0 weeks as the reference; and (2) for perinatal mortality in second twins with delayed intervals, compared with those (second twins) that were delivered contemporaneously with the first twin (0 week interval). This latter comparison allowed us to evaluate the benefit of postponing delivery of second twins as opposed to delivering both fetuses contemporaneously.

We fitted multivariable logistic regression for categorical outcomes and linear regression for continuous outcomes to adjust for potential confounders. These confounders included maternal age (categorized as <20, 20 to 24, 25 to 29, 30 to 34, 35 to 39, and ≥40 years), gravidity (primigravida or gravida ≥2), maternal education (<12 or ≥12 completed years of schooling), marital status (married or single), maternal race/ethnicity (white, black, or other race/ethnicity), and lack of prenatal care. All statistical analyses were further adjusted for gestational age at delivery of the first twin to eliminate any bias because of residual confounding by gestational age. This study was approved by the ethics review committee of the Institutional Review Board of the University of...
Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School, New Brunswick, NJ.

Results

In the United States between 1995 and 1998, 24,194 sets of twins were delivered between 22 and 28 weeks’ gestational age. Of these, there were 4257 sets of twins in which the first twin was delivered vaginally at 22 to 28 weeks. In 258 (6.1%, 95% CI 5.4, 6.8) of these sets, the second twin was delivered at least a week after the first. Group-specific demographic characteristics of the studied population are presented in Table I. Because the benefit of delaying delivery on perinatal mortality of the second twin was evident only when the first twin was delivered at 22 to 23 weeks and not at later gestational ages (the Figure), we stratified all our analyses on the basis of the gestational age at delivery of the first twin at 22 to 23 and 24 to 28 weeks.

Perinatal mortality rates based on the gestational age at delivery of the first twin from 22 to 28 weeks in relation to delivery intervals are shown in Table II. For pregnancies in which the first twin was delivered at 22 to 23 weeks, as the delivery interval increased, there was a progressively significant decline in perinatal mortality of the second twin for up to 3 weeks’ delivery interval. No statistically significant benefit existed for delivery intervals of 4 weeks or greater. The pattern of association between delayed interval delivery and stillbirths and neonatal deaths showed similar results (not shown). Analysis of infant mortality in relation to delayed interval delivery was similar to those for perinatal deaths (Table III).

With increasing delay between delivery of the twins, there was a progressive increase in mean birth weight of the second twin over the first (Table IV). However, the rates of SGA births in the second twin increased with increasing delivery interval; this reached statistical significance for ≥4 weeks’ interval (for delivery at 22 to 23 weeks) and ≥2 weeks for delivery of the first twin at 24 to 28 weeks (Table V).

Delayed delivery did not result in a reduction in the rates of respiratory distress syndrome or assisted ventilation support for over 30 minutes in the second twin. Delayed delivery led to a lower incidence of 5-minute Apgar scores <7 for the delayed twin over the first (not shown). This effect, however, reached statistical significance only for 1- to 2-week interval when the first twin was delivered at 22 to 23 weeks.

Comment

This population-based study evaluates delayed interval delivery in extremely preterm twin pregnancies and

<table>
<thead>
<tr>
<th>Table I</th>
<th>Distribution of selected maternal characteristics based on categories of delayed interval delivery: United States twin births, 1995–1998</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal characteristics</td>
<td>Delayed interval delivery group</td>
</tr>
<tr>
<td>Maternal age, y (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>13.2</td>
</tr>
<tr>
<td>20–24</td>
<td>26.4</td>
</tr>
<tr>
<td>25–29</td>
<td>21.3</td>
</tr>
<tr>
<td>30–34</td>
<td>24.4</td>
</tr>
<tr>
<td>≥ 35</td>
<td>14.7</td>
</tr>
<tr>
<td>Primigravidity (%)</td>
<td></td>
</tr>
<tr>
<td>Maternal race (%)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>66.3</td>
</tr>
<tr>
<td>Black</td>
<td>30.6</td>
</tr>
<tr>
<td>Other</td>
<td>3.1</td>
</tr>
<tr>
<td>Maternal education, y (%)</td>
<td></td>
</tr>
<tr>
<td>&lt; 12</td>
<td>17.5</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td></td>
</tr>
</tbody>
</table>

Note: SGA = small for gestational age.

Figure  Perinatal mortality for second twin by gestational age at delivery of the first twin in relation to delayed interval delivery: United States twin births 1995 to 1998. The open circles/solid line represent no delivery interval, and squares/dotted line represent any delivery interval (≥1 week). Perinatal mortality rates were significantly different (P < .05) for twins with and without delayed interval delivery when the first twin was delivered at 22 to 23 weeks but not when the first twin was delivered at ≥24 weeks.
assesses its impact on perinatal and infant outcomes. Our data demonstrate that, in this population, perinatal outcomes for second twins were improved when the first twin was delivered at 22 to 23 weeks and delivery of the second twin was delayed by up to 3 weeks. Furthermore, this improvement was proportional to the number of weeks by which the pregnancy was prolonged up to 3 weeks. Although perinatal and infant mortality was reduced in second twins when the first twin was delivered at 24 to 28 weeks, this reduction in mortality was not statistically significant when compared with pregnancies in which both twins were delivered contemporaneously.

These data suggest that delayed delivery may be a reasonable strategy when delivery of the first twin occurs at 22 to 23 weeks’ gestation. A variety of approaches have been used to postpone delivery and improve outcomes of second twins. These include aggressive tocolysis, cervical cerclage in selected cases, corticosteroids to induce fetal lung maturation, and antibiotics. Our study did not allow us to evaluate the impact of these different strategies on outcomes when delivery of the second twin was delayed. Therefore, our study does not permit recommendations as to which strategies are best for delaying delivery.

Although these initial data are promising, data on long-term outcomes of the infants who had been delivered as a consequence of delayed interval delivery are lacking. It is possible, for example, that the second-born twins may have an unacceptably high incidence of periventricular leukomalacia, intraventricular hemorrhage, and cerebral palsy. It is known that premature rupture of the membranes, especially when associated with intra-amniotic infection, is an antecedent of these conditions. One unexpected finding from this study is the higher risk for SGA in the second twin with increasing delivery intervals. This is an issue that requires examination in future studies.

Our study has some limitations. We assumed that pregnancy dating was accurate and that the recorded
gestational ages at delivery were reliable. Bleeding in early pregnancy may lead to errors in gestational age assessment. Further, in the data set available to us, the gestational age at delivery was recorded only in completed weeks. As a result, pregnancies in which the pair of twins were born a few hours apart, ie, 24 weeks 6 days and 25 weeks 0 days, would be recorded in such a manner as to give the impression that the twins were born a week apart (24 and 25 weeks). Similarly, in cases in which twins were born days apart but within the same week of gestation, the gestational ages were recorded in a manner that would suggest that there was no interval between the deliveries. Nevertheless, there would be a genuine interval of at least 1 week when the recorded interval was 2 weeks. Because our findings demonstrate a definite improved survival with increasing delivery interval between twins, the conclusion that delayed interval delivery may improve perinatal and infant survival and birth weight when the first twin is delivered at 22 to 23 weeks’ gestation and the delivery interval is ≤3 weeks is reasonable. Because we did not have data on delays of days, rather than weeks, we were unable to determine whether delays of a few days improved outcomes, as would be expected at these extremely premature gestational ages. We also did not address pregnancies in which the first twin was delivered at less than 22 weeks’ gestational age. A review of the literature suggests an improved outcome for the second twin in this situation is possible.

Another weakness of this study is that we did not obtain data on maternal morbidity and outcomes. Delayed delivery would conceivably put the mother at significant risk of infectious morbidity, deep venous thrombosis due to bed rest, coagulation disorders, and adverse effects of tocolytics and other medications; these complications could occasionally lead to maternal death. Therefore, the true risk to the mother remains unevaluated. However, there have been no reported instances of maternal death in the published literature.

It was not possible, with the data available for our study, to determine how often delayed delivery was attempted but unsuccessful. Although previous studies have been optimistic about outcomes of delayed interval delivery, the majority has suffered from selection bias. In the only study in which a uniform policy of attempting delayed delivery on all eligible multiple pregnancies was

### Table IV


<table>
<thead>
<tr>
<th>Delivery interval (wk)</th>
<th>Gestational age of first twin: 22–23 wks</th>
<th>Gestational age of first twin: 24–28 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Birth weight (mean SD)</td>
<td>Birth weight (mean SD)</td>
</tr>
<tr>
<td></td>
<td>First twin</td>
<td>Second twin</td>
</tr>
<tr>
<td></td>
<td>Number of twins</td>
<td>SGA (%)</td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td></td>
<td>(mean SD)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>527 (91)</td>
<td>578 (155)</td>
</tr>
<tr>
<td>1</td>
<td>538 (84)</td>
<td>564 (99)</td>
</tr>
<tr>
<td>2</td>
<td>555 (74)</td>
<td>674 (96)</td>
</tr>
<tr>
<td>3</td>
<td>563 (121)</td>
<td>801 (173)</td>
</tr>
<tr>
<td>≥4</td>
<td>530 (66)</td>
<td>869 (601)</td>
</tr>
<tr>
<td>Any interval ≥1 wk</td>
<td>541 (81)</td>
<td>713 (364)</td>
</tr>
</tbody>
</table>

* Relative risks denote comparison of small-for-gestational-age rates in second twins with delayed interval, relative to those with an interval of 0 weeks. Analyses were adjusted for maternal age, maternal education, marital status, maternal race/ethnicity, and gestational age at delivery of first twin.

### Table V


<table>
<thead>
<tr>
<th>Delivery interval (wk)</th>
<th>Gestational age of first twin: 22–23 wks</th>
<th>Gestational age of first twin: 24–28 wks</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of twins</td>
<td>SGA (%)</td>
</tr>
<tr>
<td></td>
<td>First</td>
<td>Second</td>
</tr>
<tr>
<td></td>
<td>Adjusted RR* (95% CI)</td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1106</td>
<td>3.2</td>
</tr>
<tr>
<td>1</td>
<td>52</td>
<td>3.6</td>
</tr>
<tr>
<td>2</td>
<td>19</td>
<td>6.7</td>
</tr>
<tr>
<td>3</td>
<td>6</td>
<td>−</td>
</tr>
<tr>
<td>≥4</td>
<td>9</td>
<td>70.0</td>
</tr>
<tr>
<td>Any interval ≥1 wk</td>
<td>86</td>
<td>−11.6</td>
</tr>
</tbody>
</table>

* Birth weight differences correspond to differences in mean birth weight in second twins with delayed interval, relative to those with an interval of 0 weeks. Analyses were adjusted for maternal age, maternal education, marital status, maternal race/ethnicity, and gestational age at delivery of first twin.
employed, Farkouh et al\textsuperscript{8} reported their experience with 24 consecutive twin and triplet pregnancies ranging between 16.4 and 28.6 weeks at delivery of the first fetus. They achieved a mean delay of 36 days (range 3 to 123 days) between delivery of the first-born and forthcoming fetuses. The mortality among the first-born infants was 84\% (21/25), compared with 37\% in the retained siblings (10/27). In contrast, a recent retrospective study of 14 cases by Livingston and colleagues\textsuperscript{11} found a high perinatal mortality of 61\% associated with delayed interval delivery. In the same study, only 1 of 19 retained fetuses survived without major sequelae.

We do not know in which cases there was a deliberate attempt to delay delivery. There have been reports of cases in which following the delivery of the first twin, the authors attempted to induce delivery of the second with oxytocin. In some of these, it was only when attempted induction was unsuccessful that attempts at delivering the second twin were aborted, with resulting delayed interval delivery.

The failure of our study in demonstrating any benefit in reducing mortality for the second twin when the first was delivered at \(\geq 24\) weeks or when the delivery interval was \(\geq 4\) weeks may be a consequence of small numbers (Table II). A post hoc analysis indicates that one would need 140 cases to demonstrate a benefit of delaying delivery of the second twin beyond 4 weeks (i.e., \(\geq 4\) weeks) when the first twin was delivered at 22 to 23 weeks. However, our analysis had a power of 76\% in being able to demonstrate a reduction in perinatal mortality in the second twin when the first was delivered at 24 to 28 weeks.

In conclusion, delayed interval delivery appears to be associated with improved outcomes for the twin whose delivery is delayed when the first twin is delivered at 22 to 23 weeks and the delivery interval is \(\leq 3\) weeks. However, we urge caution in applying the results of our study to decision making for the individual case. The management of each clinical situation must be individualized according to its own merit, after thorough counseling of the expectant couple. Prospective studies that examine the long-term outcomes of infants whose delivery is delayed are necessary, as are studies examining the risks and complications of pregnant women who undergo this management approach.

\textbf{Authors’ note}

Since the acceptance of our manuscript for publication, a study addressing delayed interval delivery in twins in the United States using the same dataset has been published in the \textit{American Journal of Obstetrics and Gynecology}.\textsuperscript{21} At the time we submitted our manuscript, we were unaware that such a paper had already been accepted for publication and was “in press” in the \textit{American Journal of Obstetrics and Gynecology}.

\textbf{References}

A randomized comparison of oral mifepristone to intravenous oxytocin for labor induction in women with prelabor rupture of membranes beyond 36 weeks’ gestation

Deborah A. Wing, MD, Cristiane Guberman, MD, Michael Fassett, MD

Objective: This study was undertaken to compare the use of oral mifepristone with intravenous oxytocin for labor induction in women with prelabor rupture of membranes (PROM) at 36 weeks’ or greater gestational age.

Study design: Sixty-five women with spontaneous PROM were randomly assigned to receive orally administered mifepristone or oxytocin infusion. Two hundred milligrams of mifepristone was administered, and subjects were observed for 18 hours, or intravenous oxytocin was administered.

Results: Thirty-three women received mifepristone and 32 received oxytocin. The average interval from start of induction to delivery was 1194.1 ± 568.7 minutes for mifepristone-treated subjects and 770.8 ± 519.9 minutes for oxytocin-treated subjects (P = .001, log-transformed data). Of 33 mifepristone-treated subjects and 32 oxytocin-treated subjects, 25 (78.1%) and 17 (51.5%), respectively, achieved successful induction (defined as vaginal delivery within 24 hours) (relative risk [RR] 0.66, 95% CI 0.45-0.96, P = .01). There was more fetal distress in the mifepristone-treated group (9 vs 2, RR 4.36, 95% CI 1.02-18.66, P = .02), and a trend toward more cesarean births (7 vs 3, RR 2.26, 95% CI 0.64-7.99, P = .19). Eleven infants of mifepristone-treated women (33.3%) and 3 infants of oxytocin-treated women (9.4%) were admitted to the neonatal intensive care unit (RR 3.56, 95% CI 1.09-11.58, P = .02).

Conclusion: Oral mifepristone administration 18 hours before oxytocin infusion did not improve labor stimulation in women with PROM near term, and was associated with more adverse fetal outcomes.

Spontaneous prelabor rupture of membranes (PROM) at term occurs in approximately 10% of pregnancies. The management of patients with PROM remains controversial. Traditionally, induction of labor is undertaken after spontaneous amniorrhexis to prevent
chorioamnionitis and neonatal sepsis, which occur with increased frequency with PROM existent more than 24 hours before delivery.1,2 Multiple studies have compared the use of expectant management to active induction of labor.3-7 A purported benefit of expectant management is fewer cesarean deliveries for failed inductions4,5; this benefit must be weighed against higher rates of maternal and neonatal infection,6,7 as well as the possibility of cord accident.

There are various approaches to labor induction in patients with PROM. Oxytocin is the most commonly used agent for labor induction in patients with PROM. Several studies have demonstrated the safety of using prostaglandin compounds in the face of PROM.8-12 A novel approach using oral misoprostol (Cytotec), a synthetic PGE1 analogue, for women with PROM near term has been described.13-15

The process of labor initiation remains a mystery. It is known, however, that progesterone is integral in the maintenance of pregnancy. It is hypothesized that antiprogestin exposure in pregnancy will enhance the initiation of parturition.16 Mifepristone is a steroid compound that possesses both antiglucocorticoid and antiprogestational activities. There are reports of the use of mifepristone as a preinductive agent in women with intact membranes.17-24 Our group studied the use of mifepristone given 24 hours before labor induction in 180 women with prolonged pregnancies. More women in the mifepristone treatment group (87.5%) than in the placebo treatment group (70.8%) were delivered vaginally 48 hours after the start of treatment, and significantly less oxytocin was administered to mifepristone-treated women compared with placebo-treated women.18 In another investigation of 120 women at term, 200 mg mifepristone on 2 consecutive days increased the number entering labor and decreased the prostaglandin requirements in the remaining women, when compared with placebo.19 The same researchers also studied the efficacy of mifepristone in a group of 32 women with previous cesarean births with similar results.20

We hypothesized that mifepristone administration to women with near-term PROM would enhance entry into active labor and minimize risks of chorioamnionitis and neonatal sepsis. This investigation was undertaken to evaluate the effect of oral mifepristone administration on the hastening of labor in women with PROM near term.

Material and methods

From November 1998 to May 2003, all women with singleton, live pregnancies at or beyond 36 weeks’ gestation (252 or more days) with PROM at Women’s and Children’s Hospital, Los Angeles County, University of Southern California Medical Center, and the Good Samaritan Hospital in Los Angeles, Calif, were considered eligible for study participation. Gestational age was determined according to the date of the last menstrual period preceded by regular cycles and confirmed by a physical examination at 20 weeks or ultrasonography at 26 weeks, or by ultrasonography at 26 weeks if the last menstrual period was uncertain. The study was approved by the Institutional Investigational Review Boards at both sites. Women meeting study criteria were invited to participate in the trial, and if they elected to do so, written informed consent was obtained. All subjects were in-patients in the labor and delivery units of these hospitals. Eighty-three women were asked to participate in the trial, and 65 agreed. Of these, 33 received mifepristone and 32 received placebo. No subjects withdrew from the study, and no subjects were excluded from data analysis. We found no statistically significant differences in outcomes between the 2 sites.

Inclusion criteria were as follows: (1) singleton gestation; (2) cephalic presentation; (3) reactive fetal heart rate (FHR) pattern; (4) gestational age of 36 weeks’ or more; (5) ruptured membranes confirmed by (a) gross pooling of amniotic fluid in the vaginal vault by sterile speculum examination, (b) positive nitrazine paper test, and (c) ferning of the amniotic fluid by microscopy; 6) maternal age of 18 years or more. Exclusion criteria included: (1) cervical dilatation in excess of 3 cm; (2) more than 12 uterine contractions per hour; (3) ultrasonographic estimated fetal weight greater than 4500 g or other evidence of cephalopelvic disproportion; (4) estimated fetal weight less than 1800 g; (5) placenta previa or other unexplained vaginal bleeding; (6) active genital herpes simplex infection; (7) previous cesarean or history of uterine surgery; (8) evidence of chorioamnionitis as evidenced by maternal temperature of 100.4°F or more and the presence of uterine tenderness and/or foul-smelling amniotic fluid; (9) parity of 6 or more; and (10) any moderate or severe preexisting medical disease.

The trial was conducted without blinding. The subjects were assigned by means of a computer-generated random number sequence to receive 200 mg mifepristone (Danco Laboratories, New York, NY, and Abortion Rights Movement, New York, NY) or intravenous oxytocin by a standardized protocol. A New Drug Application and an Investigational New Drug Application were filed by the respective sponsors with the US Food and Drug Administration. The treatment assignments were placed in opaque, sealed, and sequentially numbered envelopes. Once a subject was determined to be eligible and gave informed consent for study participation, she was assigned a sequential study number and administered mifepristone or oxytocin in accordance with her computer assignment. All mifepristone-treated women were observed to ensure ingestion of the medication.
A senior obstetrics-gynecology resident assigned the initial Bishop score, and when possible, subjects were re-examined by the same individual at the end of the 18-hour exposure to mifepristone. At this time and as necessary, labor was augmented with oxytocin. Once in active labor, routine intrapartum management of women in either group occurred without regard to treatment allocation.

Oxytocin was also administered to mifepristone-treated subjects for (1) failure to progress in the active phase of labor (less than 1 cm progression over 2 hours) and (2) after adequate cervical ripening (dilatation of 3 cm or more or Bishop score of 7 or more). Oxytocin was administered by infusion pump, using a standardized protocol with an initial dose of 1 mU/min and incremental increases every 30 minutes to a maximum of 22 mU/min. It was administered to oxytocin-treated subjects immediately after enrollment.

Continuous FHR monitoring and tocodynamometry were used. FHR patterns were classified in the manner by the National Institute of Child Health and Human Development (NICHD). Abnormal FHR patterns were defined as the presence of either fetal tachycardia or bradycardia, late decelerations, or moderate-severe FHR decelerations. The uterine activity patterns were evaluated for the frequency and duration of tachysystole, hypertonus, and hyperstimulation syndrome. Uterine hypertonus was defined as a single uterine contraction lasting 2 or more minutes, tachysystole as either hypertonus or tachysystole associated with an abnormal FHR pattern. Terbutaline 0.25 mg given intravenously or subcutaneously or intravenous magnesium sulfate was used to treat these contraction abnormalities.

Antimicrobial prophylaxis for group B streptococcal disease was administered according to 1996 American College of Obstetricians and Gynecologists risk-based guidelines in our institutions. Chorioamnionitis was diagnosed if any of the following were present: maternal temperature of 100.4°F or more, foul-smelling amniotic fluid, fundal tenderness, and/or persistent elevation of FHR baseline of 160 or more beats/min. Chorioamnionitis was treated with antipyretics and broad-spectrum antimicrobial agents. Neonatal vital signs, including blood pressure, were recorded within 24 and 48 hours of life. Standard observations were recorded and any adverse events were noted.

The primary outcome measure was the duration from start of treatment to delivery. We also tabulated the frequency of vaginal delivery within 24 hours from initiation of induction and the change in Bishop score after 18 hours of mifepristone. Other outcome variables were assessed, including route of delivery, need for oxytocin augmentation after mifepristone, and neonatal outcome measures such as Apgar scores, need for resuscitative measures beyond routine warming and drying, and number of neonatal intensive care unit (NICU) admissions.

Sample size calculations were based on the assumption that 75% of subjects would deliver vaginally within 24 hours after initiation of labor induction, we estimated that a 20% increase in this number to 90% would be clinically important, and using a type I error of 0.05 and a type II error of 0.2, we calculated that a sample size of 100 subjects in each group was necessary. The test was 2-sided. We performed an interim analysis of the data after 65 subjects were enrolled because of concerns regarding neonatal outcomes and because of poor enrollment. On the basis of these findings, we elected to terminate the study prematurely.

Differences in age, height, weight, estimated gestational age, gravidity, total oxytocin dose, average time intervals such as time from induction to delivery (log transformed) were analyzed with \( t \) tests; differences in route of delivery and frequency of complications were analyzed with \( \chi^2 \) or Fisher exact tests when appropriate. Apgar and Bishop scores were analyzed with the Mann-Whitney \( U \) test. The relationship between neonatal outcomes and time of exposure was evaluated with logistic regression. All tests were 2-sided with a .05 level of significance.

### Results

All subjects were similar with respect to age, height, weight, and estimated gestational age at entry (Table I). Fifteen (45.5%) of the mifepristone-treated women were nulliparous, and 11 (34.4%) of the oxytocin-treated women were nulliparous (\( P = .36 \)). The majority of subjects (92%) were Hispanic. The median Bishop score in each group at the start of the induction was 4 in both groups.

After the initial 18-hour observation period after mifepristone, 8 subjects failed to achieve adequate cervical ripening or enter active labor. The median Bishop score at the initiation of oxytocin for these 8

<table>
<thead>
<tr>
<th>Table I: Maternal characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Maternal age (y)</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
</tr>
<tr>
<td>Gravidity</td>
</tr>
<tr>
<td>Parity</td>
</tr>
<tr>
<td>Height (in)</td>
</tr>
<tr>
<td>Weight (lb)</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD.
women was 6 (range 1-13). Of all mifepristone-treated subjects, 20 (60.6%) required oxytocin. This was given on average 1018.9 \pm 158.5 minutes after mifepristone administration. The indications for oxytocin administration in the mifepristone subjects were as follows: failure to achieve adequate ripening or active labor after 18 hours after mifepristone administration (8/20), adequate cervical ripening after mifepristone administration (5/20), or inadequate progress in the active phase of labor (7/20).

The mean time interval from treatment to delivery regardless of the route was shorter for oxytocin-treated subjects than for mifepristone-treated subjects (Table II). The difference between the 2 groups was nearly 7 hours, due in part to the 18-hour observation period after mifepristone administration. The mean time interval from treatment to delivery was 1194.1 \pm 568.7 minutes for mifepristone-treated women, and 770.8 \pm 519.9 minutes for oxytocin-treated women, regardless of route of delivery (\( P = .001^* \)). Of those women who delivered vaginally, the mean time from treatment to delivery was 1133.0 \pm 592.9 minutes for the mifepristone-treated women and 740.3 \pm 522.6 minutes for the oxytocin-treated women (\( P = .004, \) log transformed data).

Ten women were delivered by cesarean section. One oxytocin-treated woman underwent cesarean section for failed induction. One mifepristone-treated and 2 oxytocin-treated women underwent cesarean section for arrest disorders of labor. All 6 cesarean sections performed for fetal distress occurred in the mifepristone-treatment group.

We analyzed the effect of parity on outcomes. Of the 55 women who delivered vaginally within 24 hours, there were 6 oxytocin-treated nulliparous women and 6 mifepristone-treated nulliparous women (\( P = .42 \)). Not unsurprisingly, the duration of labor was longer for nulliparous women compared with the parous subjects. For example, the mean time from induction to delivery for mifepristone-treated nulliparous women was 1398.5 \pm 620.4 minutes and for mifepristone-treated parous women was 938.4 \pm 506.9 minutes. The duration from induction to delivery in oxytocin-treated subjects differed between the nulliparous women and the parous women by approximately 4.5 hours.

The total amount of oxytocin administered and maximum oxytocin infusion rates were greatest in the

<table>
<thead>
<tr>
<th>Table II Delivery data</th>
<th>Mifepristone (n = 33)</th>
<th>Oxytocin (n = 32)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean interval between start of induction and delivery (min)</td>
<td>1194.1 \pm 568.7</td>
<td>770.8 \pm 519.9</td>
<td>.001*</td>
</tr>
<tr>
<td>Mean interval between start of induction and vaginal delivery (min)</td>
<td>1133.0 \pm 592.9</td>
<td>740.3 \pm 522.6</td>
<td>.004*</td>
</tr>
<tr>
<td>Mean interval between start of induction and active labor (min)</td>
<td>855.7 \pm 564.8</td>
<td>437.2 \pm 407.9</td>
<td>.004*</td>
</tr>
<tr>
<td>Mean interval between rupture of membranes and initiation of induction (min)</td>
<td>990.6 \pm 1403.6</td>
<td>450.9 \pm 593.0</td>
<td>.005*</td>
</tr>
<tr>
<td>Vaginal delivery in 12 h</td>
<td>7 (21.2)</td>
<td>17 (53.1)</td>
<td>.005†</td>
</tr>
<tr>
<td>Vaginal delivery in 24 h</td>
<td>17 (51.5)</td>
<td>25 (78.1)</td>
<td>.01†</td>
</tr>
</tbody>
</table>

Data represented as mean \( \pm \) SD, or N (%).
* Student t test, based on log transformed data.
† \( \chi^2 \) test.

<table>
<thead>
<tr>
<th>Table III Intrapartum characteristics</th>
<th>Mifepristone (n = 33)</th>
<th>Oxytocin (n = 32)</th>
<th>( P )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean oxytocin dose (mU)</td>
<td>2511.9 \pm 4327.9</td>
<td>5648.0 \pm 8233.5</td>
<td>.13*</td>
</tr>
<tr>
<td>Maximum oxytocin infusion rate</td>
<td>6.9 \pm 5.2</td>
<td>9.9 \pm 6.6</td>
<td>.09*</td>
</tr>
<tr>
<td>Abnormal FHR Patterns</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moderate-severe variable decelerations</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Late decelerations</td>
<td>4</td>
<td>2</td>
<td>.02†</td>
</tr>
<tr>
<td>Prolonged decelerations</td>
<td>4</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Epidural analgesia</td>
<td>23 (70.0)</td>
<td>19 (59.4)</td>
<td>.38†</td>
</tr>
</tbody>
</table>

Data represented as mean \( \pm \) SD, or N (%).
* Student t test.
† \( \chi^2 \) test.
oxytocin treatment group (Table III). Antimicrobial prophylaxis for group B streptococcal disease was given to 10 (30.3%) of the mifepristone-treated women and 11 (34.4%) of the oxytocin-treated women (RR 0.88, 95% CI 0.44-1.78, P = .73). Chorioamnionitis was diagnosed in 5 (15.2%) of the mifepristone-treated women and 1 (3.1%) oxytocin-treated subject (RR 0.48, 95% CI 0.60-39.2, P = .20, Fisher exact test). Endometritis was diagnosed in 4 (12.1%) mifepristone-treated women and none of the oxytocin-treated women (P = .11, Fishers exact test).

Meconium passage occurred in 3 (9.1%) infants of mifepristone-treated women and 6 (18.8%) oxytocin-treated women (RR 0.48, 95% CI 0.13-1.78, P = .26). Abnormal FHR patterns were found in 9 mifepristone-treated women and 2 placebo-treated women (RR 4.36, 95% CI 1.02-18.66, P = .02). The distribution of the types of FHR abnormalities can be found in Table III.

One mifepristone-treated woman experienced tachysystole, and another experienced uterine hyperstimulation. Both uterine contractile abnormalities occurred many hours after the administration of mifepristone and in the active phase of labor.

Some birth outcomes differed markedly between the 2 treatment groups (Table IV). The numbers of women who required any degree of resuscitation beyond usual warming and drying were (13 [39.4%] and 8 [25.0%]) infants born to mifepristone- and oxytocin-treated women, respectively (RR 1.58, 95% CI 0.76-3.28, P = .21). Eleven (33.3%) infants born to mifepristone-treated mothers and 3 (9.4%) born to oxytocin-treated mothers were admitted to the NICU (RR 1.58, 95% CI 1.09-11.58, P = .02). More infants in the mifepristone-treated group were diagnosed with suspected or confirmed sepsis (11 vs 2, RR 5.33, 95% CI 1.28-22.20, P = .04). Only 1 sepsis was confirmed (in the mifepristone-treated group).

We were concerned that the increased observation period after mifepristone administration increased the likelihood of suspected or confirmed sepsis infants born to mifepristone-treated mothers. We performed logistic regression analyses combining both groups that revealed statistically significant associations between admissions to the NICU and suspected or confirmed sepsis with 4 time variables: the time from rupture of membranes to delivery, the time from rupture of membranes to induction initiation, the time from induction initiation to active labor, and the time from induction initiation to delivery. Multivariate logistic regression was performed to determine which of the time variables bore the strongest association with NICU admissions and suspected or confirmed sepsis. Only the time from rupture of membranes to delivery had a statistically significant relationship. No similar relationships existed for the oxytocin-treated women. The relationship is derived predominantly from the mifepristone-treated group. In the mifepristone-treated group, 16.7% had NICU admission and sepsis (suspected or confirmed) for the time interval from rupture of membranes to delivery less than 1100 minutes (first tertile of the distribution); the percentages were 25% for the time interval from rupture of membranes to delivery between 1100 to 1700 minutes (second tertile) and 46.7% for greater than 1700 minutes (third tertile). In the oxytocin-treated group, the analogous percentages were 0%, 11.1%, and 14.3%, respectively.

Comment

There are a few reports in the literature describing labor induction with preinduction mifepristone, and to our knowledge, none describing the use of mifepristone in women with PROM. We focused on PROM because we believed that these women would be better primed to enter labor spontaneously when compared with women with intact membranes, and thus, more responsive to the antiprogestational effects of mifepristone. Our past experience with mifepristone in women with prolonged pregnancies found reduced oxytocin requirements and less need for prostaglandin administration in those women treated with the antiprogestin compared with placebo.18

Because the time from induction to delivery was longer in the mifepristone group than in the oxytocin group, we reject our null hypothesis that there would be no difference in this outcome measure between the groups. The lack of efficacy of mifepristone as an initiator of labor in this study may be due to its theorized method of action, ie, that we allowed inadequate time for mifepristone to trigger a decline in the systemic level of progesterone or decrease in the local actions of progesterone on myometrium. Other researchers have allowed pretreatment periods of 24 to 96 hours after mifepristone but in women with intact membranes.19,20,22 Our results with 18 hours of

<table>
<thead>
<tr>
<th>Table IV Neonatal outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
</tr>
<tr>
<td>Birth weight (g)</td>
</tr>
<tr>
<td>Apgar score &lt;7</td>
</tr>
<tr>
<td>5 min</td>
</tr>
<tr>
<td>NICU Admission</td>
</tr>
<tr>
<td>Mean NICU stay, if admitted</td>
</tr>
<tr>
<td></td>
</tr>
</tbody>
</table>
* Student t test.  † Fisher exact test.  ‡ χ² test.
pretreatment are similar to those previously reported: for example, Frydman et al reported that only 3% of women went into labor within 24 hours of receiving 200 mg mifepristone. Both the dosage of mifepristone and pre-treatment time interval were selected arbitrarily in the study by Frydman et al.

We are gravely concerned about the higher frequency of neonates born with either suspected or clinical sepsis to mothers treated with mifepristone during this trial. Our multiple logistic regression analysis confirms what is widely known in obstetric practice: The longer a woman remains undelivered with ruptured membranes, the higher the likelihood of chorioamnionitis and subsequent neonatal infection. Our findings reflect of our study design that required an 18-hour observation period after mifepristone ingestion and before oxytocin administration. We do not believe that mifepristone potentiates intrauterine infection and neonatal infection. We also found that the duration from the time of rupture of membranes to initiation of induction (regardless of treatment arm) was statistically longer in the mifepristone-treated subjects by nearly 10 hours; this likely compounded this clinical outcome. We have no good explanation for this time difference between groups. We also found more fetal distress in women who received mifepristone than in those who received oxytocin, but no differences in the frequency of uterine contractile abnormalities between groups. It is known that there is an increase in sustained uterine activity in both humans and rhesus macaques after mifepristone administration. For safety reasons, we elected to terminate the trial prematurely, and acknowledge criticisms for this. In doing so, we acknowledge that we failed to meet our expectations for statistical power.

A heightened sensitivity to oxytocin has been seen after mifepristone administration after the second trimester pregnancy termination and term labor induction. We could not assess a heightened responsiveness to oxytocin in our trial because we did not use a placebo control. We acknowledge this limitation in study design as well as the limitations posed by lack of blinding. However, we believe that if bias were to have been encountered because of lack of blinding, that we would have expected shorter time intervals from induction to delivery in mifepristone-treated subjects compared with those of placebo-treated women.

We found that a 200-mg dose of mifepristone given to women with PROM near term had no benefit compared with the common practice of intravenous oxytocin administration, and in fact, had some detriment in terms of neonatal outcomes such as suspected or confirmed sepsis. We could find no compelling reason by which to recommend the use of this agent with an 18-hour period of observation for those women with PROM.

References


32. Rodger MW, Baird DT. Pretreatment with mifepristone (RU 486) reduces the interval between prostaglandin administration and expulsion in second trimester abortion. BJOG 1990;97:41-5.


Severe fetal placental vascular lesions in term infants with neurologic impairment

Raymond W. Redline, MD

Departments of Pathology and Reproductive Biology, Case School of Medicine and University Hospitals of Cleveland, Case Western Reserve University, Cleveland, Ohio

Received for publication May 19, 2004; revised June 22, 2004; accepted July 19, 2004

KEY WORDS
Brain damage
Cerebral palsy
Neonatal encephalopathy
Placenta

Objective: This study tests the hypothesis that placental disease can identify antepartum processes that either progress into the intrapartum period or predispose to intrapartum brain injury.

Study design: Lesions that affect large fetal vessels were compared in the placentas of 125 neurologically impaired term infants who were the focus of clinical negligence litigation and 250 consecutive singleton deliveries of ≥36 weeks of gestation.

Results: One or more of 4 severe placental fetal vascular lesions (fetal thrombotic vasculopathy, chronic villitis with obliterative fetal vasculopathy, chorioamnionitis with severe fetal vasculitis, and meconium-associated fetal vascular necrosis) were found in 51% of index cases versus 10% of the comparison group (P < .0001). Prevalence of these lesions in the 64 infants with cerebral palsy was 52% (P < .0001).

Conclusion: Severe fetal placental vascular lesions are correlated highly with neurologic impairment and cerebral palsy. Their nature, duration, and anatomic location make them strong candidates for the antepartum processes that place fetuses at risk for brain injury during the intrapartum period.

© 2005 Elsevier Inc. All rights reserved.

The problem of litigation related to cerebral palsy (CP) and other long-term neurologic disorders threatens health care delivery to pregnant women in several countries. Two opposing theories of cause have been proposed: intrapartum hypoxia and antepartum injury. An international consensus statement based on several large epidemiologic studies concluded that intrapartum hypoxia could not be the cause of CP in at least 90% of affected children. However, recent studies that used early brain imaging suggest injury in the immediate perinatal period in most cases.

The rationale for this study rests on 2 theses: First, the thesis that neither intrapartum hypoxia nor antepartum injury alone adequately explain most cases of CP. Alternatively, antenatal processes might lower the threshold for injury by impairing reserve, altering fetal physiologic condition, and generating potentially neurotoxic mediators. Second, the thesis that placental disease has not been fully exploited as a window into the antenatal environment. Previous placental studies have been limited by an emphasis on gross structural abnormalities, a lack of consensus on histologic lesions, and the small study size. Recent data have highlighted the importance of placental lesions that affect the fetal
vasculature. This study tests the hypothesis that severe fetal placental vascular lesions are a biologically plausible cause of increased susceptibility to brain injury, by evaluating a large number of placentas from infants who are subject to clinical negligence litigation and comparing them to placentas that have been submitted for routine examination.

Patients and methods

Patients

A medicolegal case registry was used to identify 125 index cases with neonatal encephalopathy (NE), CP, or other well-documented related forms of long-term neurologic impairment after singleton birth at ≥36 weeks gestation. Cases from 32 states were entered into the registry without patient identifiers and represent, with 1 exception, births that occurred after 1990. Approximately 80% of cases were reviewed at the request of defense attorneys and 20% for plaintiffs. Infants with major congenital malformations, congenital infections, or chromosomal/genetic syndromes were excluded. NE was diagnosed by the recently published criteria of Cowan et al5 (abnormal tone, feeding difficulties, and altered alertness plus ≥3 of the following criteria: late decelerations or meconium, delayed onset of respiration, cord pH <7.1, Apgar 5-minute score <7, and multiorgan failure). This objective definition, although suboptimal, was used due to the wide variation in quality of clinical data that were available in the index cases. Among the 83 cases of NE, 14 infants died before discharge from the nursery, 36 infants had CP, and 33 infants were neurologically abnormal at discharge and developed neurologic disabilities that resulted in litigation but did not carry a diagnosis of CP at the time of the review. CP without NE was diagnosed in 28 cases. Fourteen infants without NE had other related neurologic impairments at long-term follow-up that included seizures, severe developmental delay, mental retardation, neuromotor deficits, and motor disorders (such as hypotonia or hypertonia). Among the 64 cases of CP, 15 infants had spastic quadriplegia or dyskinetic type alone; 2 infants had spastic hemiplegia alone; 1 infant had spastic diplegia alone, and the remainder of the infants had spastic quadriplegia/dyskinetic type plus additional severe mental, developmental, and/or sensory deficits.

The comparison group consisted of 250 consecutive singleton deliveries of ≥36 weeks of gestation at the University MacDonald Women’s hospital serves a racially mixed population of high- and low-risk patients from the Cleveland metropolitan area (population, 2 million) and performs approximately 3500 deliveries per year. Placental submission rate varies between 15% and 20%. Most cases (65%) were submitted for ≥1 of the indications recommended by the College of American Pathologists joint task force of pathologists, obstetricians, and neonatologists.12 Four percent of the cases were submitted after repeat cesarean delivery. Most of the remaining cases were submitted for intrapartum abnormalities (such as worrisome fetal monitoring). Hospital policy requires retrieval and review of placental slides whenever copies of the medical record are requested by the family or their medical representatives. By this criterion, none of the cases in the comparison group is currently the subject of clinical negligence litigation.

Procedures

Demographic features were available for both index cases and the comparison group (Table I). Maternal records from index cases were reviewed for Kleihauer-Betke testing, uterine rupture, and abruptio placenta. Infant records were reviewed for diagnostic criteria of NE by umbilical blood gas testing. Long-term outcomes were ascertained from a review of reports by occupational or physical therapists or child neurologists who were caring for the child.

Placental slides from index cases and control subjects were examined by the author using diagnostic criteria that were developed in previous studies (Table III).9,13-15 Blinded re-review of a subgroup of placentas from the index cases (n = 20) and comparison group (n = 38) showed high comparable rates of intraobserver agreement (87.5% for cases vs 90% for comparison group). Comparison of the prevalence of each lesion in cases that were reviewed for plaintiff versus defendant showed no statistically significant differences.

Statistical analysis

The study hypothesis was that lesions that affect large fetal vessels in the placenta might be important contributors to intrauterine brain damage that leads to abnormal neurologic status at birth and long-term neurologic impairments. Chi-squared or Fisher exact tests were used for categoric variables. Continuous variables were compared with the use of the 2-tailed t-test. Z-scores (standard deviations from the mean for gestational age) for birth weight were calculated from published tables.16

Results

Mean maternal age, mean infant birth weight for gestational age (Z-score), and the proportion of infants
who were male were similar in the 125 index cases with neurologic impairment and the 250 children in the comparison group (Table I). Gestational age at delivery was slightly but significantly higher (39.5 ± 1.6 weeks vs 39.1 ± 1.4 weeks) in the index cases, in part because of a higher proportion of mothers delivering after 42 weeks (8% vs 4% in comparison group). Index cases were born more commonly to primiparous mothers, were more likely to be growth restricted as defined by a birth weight $\leq 1.5$ standard deviations below mean birth weight for gestational age, and were assigned more frequently a 5-minute Apgar score $\leq 7$. Acute (sentinel) events, abruptio placentae and uterine rupture, were identified in 15% of index cases and were more frequent with NE. Fetal hemorrhages, either fetomaternal or large parenchymal bleeds into the placenta or fetus, were seen in 11% of cases and were more frequent without NE (results not shown). Early postpartum parameters indicated a high frequency of findings that were indicative of severe birth depression (16% with 10-minute Apgar score $\leq 4$) and antepartum hypoxemia (31% with initial nucleated red blood cell count $> 7500/\text{mm}^3$).

Four major pathologic processes that affected large fetal placental vessels were significantly increased in index cases (Table II): fetal thrombotic vasculopathy, villitis of unknown cause (VUE) with obliterator fetal vasculopathy, chorioamnionitis with severe fetal vasculitis, and meconium-associated vascular necrosis. VUE without obliterator fetal vasculopathy, chorioamnionitis with mild-to-moderate fetal vasculitis, and meconium deposition without vascular necrosis were equivalent in the 2 groups (data not shown). Overall, $\geq 1$ of the 4 severe placental vascular lesions was identified in 51% of index cases versus 10% for the comparison group. The prevalence of these lesions was equivalent for infants with NE or without NE, infants with 5-minute Apgar scores above and below 6, and umbilical artery pH values above and below 7.00 (data not shown). Among the 64 infants with CP, severe fetal vascular lesions were found in 52% of placentas ($P < .0001$).

Comment

The primary finding of this study is that more than one half of placentas from children referred for medicolegal consultation to evaluate possible causes of neurologic impairment had severe fetal vascular lesions. This represents a >5-fold increase compared with term placentas that were sent for pathologic evaluation in a university hospital setting. The similar prevalence in cases with or without NE and the lack of variation with respect to umbilical artery pH values and 5-minute Apgar scores suggest that these lesions are more likely related to the eventual outcome than to the alleged intrapartum events that led to litigation.

The study group was composed of children with neurologic impairment who either died shortly after birth or had clinically confirmed neurodevelopmental handicaps. Over one half of the children had CP. All of the children were involved in clinical negligence litigation that alleged inappropriate intrapartum treatment. Three potential biases should be considered. First, cases that are subject to litigation might constitute a small unrepresentative subset of neurologic impairment at term. The few available studies indicate that 10% to 26% of term infants with severe neurologic impairment are the focus of clinical negligence actions.1,2 In the more recent paper, this proportion increased to two-thirds in the last 4 years of the study. It is likely that cases with suspicion of so-called birth asphyxia are over represented. Any bias toward cases that were attributable to intrapartum hypoxia alone should decrease the chance of the observation of placental lesions that evolve over days to weeks. Second, placentas

<table>
<thead>
<tr>
<th>Table I</th>
<th>Comparison of the study populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Long-term outcome</td>
<td>NE</td>
</tr>
<tr>
<td>N</td>
<td>14</td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td>26.6</td>
</tr>
<tr>
<td>Gestational age</td>
<td>39.6</td>
</tr>
<tr>
<td>Birth weight: Z-score</td>
<td>0.7</td>
</tr>
<tr>
<td>Birth weight: Z-score $&lt; 1.5$</td>
<td>0</td>
</tr>
<tr>
<td>Primiparity</td>
<td>9/14 (64%)*</td>
</tr>
<tr>
<td>Male sex</td>
<td>11/14 (79%)</td>
</tr>
<tr>
<td>5-min Apgar score $&lt; 7$</td>
<td>13 (93%)</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.
† P < .0500.
‡ No. positive (% positive).
might only be submitted for pathologic evaluation in certain types of medicolegal cases. Although the occasional case of neurologic impairment after an apparently normal pregnancy would be missed, few placentas from births with significant antenatal or intrapartum abnormalities in the present medicolegal environment are not submitted for pathologic evaluation. Third, there could be a referral bias in which only cases that are known to have significant placental lesions are submitted for expert review. This is unlikely because few of the severe fetal vascular lesions that were found in this study were mentioned in the original placental reports. To a large extent, this reflects their recent delineation and can be expected to improve in the near future, as their importance becomes better established. Advantages of the study group are that it is well characterized, geographically disperse, and drawn from a variety of care settings.

Index cases were compared with term placentas that were examined by the author over a 13-month period. They were comparable to index cases in terms of maternal age, overall birth weight, and proportion of male births. Index cases had a higher mean gestational age and increased proportions of primiparous mothers, small for gestation infants, and low 5-minute Apgar scores. These differences are all consistent with previous studies of neurologic impairment after term birth.\textsuperscript{17,18}

Placentas that were submitted for pathologic examination may be more likely than a population-based control group to show placental lesions. This should decrease the likelihood of seeing a significant increase in fetal vascular disease in the index cases. Strengths of the comparison group are that it was not further selected and was contemporaneous with the study group.

Descriptions of placental disease have concentrated on structural abnormalities and so-called uteroplacental insufficiency that affect the maternal vascular supply line.\textsuperscript{19} Although these abnormalities are important, they often can be compensated for by placental reserve, which has been estimated at between 30% and 50%. Only recently has attention been directed to lesions that affect the “fetal supply line” including fetal thrombosis, inflammation of the fetal vessel wall, and hemodynamically significant umbilical cord abnormalities. These lesions affect a vascular bed that receives up to 55% of the total fetal cardiac output. They could act by a variety of mechanisms including impaired fetoplacental vascular regulation, decreased gas and metabolite exchange, activation of platelets and leukocytes, generation of cytokines and other thromboinflammatory mediators, release of heat shock proteins from ischemic placental tissue, and embolism of placental thrombi to other fetal vascular beds. In the present study, 4 severe fetal vascular lesions were studied. Two lesions (fetal thrombotic vasculopathy and VUE with obliterative fetal vasculopathy) represent chronic processes beginning weeks before delivery. The other lesions (chorioamnionitis with severe acute fetal vasculitis and meconium-associated fetal vascular necrosis) occur when common acute processes that are generally terminated by delivery within hours of onset are extended over a period of days (subacute lesions).

Fetal thrombotic vasculopathy has been linked to CP, fetal and neonatal thromboembolic disease, perinatal liver disease, and discordant twin growth.\textsuperscript{20-23} Although inherited fetal coagulation disorders may result in thrombotic vasculopathy in occasional cases,\textsuperscript{24}

\begin{table}[ht]
\centering
\caption{Prevalence of placental lesions in the study populations}
\begin{tabular}{lllllll}
\hline
\textbf{Long-term outcome} & \textbf{NE} & \textbf{Death} & \textbf{CP} & \textbf{Abnormal (not otherwise specified)} & \textbf{No NE} & \textbf{Total neurologic impairment} & \textbf{Comparison group} \\
\hline
N & 14 & 36 & 33 & 28 & 14 & 125 & 250 \\
Fetal thrombotic vasculopathy* & 2 (14) & 8 (22) & 7 (21) & 5 (18) & 1 (7) & 23 (18) & 6 (2) \\
VUE with obliterative fetal vasculopathy\textsuperscript{1} & 0 & 6 (17) & 7 (21) & 4 (14) & 5 (36) & 22 (18) & 7 (3) \\
Chorioamnionitis with severe fetal vasculitis\textsuperscript{2} & 2 (14) & 1 (3) & 4 (12) & 4 (14) & 0 & 11 (9) & 8 (3) \\
Meconium-associated vascular necrosis\textsuperscript{3} & 2 (14) & 5 (14) & 5 (15) & 4 (14) & 1 (7) & 17 (14) & 8 (3) \\
One or more of the above & 5 (36) & 18 (50) & 20 (61) & 15 (54) & 6 (43) & 64 (51) & 26 (10) \\
\hline
\end{tabular}
\end{table}

* Upstream vascular occlusion resulting in an average of >15 avascular villi per slide.
\textsuperscript{1} No. positive (% positive).
\textsuperscript{2} P < .0001.
\textsuperscript{3} Terminal VUE extending to stem villi with occlusion of stem vessels and downstream avascular villi.
\textsuperscript{4} Near-confluent acute inflammation of chorionic plate vessels with attenuation/degeneration of vascular smooth muscle cells.
\textsuperscript{5} P < .0050.
\textsuperscript{6} Cytoplasmic eosinophilia and nuclear pyknosis (apoptosis) of peripheral vascular smooth muscle cells.

\textsuperscript{1} Redline
large studies have failed to show a significant association between these 2 entities.\textsuperscript{21,25,26} Other factors (such as right-sided heart failure, polycthemia, and the transmitted effects of maternal diabetes mellitus or antiphospholipid antibodies) have been implicated, but most cases remain unexplained. It has also been suggested that chronic umbilical cord obstruction might be a contributing factor. We have found recently that clinical cord loops and pathologic cord abnormalities are both significantly increased in placentas with fetal thrombotic vasculopathy (unpublished data).

VUE is caused by infiltration of activated maternal T lymphocytes into the terminal villi of the placenta.\textsuperscript{27} It is a common and relatively benign finding in term placentas that has been associated with fetal growth restriction, abnormal nonstress testing, and elevated serum alpha-fetoprotein levels.\textsuperscript{28,29} A subgroup of patients have more severe outcomes that include stillbirth, recurrent reproductive failure, neonatal seizures, and CP.\textsuperscript{8,30-32} This study demonstrates that neurologic impairment is increased significantly with a recently described pattern of VUE characterized by the spread of the inflammatory infiltrate into stem villi with associated stem villous vasculitis or perivasculitis, fetal vascular occlusion, and downstream avascular terminal villi (obliterative fetal vasculopathy).\textsuperscript{14} Fetal complications of VUE with obliteratorive fetal vasculopathy might be attributable to the activation of the coagulation cascade, the release of cytokines from activated maternal lymphocytes, or even the spread of maternal lymphocytes into the fetus, which has been documented for some childhood connective tissue diseases.\textsuperscript{33}

The fetal inflammatory response to chorioamnionitis has been associated with susceptibility to CP and other neurodevelopmental disorders in many previous studies of both premature and term infants.\textsuperscript{34} In 1 study, a link to local thrombosis in severely inflamed vessels was demonstrated.\textsuperscript{35} In other studies, an increase in cytokines previously linked to brain injury has been found.\textsuperscript{36} Our data demonstrate a dose-response relationship with only the most severe fetal inflammatory responses being significantly increased with neurologic impairment. Lesser degrees of funisitis or chorionic vasculitis were elevated only mildly with neurologic impairment (9\% in cases vs 6\% in the comparison group).

Prolonged exposure to meconium has been recognized as a risk factor for meconium aspiration syndrome, persistent fetal circulation, and birth depression.\textsuperscript{37} Constituents of meconium can cause necrosis of fetal tissues and vasospasm of large fetal arteries.\textsuperscript{38,39} A subgroup of meconium-stained placentas show meconium-associated necrosis of peripheral myocytes in umbilical and chorionic plate vessels.\textsuperscript{9} This lesion has been associated with a very high rate of perinatal morbidity and mortality. It was shown recently to represent apoptosis of vascular smooth muscle cells.\textsuperscript{15} Why apoptosis occurs in only a few cases with meconium exposure is not clear. Increased concentration, duration of exposure, or increased host susceptibility to apoptosis could all play a role.

In conclusion, this study identifies 4 placental lesions that may correspond to some of the heretofore poorly understood antenatal factors that contribute to perinatal brain injury. These lesions could act in 2 distinct scenarios: (1) by decreasing the ability of normal infants to withstand the inherent stresses of routine labor and delivery and (2) by increasing the proportion of genetically susceptible patients who express the disease independent of the events of labor and delivery. In both situations, strategies to identify, treat, andatraumatically deliver patients with these clinically silent pathologic processes might reduce significantly the later development of neurologic impairment.

References


Progesterone enhances the tocolytic effect of ritodrine in isolated pregnant human myometrium

Boonsri Chanrachakul, MD,a Fiona Broughton Pipkin, DPhil,b Averil Y. Warren, MPhil,a Sabaratnam Arulkumaran, MD, PhD,c Raheela N. Khan, PhDa,*

Objective: To evaluate the effect of natural progesterone on the relaxant effect of ritodrine on pregnant human oxytocin-induced myometrial contractility.

Study design: Isometric tension recordings were performed under physiologic conditions on isolated myometrial strips taken from low-risk term pregnant women undergoing elective cesarean section. Cumulative effects of natural progesterone (10^{-11} to 10^{-5} mol/L) on oxytocin-induced myometrial contractility were evaluated. Contractile activity following ritodrine exposure was also investigated in myometrium pretreated with natural progesterone.

Results: Natural progesterone alone exerted a concentration-dependent relaxant effect on myometrial contractions. The concentration-response curve for ritodrine from natural progesterone pretreated myometrium was shifted to the left with a significant reduction ($P < .01$) of 50% of the maximal response, contraction amplitude ($P < .05$), and frequency ($P < .05$). However, there was no significant difference in the mean maximal inhibition achieved ($P = .95$).

Conclusion: Natural progesterone increased the relaxant effect of ritodrine by reducing 50% of the maximal response, amplitude, and frequency of myometrial contraction, most likely through nongenomic actions.

These results suggest that natural progesterone may be beneficial for preventing preterm birth in a low-risk population.

Preterm birth remains a leading cause of perinatal mortality and morbidity. Despite advances in obstetric and pediatric care, the incidence of preterm birth has not declined over the past 4 decades. Preterm birth and its sequelae are major burdens on health and educational resources. Furthermore, the attendant emotional and personal costs to families and individuals affected by prematurity are considerable. There is still no effective...
prevention or treatment for preterm birth due to our poor understanding of the mechanisms leading to preterm delivery.

Among the theories regarding the pathogenesis of preterm labor, the progesterone withdrawal theory founded by Csapo is well established in many species, but its role in human parturition is debatable. Although associated with uterine quiescence, clinical use of prostogestogens in alleviating preterm delivery has not been fully realized. Progesterone treatment appears devoid of potentially deleterious effects at the therapeutic concentrations used, but continuous exposure to steroid hormones remains a concern. Previous clinical trials using synthetic progestrone to reduce preterm delivery rates have been reported with mixed results. Natural progesterone has greater benefit over its synthetic equivalents in view of cost, availability, and biological safety. Recently Da Fonseca et al first reported on the promising effect of natural progesterone in preventing preterm birth in pregnant women with a history of previous preterm birth, uterine anomalies, and cervical incompetence. However, it is not clear whether this dramatic reduction in the preterm delivery rate resulted from either the effect of progesterone itself or the modulatory effect of progestrone via interactions involving β2-agonists, which were used as tocolytics in the particular study.

An effective treatment for preterm labor should not only benefit the high-risk group but also be widely applicable to women generally. In view of the beneficial effects of natural progesterone on preterm delivery, we hypothesized that natural progesterone acts via mechanisms that involve β2-agonists to promote relaxation. The specific aim of our study was to determine the effect of natural progesterone on the relaxant effect of ritodrine on oxytocin-induced myometrial contractility in women at low risk of preterm delivery.

Material and methods

Tissue collection

Myometrial samples were taken from the mid-upper margin of the uterine incision of normal singleton term pregnant women (37-42 weeks of gestation) undergoing elective cesarean section. All cesarean sections were performed before the onset of labor for breech presentation, previous cesarean section, or maternal request. This study was approved by the Southern Derbyshire Ethics Committee, and written informed consent was obtained from each participant. Biopsies were immediately placed in physiological salt solution (CaCl₂ 1.6 mM, NaCl 119 mM, NaHCO₃ 25 mM, glucose 5.5 mM, KH₂PO₄ 1.18 mM, MgSO₄ 1.17 mM, KCl 4.7 mM, pH 7.4) and transported to the laboratory. Tissues were stored at 4°C and used within 24 hours of collection.

Isometric tension recordings

Longitudinal myometrial strips, approximately 2 × 2 × 10 mm, were dissected free from adherent nonmyometrial tissue and mounted in an organ bath (Letica, AD Instruments, Oxford East Sussexshire, UK) under 2 g tension at 37°C in aerated (95% O₂ + 5% CO₂) physiological salt solutions as previously described. Contractile activity for each myometrial tissue strip was recorded using isometric force transducers (range 0-25 g) connected to a bridge amplifier, which was in turn connected to a dedicated data acquisition system (Powerlab/8SP, AD Instruments). After equilibration, myometrial contractions were stimulated with oxytocin (10⁻⁹ mol/L; Sigma-Aldrich, Poole, UK) until regular phasic contractions were achieved. Time taken to achieve equilibration varied between 60 and 90 minutes following which control contractile performance was assessed. Cumulative additions (10⁻¹¹ to 10⁻⁵ mol/L) of progesterone (4-pregnene-3, 20-dione [P₄]; Sigma-Aldrich) were then applied at 20-minute intervals. The resultant contractile activity was measured and analyzed for each period.

In a separate group of experiments, paired strips from the same group of term pregnant women (n = 11) were mounted. One strip was preincubated with 10⁻⁸ mol/L of P₄ for 1 hour, and the other strip from the same subject, serving as a control, was tested against the corresponding vehicle (70% alcohol) dilutions. All tissues for isometric tension recordings were stimulated with oxytocin. Once equilibrated, as noted by the generation of stable, reproducible contractions, ritodrine (Sigma-Aldrich), a β₂-agonist, was then added to the bath in a cumulative manner from 10⁻⁹ to 10⁻³ mol/L every 20 minutes. The activity integral (area under the time-force curve), peak force (maximum tension above basal force), and frequency of contractions, were measured during the 20 minute period following each drug addition. The mechanical responses of all myometrial strips tested were as measured (Quadbridge (PowerLab, AD Instruments) and analyzed using recorded by Chart software (version 4.2; PowerLab, AD Instruments).

Statistical analysis

The concentration-response curves were analyzed by fitting to the equation:

\[ y = (y_{\text{min}}) + (y_{\text{max}} - y_{\text{min}})/1 + 10^{(\log EC_{50} - X) \times \text{Hill slope}} \]

where \(y\) is the observed response, \(y_{\text{max}}\) is the maximum relaxation achieved, \(y_{\text{min}}\) is the minimum relaxation achieved, and EC₅₀ is the concentration giving the half maximal response. Data are expressed as a percentage of
the results obtained before any drug application for each individual strip and presented as mean ± SEM. Significance was determined by F test and paired t test as appropriate. P < .05 was considered as statistically significant.

Results

Effects of progesterone on myometrial strips

Application of oxytocin induced constant regular myometrial contractions in control strips over the 4-hour course of experiments (n = 11). A representative recording (Figure 1) demonstrates a concentration dependent, relaxant effect of natural progesterone on oxytocin-induced myometrial contractions. This effect is mediated principally via a reduction in the amplitude, which is concentration dependent on myometrial contractions rather than any changes in frequency or duration of contractions. The concentration required to achieve 50% of the maximal response (EC50) to progesterone is 3.9 × 10⁻⁶ mol/L (Figure 1).

Effects of progesterone on the relaxant effect of ritodrine on myometrial strips

This separate group of experiments investigated the effect of a physiological concentration of progesterone on myometrial contractions and its influence on the tocolytic effect of ritodrine. Overnight incubation with progesterone, at a fixed concentration of 10⁻⁸ mol/L, significantly inhibited myometrial contractility (data not shown). We therefore chose to preincubate tissues for 1 hour with 10⁻⁸ mol/L progesterone for comparison with paired myometrial strips from the same women untreated before exposure to ritodrine (n = 11). Application of ritodrine alone exerted a concentration-dependent relaxant effect on oxytocin-induced myometrial contractions (Figure 2, B) with an EC50 of \(1.52 \times 10^{-7}\) mol/L (Figure 3, A). Although progesterone (10⁻⁸ mol/L) had little effect on sustained oxytocin-induced myometrial contractility (n = 11; Figure 2, A), it significantly enhanced the relaxant action of ritodrine (Figure 2, C). The concentration response curve for ritodrine was shifted to the left for progesterone-pretreated strips.
The EC$_{50}$ of ritodrine was also significantly different when compared in the presence and absence of 10^{-8} mol/L progesterone (5.7 \times 10^{-9} and 1.52 \times 10^{-7} mol/L, respectively; n = 11; P < .01; Figure 3, A). This marked effect of progesterone on the activity integral of ritodrine was associated with a significant reduction in both amplitude (P < .05) and frequency (P < .05) of contractions (Figure 3, B and C). However, there was no significant difference in the maximum relaxation achieved in both groups (n = 11; Figure 3, A). Control experiments demonstrated that pretreatment of myometrial tissues with progesterone at a concentration of 10^{-9} mol/L did not display a significant effect on oxytocin-induced myometrial contractility (Figure 2, A).

**Comment**

Preterm birth is a leading cause of neonatal mortality and associates with both short- and long-term morbidity.\(^8\) Spontaneous preterm labor leads to more than half of preterm births.\(^9\) However, lack of detailed information on the mechanisms of preterm labor has hindered the development of novel treatments and preventative measures. Progesterone plays an important role in the maintenance of pregnancy, and antiprogestogens have been used to initiate uterine contractions.\(^10\) Although maternal, fetal, and amniotic progesterone levels do not show an apparent decline with labor and delivery,\(^11,12\) functional progesterone withdrawal may occur as a consequence of altered progesterone receptor isoforms, progesterone receptor regulation through transcription factors, or nongenomic effects of progesterone.\(^13,14\)

Over the years, there has been renewed interest in the use of synthetic progesterone in different forms for prevention and treatment of preterm labor, but the results are inconsistent.\(^4,5\) Although a recent randomized controlled trial of natural progesterone\(^6\) and 17\alpha-hydroxyprogesterone caproate\(^15\) revealed a significant decrease in the incidence of preterm birth, there was no statistically significant difference in the admissions for preterm labor. In addition, these results\(^6\) raise the
2 main question. First, does the reduction in preterm delivery result from the action of progesterone itself or does progesterone enhance the effectiveness of the β₂-mimetics that were used as tocolytics in that study? For this reason, for the preincubation studies, we selected to use progesterone at a submicromolar concentration (10⁻⁸ M) that had no obvious effect on oxytocin-induced myometrial contractions to delineate the effect of ritodrine from progesterone inhibition of contractions. Second, can the benefit of progesterone be extended to pregnant women at lower risk of preterm delivery? Our results demonstrate the relaxant effect of natural progesterone on oxytocin-induced myometrial contractility, which could be beneficial. This effect of progesterone could arise from the repression of contraction-associated proteins such as oxytocin, prostaglandins, estrogen receptor α, ion channels, and gap junctions. Although the relaxant effect of progesterone on myometrium at the nanomolar level is minimal (Figure 2, A), it significantly increases the relaxant effect of ritodrine by reducing both amplitude and frequency of oxytocin-induced myometrial contractions.

The effects of steroid hormones can be mediated by the modulation of gene expression, which take hours or even days, or through nontranscriptional effects occurring within minutes by direct interactions with cellular targets. The appreciable myometrial relaxation observed in this study is more likely due to the action of progesterone acting directly on β₂-andrenergic receptor (AR). Progesterone increases β-AR activity in rat uteri by increasing adenylylate cyclase coupling and β-AR density. Vivat et al. have shown that progesterone selectively up-regulated β₂-AR in rat myometrium by increasing its gene transcription rate. However, this effect appears to take at least 18 hours following progesterone exposure before this change becomes significant. The transcriptional actions of progesterone on inhibiting expression of contraction-associated proteins or stimulating β₂-AR synthesis are unlikely, given the relatively short preincubation time (1 hour) with the steroid used in the present study.

Interestingly, progesterone is more effective when applied outside the cell than when injected into cytoplasm. Because all the results from the present study were obtained from in vitro studies of oxytocin-induced contractility, an interaction involving progesterone and oxytocin may underlie these effects. However, oxytocin was present throughout the experiments, yet significant differences were obtained with ritodrine with or without progesterone, suggesting little influence of oxytocin on the reported findings. An earlier report suggested that oxytocin receptor function was directly inhibited by natural progesterone, P₄. Moreover, the P₄ metabolite, 5β-dihydroprogesterone (but not P₄) inhibited both oxytocin binding to its receptor and oxytocin-induced inositol phosphate production. Recent findings did not

![Figure 3](cumulative-increases-in-ritodrine-10⁻⁹-to-10⁻³-mol-l-were-applied-to-paired-pregnant-human-myometrial-strips-in-the-absence-solid-line-or-presence-dashed-lines-of-progesterone-n-11-pairs-data-of-activity-integral-a-maximum-tension-b-and-frequency-c-were-presented-as-mean-sem-percentage-of-the-results-obtained-before-any-drug-application-for-each-individual-strip-the-concentration-response-curves-were-analyzed-by-fitting-with-the-equation-y-(y-min)+(y-max-y-min)/1+10(log EC₅₀-X)×Hill-slope-where-y-is-the-response-y-max-is-the-maximum-relaxation-achieved-and-y-min-is-the-minimum-relaxation-achieved)
demonstrate effects of 5β-dihydroprogesterone on the native oxytocin receptor in human myometrial membranes or when expressed in Chinese hamster ovary cells, casting doubt on a direct interaction between progesterone and the oxytocin receptor.

Changes in gene transmission are unlikely to underlie these results. Interestingly, progesterone is more effective when applied outside the cell than when injected into cytoplasm.20 These present data, taken together with our previous findings23 demonstrating the plasma-membral distribution of β2-ARs in human pregnant myometrial cells, suggest that progesterone interacts directly with β2-AR through a nongenomic pathway. Our novel findings provide encouraging evidence that natural progesterone might be of benefit in preventing preterm birth in pregnant women in a low- as well as high-risk population. Further studies will seek to investigate the physiological mechanisms underlying this modulatory effect of progesterone on ritodrine-mediated relaxation.

Acknowledgments
We are grateful to the consultants, midwives, theatre staff, and patients at Derby City General Hospital for their assistance with this study.

References

Anthony M. Vintzileos, MD,a Cande V. Ananth, PhD, MPH,b Eftichia Kontopoulos, MD,a John C. Smulian, MD, MPHa

Department of Obstetrics, Gynecology and Reproductive Sciences, Division of Maternal-Fetal Medicine,a Section of Epidemiology and Biostatistics,b University of Medicine and Dentistry of New Jersey, Robert Wood Johnson Medical School/Robert Wood Johnson University Hospital, New Brunswick, NJ

Received for publication July 6, 2004; revised July 29, 2004; accepted August 13, 2004

Objective: The purpose of this study was to estimate the risks of stillbirth and neonatal and infant deaths in triplets, according to mode of delivery.

Study design: We used the “matched multiple birth” data file that was comprised of triple births that were delivered in the United States in the years 1995 through 1998. Analyses were restricted to fetuses that were delivered at ≥24 weeks of gestation. Based on the order of the birth of the fetuses within the triplet set, the mode of delivery of triplets was assigned as cesarean-cesarean-cesarean (all cesarean), vaginal-vaginal-vaginal (all vaginal), and vaginal-cesarean-cesarean or vaginal-vaginal-cesarean (other). Associations between mode of delivery and stillbirth, neonatal deaths (within 28 days), and infant deaths (up to 1 year) were expressed as relative risks with 95% confidence intervals and population attributable risks, which were derived from multivariate logistic regression models that were based on the method of generalized estimated equations (with all cesarean deliveries serving as the reference). All analyses were adjusted for several confounding factors.

Results: Ninety-five percent of all triplets were delivered by cesarean delivery. Vaginal delivery (all vaginal) was associated with an increased risk for stillbirth (relative risk, 5.70; 95% CI, 3.83, 8.49) and neonatal (relative risk, 2.83; 95% CI, 1.91, 4.19) and infant (relative risk, 2.29; 95% CI, 1.61, 3.25) deaths. The population-attributable risks were 15.9% for neonatal and 12.4% for infant deaths, which implied that these proportions of deaths were potentially avoidable had these triplet fetuses all been delivered by cesarean delivery rather than all fetuses being delivered vaginally.

Conclusion: Cesarean delivery of all 3 triplet fetuses is associated with the lowest neonatal and infant mortality rate. Vaginal delivery among triplet gestations should be avoided.

© 2005 Elsevier Inc. All rights reserved.

The rate of multiple pregnancies has increased significantly since the introduction of assisted reproductive techniques; triplet pregnancies are the most common type of multiple pregnancy of high fetal order.
Reported cesarean delivery rates for triplet gestations range from 42% to 85%. Although there is an increasing number of cesarean deliveries in triplet gestations, the evidence for the optimal mode of delivery has not been established in the literature. Four studies suggested improved perinatal outcomes by cesarean delivery, but 4 studies found no benefit. In fact, 2 of these studies suggested that the outcome may be better if the triplets are delivered vaginally. One of the chief limitations of all these studies is the small number of triplet births.

The objective of our study was to estimate the rate of cesarean delivery among triplet gestations in the United States and to document the association between the mode of delivery and stillbirth, neonatal, and infant mortality rates in triplet gestations.

Material and methods

Data for this study were derived from the United States “matched multiple birth” data file for the years 1995 to 1998, which were assembled and provided by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention. These data correspond to fetal deaths, live birth, and infant death for twins and higher-order multiple births.

Gestational age in these data files was calculated as the interval between the date of the delivery and the date of the last menstrual period (LMP). Records with a missing date of LMP, but with valid month and year of the LMP, had gestational ages imputed. When the LMP date (but not the month and year) was missing or when the LMP-based gestational age was inconsistent with birth weight, a clinical estimate of gestational age, which also was contained on these data files, was used instead (in approximately 5% of records). The clinical estimate of gestation is based on the birth attendant’s estimate of gestation, which usually is based on either the Dubowitz or the Ballard assessment of gestational age. These imputations and replacements of gestational age by clinical estimates were performed by the NCHS before the release of the public data files. Records with missing or incomplete data after the imputations by NCHS were excluded. All antenatal, medical, and obstetric high-risk conditions are recorded by the use of a check-box format on birth certificates and indicate the presence or absence of the condition.

We restricted the analyses to triplet births that were delivered at ≥24 completed weeks of gestation. Rates of stillbirth per 1000 total triplet births were derived on the basis of the modes of triplet deliveries, and the adjusted relative risk (RR) with 95% CI were derived as measures of effect. In a similar fashion, we also calculated the rates of neonatal (deaths within the first 28 days) and infant (deaths within the first year) mortality (per 1000 triplet live births) in relation to mode of triplet delivery.

The data were analyzed with the SAS software (version 8.2; SAS Institute Inc, Cary, NC) on the UNIX system. This study was approved by the ethics committee of the Institutional Review Board of the Robert Wood Johnson Medical School, University of Medicine and Dentistry of New Jersey.
Results

In the United States, of the 23,381 triplet births that were delivered between 1995 and 1998, we excluded missing data on gestational age after the imputations that were performed by the NCHS (n = 357 births), gestational age <24 completed weeks (n = 881 births), missing information on mode of delivery (n = 188 births), which left 21,955 triplet fetuses. Of these, we further excluded 754 fetuses from incomplete triplet sets, which left 21,201 fetuses from 7067 triplet pregnancies (mothers) for analysis. The overall cesarean delivery rate among triplets in the United States was 95% (6680/7067 women).

Table I shows the distribution of selected demographic characteristics of women in relation to the mode of triplet births. The distribution of maternal age was fairly similar across all 3 modes of delivery. However, women with cesarean delivery of all 3 fetuses were less likely to be single, more likely to be educated, less likely to have no prenatal care, and more likely to be white compared with the women in other modes of delivery.

Stillbirths and neonatal and infant mortality rates were consistently lower among those women who were delivered of all 3 fetuses by cesarean delivery compared with those women who were delivered vaginally (Figure 1). As can be seen, most of the benefit of cesarean delivery in terms of infant death is derived from the improvement in neonatal mortality rates.

Table II shows the relationship among the 3 birth order groups and stillbirth and neonatal and infant mortality rates. Vaginal delivery for all 3 triplets was associated with the highest gestational age-adjusted RRs for stillbirth and neonatal and infant death rates. The group with combined vaginal-cesarean delivery was also associated with increased risk for stillbirth, but definite conclusions cannot be made because some of these stillbirths may have occurred antepartum.

Compared with women who were delivered of all 3 fetuses by cesarean delivery, those women who were delivered of all 3 fetuses vaginally carried a 2.83-fold increased (adjusted) RR for neonatal and 2.29-fold increased risk for infant deaths (Table III). These RRs were also fairly similar when the analysis was restricted to nonmalformed fetuses. The population-attributable risks were approximately 12.4% to 22.4% for deaths among fetuses that were all delivered vaginally, which implies that 15.9% of neonatal and 12.4% of infant deaths among triplets potentially are avoidable, had these triplet fetuses been delivered by cesarean delivery rather than by vaginal delivery.

There was no association between birth order and neonatal or infant mortality rate (Table IV). However, the rate of stillbirth was increased for the third fetus, regardless of mode of delivery, but again no definite conclusion can be made because some of the stillbirths may have occurred antepartum.

Comment

Despite the increasing rates of cesarean delivery for triplet gestations, there are scarce data that relate perinatal outcome to the mode of delivery. In previous studies, the range of cesarean deliveries has been between 29% and 85%. In the present study, which used US data, the cesarean delivery rate in triplet gestations was 95%. Previous studies that used a small number of patients had conflicting results. Some studies suggested that cesarean delivery may be of benefit; other studies showed that vaginal delivery of triplets was beneficial. The study by Ron-El, which involved only 19 triplet gestations, was an observational study that compared perinatal outcomes between patients who were delivered by vaginal delivery and patients who...
were delivered by cesarean delivery. Perinatal outcomes were similar between the 2 groups, and the authors concluded that the preferred mode of delivery cannot be stated dogmatically in triplet gestations. However, this was a nonrandomized observational study that included a very small number of patients. The study by Clarke and Roman also included 19 triplet gestations. The perinatal outcome was better among fetuses who were delivered vaginally compared with those who were delivered by cesarean delivery; however, the vaginally

![Figure 1](image)

**Figure 1** Gestational age-specific rates of stillbirth and neonatal and infant mortality in relation to the mode of triplet delivery: United States 1995 through 1998.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Total triplet births (n)</th>
<th>Gestational age (y)*</th>
<th>Mortality rate per 1000 births (%)</th>
<th>Gestational age-adjusted RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stillbirth</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cesarean</td>
<td>19,599</td>
<td>32.7 ± 3.1</td>
<td>7.8</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>All vaginal</td>
<td>1,161</td>
<td>31.6 ± 4.0</td>
<td>57.7</td>
<td>6.04 (4.10, 8.97)</td>
</tr>
<tr>
<td>Other†</td>
<td>441</td>
<td>30.1 ± 4.0</td>
<td>29.5</td>
<td>2.45 (1.20, 5.04)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cesarean</td>
<td>19,446</td>
<td>32.7 ± 3.1</td>
<td>17.8</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>All vaginal</td>
<td>1,094</td>
<td>31.8 ± 3.9</td>
<td>96.6</td>
<td>2.92 (2.00, 4.27)</td>
</tr>
<tr>
<td>Other†</td>
<td>428</td>
<td>30.0 ± 4.0</td>
<td>67.8</td>
<td>0.89 (0.51, 1.53)</td>
</tr>
<tr>
<td>Infant death</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cesarean</td>
<td>19,446</td>
<td>32.7 ± 3.1</td>
<td>24.5</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>All vaginal</td>
<td>1,094</td>
<td>31.8 ± 3.9</td>
<td>104.2</td>
<td>2.38 (1.70, 3.35)</td>
</tr>
<tr>
<td>Other†</td>
<td>428</td>
<td>30.0 ± 4.0</td>
<td>86.4</td>
<td>0.90 (0.57, 1.42)</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.
† Includes vaginal-cesarean-cesarean or vaginal-vaginal-cesarean modes of delivery.
delivered group had greater maturity, which seemed to be the major factor for the observed lower neonatal morbidity and mortality rates. The study by Wildschut et al involved a comparison of 30 women who had planned abdominal delivery and 39 women who had planned vaginal delivery. Compared with vaginal delivery, planned abdominal delivery was associated with a significantly higher perinatal mortality rate, primarily because of respiratory distress syndrome and a higher neonatal complication rate that was related to sepsis, respiratory distress syndrome, and necrotizing enterocolitis. Although the authors concluded that a policy of planned abdominal delivery in triplets is not superior to the policy of planned vaginal delivery, it should be noted that the 2 groups of women were treated by different physicians in 2 different institutions in The Netherlands (1 institution in Leiden and the other institution in Amsterdam). Recently, Alran et al suggested that offering vaginal delivery in triplet gestations is a reasonable option. However, there were 9 neonatal deaths among the 78 patients for whom a trial of labor was allowed, and no deaths for the 15 patients who had planned cesarean delivery.

Some observational, nonrandomized, and historical-control studies that have used larger study samples (range, 30-105 study samples) have favored cesarean delivery. Our observational, nonrandomized study, which was based on national data, is the largest series on triplet births to be reported to date and establishes that the cesarean delivery rate for triplets in the United States is extremely high (95%). This approach may be supported by the increased neonatal and infant mortality rates that are associated with vaginal delivery. We found no association, however, between birth order and mortality rates, except that the stillbirth rate was increased for the third fetus, regardless of the mode of delivery.

One of the major strengths of our study is the larger number of analyzed triplet gestations that were based on the national data. Another strength is that a robust statistical analysis was used that controlled for confounding factors, such as gestational age at delivery, maternal age, gravidity, maternal education, marital status, maternal race/ethnicity, smoking and alcohol use, lack of prenatal care, and high-risk pregnancy.

### Table III

<table>
<thead>
<tr>
<th>Variable</th>
<th>Adjusted RR (95% confidence interval) *</th>
<th>Population attributable risk (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Overall</td>
<td>Nonmalformed fetuses</td>
</tr>
<tr>
<td>Stillbirth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cesarean</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>All vaginal</td>
<td>5.70 (3.83, 8.49)</td>
<td>5.99 (3.97, 9.03)</td>
</tr>
<tr>
<td>Other</td>
<td>2.58 (1.26, 5.28)</td>
<td>2.69 (1.27, 5.69)</td>
</tr>
<tr>
<td>Neonatal death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cesarean</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>All vaginal</td>
<td>2.83 (1.91, 4.19)</td>
<td>2.95 (1.97, 4.42)</td>
</tr>
<tr>
<td>Other</td>
<td>0.91 (0.52, 1.57)</td>
<td>0.85 (0.48, 1.51)</td>
</tr>
<tr>
<td>Infant death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>All cesarean</td>
<td>1.00 (Reference)</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>All vaginal</td>
<td>2.29 (1.61, 3.25)</td>
<td>2.36 (1.65, 3.40)</td>
</tr>
<tr>
<td>Other</td>
<td>0.91 (0.58, 1.43)</td>
<td>0.88 (0.55, 1.39)</td>
</tr>
</tbody>
</table>

* RRrs were derived from fitting multivariate logistic regression models that are based on the method of generalized estimating equations. All analyses were adjusted for the confounding effects because of gestational age, maternal age, gravidity, maternal race/ethnicity, and chronic hypertension. Population attributable risk is based on the “overall” analysis.

### Table IV

<table>
<thead>
<tr>
<th>Variable</th>
<th>Overall mortality rate per 1000 births (%)</th>
<th>Mortality rate per 1000 births, based on order of triplet birth (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>First fetus</td>
<td>Second fetus</td>
</tr>
<tr>
<td>Stillbirth</td>
<td>7.8</td>
<td>2.0</td>
</tr>
<tr>
<td>All cesarean</td>
<td>7.8</td>
<td>2.0</td>
</tr>
<tr>
<td>All vaginal</td>
<td>57.7</td>
<td>38.8</td>
</tr>
<tr>
<td>Other*</td>
<td>29.5</td>
<td>20.4</td>
</tr>
<tr>
<td>Neonatal death</td>
<td>17.8</td>
<td>17.9</td>
</tr>
<tr>
<td>All cesarean</td>
<td>17.8</td>
<td>17.9</td>
</tr>
<tr>
<td>All vaginal</td>
<td>96.6</td>
<td>96.8</td>
</tr>
<tr>
<td>Other*</td>
<td>67.8</td>
<td>83.3</td>
</tr>
<tr>
<td>Infant death</td>
<td>24.5</td>
<td>24.4</td>
</tr>
<tr>
<td>All cesarean</td>
<td>24.5</td>
<td>24.4</td>
</tr>
<tr>
<td>All vaginal</td>
<td>104.2</td>
<td>104.8</td>
</tr>
<tr>
<td>Other*</td>
<td>86.4</td>
<td>97.2</td>
</tr>
</tbody>
</table>

* Includes vaginal-cesarean-cesarean or vaginal-vaginal-cesarean modes of delivery.
In addition, we derived all estimates of risk after accounting for the nonindependent nature of outcomes that were examined. Failure to account for this phenomenon during statistical analysis likely may result in an imprecise estimate of variances of RRs, which could lead to incorrect inferences.

This study, as with any population-based study, has a few limitations. Some of these limitations include the possible underreporting of certain risk factors and complications and the unavailability of some known risk factors in the vital statistics data.15,16 Such factors include suboptimal weight gain during pregnancy, drug use, home environment, the suboptimal use of pediatric medical resources, and other factors that may be associated with increased perinatal and infant mortality rates. In addition, the lack of information on financial status or health care provider and hospital type may have impacted our results. Another limitation of the study pertains to the association between the mode of triplet delivery and perinatal outcomes in nonmalformed fetuses. We initially hypothesized that, if a malformation was diagnosed prenatally, then this may have precipitated a (planned) cesarean delivery. However, given that the association between the mode of delivery and perinatal outcomes in all fetuses and those among nonmalformed fetuses were fairly similar (Table III), we believe that our conclusions are robust. Finally, because the risk of stillbirth was substantially higher among the vaginal deliveries than the risk of neonatal and infant death, it is possible that some of the stillbirths may have occurred in the antepartum period. However, the vital statistics data did not make it possible for us to differentiate antepartum from intrapartum fetal deaths. Thus, no firm conclusion can be made with respect to stillbirths and mode of delivery.

Although we recognize these limitations, it is our opinion that vaginal delivery should be avoided in the case of triplets because it is associated with increased stillbirth and infant deaths. Because randomized controlled trials on this topic are not likely in the near future, the present study provides the best evidence that perinatal outcome in triplet gestations is improved by cesarean delivery as the mode of delivery.

References

Is bacterial vaginosis a stronger risk factor for preterm birth when it is diagnosed earlier in gestation?

Mark A. Klebanoff, MD, MPH,a,* Sharon L. Hillier, PhD,b Robert P. Nugent, PhD,a Cora A. MacPherson, PhD,c John C. Hauth, MD,d J. Christopher Carey, MD,e Margaret Harper, MD, MS,f Ronald J. Wapner, MD,g Wayne Trout, MD,h Atef Moawad, MD,i Kenneth J. Leveno, MD,j Menachem Miodovnik, MD,k Baha M. Sibai, MD,l J. Peter VanDorsten, MD,m Mitchell P. Dombrowski, MD,n Mary J. O’Sullivan, MD,o Michael Varner, MD,p Oded Langer, MD,q and the National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network

National Institute of Child Health and Human Development, National Institutes of Health, Department of Health and Human Services, Bethesda, Md,a Department of Obstetrics, Gynecology and Reproductive Sciences, University of Pittsburgh, Pittsburgh, Pa,b Biostatistics Center, George Washington University, Rockville Md,c Department of Obstetrics and Gynecology, University of Alabama, Birmingham, Ala,d Department of Obstetrics and Gynecology, University of Oklahoma, Oklahoma City, Okla,e Department of Obstetrics and Gynecology, Wake Forest University, Winston-Salem, NC,f Department of Obstetrics and Gynecology, Thomas Jefferson University, Philadelphia, Pa,g Department of Obstetrics and Gynecology, Ohio State University, Columbus, Ohio,h Department of Obstetrics and Gynecology, University of Chicago, Chicago, Ill,i Department of Obstetrics and Gynecology, University of Texas Southwestern Medical Center, Dallas, Tex,j Department of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, Ohio,k Department of Obstetrics and Gynecology, University of Tennessee, Memphis, Tenn,l Department of Obstetrics and Gynecology, Medical University of South Carolina, Charleston, SC,m Department of Obstetrics and Gynecology, Wayne State University, Detroit, Mich,n Department of Obstetrics and Gynecology, University of Miami, Miami, Fla,o Department of Obstetrics and Gynecology, University of Utah, Salt Lake City, Utah,p Department of Obstetrics and Gynecology, University of Texas at San Antonio, Tex,q

Received for publication April 6, 2004; revised June 17, 2004

**KEY WORDS**

Bacterial vaginosis
Preterm birth
Gram stain

**Objective:** It is stated commonly that the earlier in pregnancy bacterial vaginosis is diagnosed, the greater is the increase in risk of preterm birth compared with women without bacterial vaginosis. However, this contention is based on small numbers of women.

Supported by grants No. U10 HD21410, U10 HD21414, U10 HD22760, U10 HD27861, U10 HD27869, U10 HD27883, U10 HD27889, U10 HD27905, U10 HD27915, U10 HD27917, U10 HD34116, U10 HD34122, U10 HD34136, U10 HD34208, U10 HD34210, and U01 HD36801 from the National Institute of Child Health and Human Development and by grant AI 38514 from the National Institute of Allergy and Infectious Diseases.

* Reprint requests: Mark A. Klebanoff, MD, DESPR, NICHD, NIH, Bldg 6100, Room 7B05, Bethesda, MD 20892-7510.

E-mail: mk90h@nih.gov
**Study design:** In this analysis of 12,937 women who were screened for bacterial vaginosis as part of a previously conducted clinical trial, the odds ratio of preterm birth (<7 weeks of gestation) for asymptomatic bacterial vaginosis-positive versus bacterial vaginosis-negative women was evaluated among women who were screened from 8 to 22 weeks of gestation.

**Results:** The odds ratio of preterm birth among bacterial vaginosis-positive versus bacterial vaginosis-negative women ranged from 1.1 to 1.6 and did not vary significantly according to the gestational age at which bacterial vaginosis was screened. The odds ratio for preterm birth did not vary significantly by gestational age at diagnosis when bacterial vaginosis was subdivided into Gram stain score 7 to 8 or 9 to 10.

**Conclusion:** Although bacterial vaginosis was associated with an increased risk of preterm birth, the gestational age at which bacterial vaginosis was screened for and diagnosed did not influence the increase.

© 2005 Elsevier Inc. All rights reserved.

Bacterial vaginosis (BV) is a syndrome in which the normal vaginal hydrogen peroxide—producing lactobacilli are replaced by a mixed flora with high concentrations of anaerobic bacteria, *Gardnerella vaginalis* and *Mycoplasma hominis.* The condition is relatively common in pregnant women; 23% of black women and 9% of white women were reported to be affected. Multiple case-control and prospective cohort studies have demonstrated that BV is associated with an increased risk of preterm birth. However, results of clinical trials of treatment of BV to prevent preterm birth have been mixed.

Some authors have stated that the earlier in gestation at which BV is detected, the greater is the risk of an adverse outcome. A recent meta-analysis concluded that BV that was diagnosed at <16 and <20 weeks of gestation was associated with odds ratios for preterm birth of 7.55 and 4.20, respectively; the odds ratio for diagnosis was 1.53. However, the studies on which this contention is based were conducted in different countries, had different definitions of BV, and used different definitions of adverse pregnancy outcome. In addition, the studies that reported on BV that was diagnosed at <20 weeks of gestation had relatively small sample sizes, and the odds ratios in the meta-analysis had wide confidence limits.

Recently conducted randomized clinical trials of treatment of BV and *Trichomonas vaginalis* gave us the opportunity to study, in a large population of women who were evaluated for BV at a wide range of gestational ages according to a single common protocol, whether BV that was diagnosed earlier in pregnancy was associated with a higher risk of preterm birth than BV that was diagnosed later in pregnancy.

**Material and methods**

The data for this study are from the recently completed National Institute of Child Health and Human Development Maternal-Fetal Medicine Units Network BV and *T vaginalis* clinical trials, in which women with these conditions were assigned randomly to metronidazole or placebo treatment to prevent preterm birth. These 2 trials required that large numbers of women be screened for BV and *T vaginalis* at 8 to 22 completed weeks of gestation. Normally, we did not ascertain the outcome of pregnancies to women who were screened but not enrolled in the clinical trials. However, as part of an ancillary protocol, we obtained pregnancy outcomes for women who were screened from November 1995 to February 1998; during this limited time 15,864 women were screened and are included in this protocol. Not all Maternal-Fetal Medicine Unit centers participated in this ancillary study. The Institutional Review Board at each clinical site approved the protocol, which included screening and obtaining pregnancy outcomes on screened women.

The inclusion and exclusion criteria for screening have been described previously. Women were eligible to be screened if they were from 8 to 22 weeks of gestation inclusive; did not report genital itching, burning, or malodor in response to nondirected questioning; had no contraindications to receiving metronidazole; had no major medical or obstetric complications in the current pregnancy; had not received antibiotics within the past 14 days and were not expected to receive them before the intrapartum period; and could be followed up to delivery.

We defined BV as a vaginal Gram-stain Nugent score of ≥7 in conjunction with a vaginal pH of >4.4. This is the same definition that was used for the clinical trial and in the Vaginal Infections and Prematurity Study. One Dacron swab sample, which was taken from the junction of the upper third and lower two thirds of the lateral vaginal wall, was rolled on a glass slide and then touched to a ColorpHast pH stick (Curtin Matheson, Grand Prairie, Tex). Slides from women whose vaginal pH was >4.4 were shipped to the laboratory of one of the authors (S.L.H.), where they were stained and interpreted by technicians who were masked to the clinical data. Therefore, according to the study protocol, women with vaginal pH values of ≤4.4...
were considered not to have BV, although they did not have Gram stains that were read.

Women received routine prenatal care at their institutions. Study personnel abstracted delivery records to determine the date, method, and indication of delivery, and birth weight. In this analysis, we defined BV from the initial screening, without regard to Gram stains that were obtained later in pregnancy, because the latter were available only for those women who entered into the trials. The physicians of women who were enrolled in the clinical trial were required to follow a specific protocol, which included sonography, to determine gestational age.9 However, there was no protocol to determine gestational duration of pregnancies for women who were screened but not enrolled in the trial; therefore, the gestational age that was used was the best obstetric estimate at delivery. We considered all pregnancy losses after the screening tests, plus all preterm deliveries (<37 completed weeks of gestation) as the outcome of interest. Secondary outcomes included deliveries that were stratified as <23 weeks of gestation and 23 to 36 weeks of gestation inclusive. The former stratum evaluates the association between BV and miscarriage at a time early in pregnancy when women were still being screened and would have a variable time of follow-up, depending on the gestation at which screening occurred. Because all women were screened at <23 weeks of gestation, the latter definition avoided the need to account for the fact that women who were screened earlier in pregnancy had more time than women who were screened later to have had a pregnancy loss. We also studied spontaneous preterm birth, which is defined as preterm birth after spontaneous onset of labor or spontaneous membrane rupture, regardless of the ultimate method of delivery.

Categorical variables were compared with the use of the chi-squared test. When categories were ordered (eg, no BV, Gram stain 7-8, Gram stain 9-10), significance was assessed with the Cochran-Armitage test for trend.15 To determine whether BV that was diagnosed earlier in gestation carried a greater relative risk of preterm birth than BV that was diagnosed later, we present gestational age at screening from a start of 8 to 12 weeks of gestation to 21 to 22 weeks of gestation, in 2-week intervals. For women who were screened in each gestational age window, we then calculated the odds ratio for preterm birth among BV-positive compared with BV-negative women. If BV that was diagnosed earlier in gestation carried a greater relative risk of preterm birth than BV that was diagnosed later, the odds ratio for BV would be greater the earlier in gestation when women were screened. To test this hypothesis, we used a multiple logistic regression model that included terms for BV (yes/no), gestational age at screening (in weeks, as a continuous variable), and an interaction term between BV and gestational age.

Determination of the actual probability value for the interaction was based on gestational age as a continuous variable. When we assessed the degree of BV, we defined 2 interaction terms, 1 for Gram stain scores of 7 to 8 and 1 for Gram stain scores of 9 to 10. In either case, if BV that was diagnosed earlier in pregnancy carried a greater relative risk of preterm birth than BV that was diagnosed later, the interaction term would be statistically significant and have a negative value. This assumes that the log of the odds ratio for preterm birth among BV-positive versus BV-negative women decreases linearly with advancing gestation, which appears reasonable on the basis of previous work.6 Finally, the association between BV and pregnancy loss at <23 weeks of gestation was assessed by a proportional hazards model.16 This allowed for staggered entry into the study (ie, women who were screened at different gestational ages); the model estimates a hazard ratio, which is akin to a relative risk. Statistical analysis was performed with SAS statistical software (version 8.2; SAS Institute Inc, Cary, NC). In all analyses, 2-tailed probability values of <.05 were considered statistically significant.

**Results**

There were 15,864 women who were screened for BV, 5449 (34.4%) of whom had BV. Pregnancy outcome was available for 12,937 of these women, and 1704 pregnancies (13.2%) lasted <37 weeks. The exclusion of the 263 pregnancy losses at <23 weeks of gestation reduced the preterm birth fraction to 11.4%. The characteristics of the women in the study are given in Table I. Most women

<table>
<thead>
<tr>
<th>Table I Characteristics of study population</th>
<th>Women who were delivered, from screening to &lt;37 weeks of gestation (%)</th>
<th>Women with BV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>N (%)</td>
<td></td>
</tr>
<tr>
<td>Race/ethnicity*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>7209 (56%)</td>
<td>15</td>
</tr>
<tr>
<td>Other</td>
<td>2765 (21%)</td>
<td>10</td>
</tr>
<tr>
<td>White</td>
<td>2955 (23%)</td>
<td>11</td>
</tr>
<tr>
<td>Nulliparous*</td>
<td>4913 (39%)</td>
<td>13</td>
</tr>
<tr>
<td>Multiparous*</td>
<td>7743 (61%)</td>
<td>13</td>
</tr>
<tr>
<td>Gestational age at screening (wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;13</td>
<td>5032 (39%)</td>
<td>15</td>
</tr>
<tr>
<td>13-14.9</td>
<td>1633 (13%)</td>
<td>15</td>
</tr>
<tr>
<td>15-16.9</td>
<td>1448 (11%)</td>
<td>13</td>
</tr>
<tr>
<td>17-18.9</td>
<td>1737 (13%)</td>
<td>11</td>
</tr>
<tr>
<td>19-20.9</td>
<td>1784 (14%)</td>
<td>11</td>
</tr>
<tr>
<td>21-22.9</td>
<td>1303 (10%)</td>
<td>11</td>
</tr>
</tbody>
</table>

* Numbers do not equal 12,937 because data were missing.
were screened at <15 weeks of gestation; slightly more than one half of the women were black, and 39% of the women were nulliparous. No other descriptive data were collected about the women. As has been described in other studies, the prevalence of BV was lower among women who are screened later in pregnancy, and the 24% prevalence of BV among women who are screened at 21 to 22.9 weeks of gestation is only slightly greater than the 16% that was observed in the Vaginal Infections and Prematurity Study,14

In the entire population, delivery at <37 weeks of gestation was more frequent among the 4634 women with BV (15.1%), than among the 8303 women without BV (12.1%; P < .001). Furthermore, there was a significant trend (P < .001) for women with more abnormal Gram-stain values to have a higher incidence of preterm birth; 13.9% of the 2523 women with scores 7 to 8 were delivered preterm compared with 16.5% of the 2111 women with scores 9 to 10. Results were similar for spontaneous preterm birth, for total and spontaneous delivery from 23 to 36 weeks of gestation, and for both black women and non-black women (data not shown). The hazard ratio for pregnancy loss at <23 weeks of gestation was 1.4 (95% CI, 1.1-1.8), which was comparable to the odds ratio for birth at 23 to 36 weeks (odds ratio, 1.2; 95% CI, 1.1-1.4).

The probabilities of all preterm birth, delivery from screening to 23 weeks of gestation, and delivery at 23 to 36 weeks of gestation are presented by BV status and gestational age at screening in Table II. Although there was a trend for the probabilities of all the outcomes to decrease, the later in gestation screening occurred, the trends were similar in BV-positive and BV-negative women. The odds ratios for preterm and spontaneous preterm birth, along with their 95% CIs, for BV-positive compared with BV-negative women are presented in Figure 1. BV was associated with an elevated risk of both outcomes, although there was no evidence that the odds ratio that was associated with BV was higher when screening occurred earlier in gestation than when it occurred later. Figure 2 shows the odds ratios for delivery at <23 weeks of gestation and 23 to 36 weeks of gestation according to the time of screening. In Figures 1 and 2, there were trends of borderline significance for the odds ratio of all preterm births (P = .06) and for all deliveries at 23 to 36 weeks of gestation (P = .0502) to be higher when BV was diagnosed later in gestation; the probability values increased to .09 and .08, respectively, when there was adjustment for maternal race and parity, which were the only potentially confounding factors that we collected about women who were not in the clinical trial. However the probability values for trend in odds ratios with

### Table II  Pregnancy outcome by gestational age at screening among women with and without BV

<table>
<thead>
<tr>
<th>Gestation at screening</th>
<th>No. screened</th>
<th>&lt;37 weeks (%)</th>
<th>Birth from screening to &lt;37 weeks of gestation</th>
<th>Women who were delivered from screening to &lt;23 weeks of gestation (%)</th>
<th>Women who were delivered from 23 to &lt;37 weeks of gestation*</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;13 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV—positive</td>
<td>2036</td>
<td>15.6</td>
<td>4.1</td>
<td>12.0</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis—negative</td>
<td>2996</td>
<td>14.0</td>
<td>2.9</td>
<td>11.4</td>
<td></td>
</tr>
<tr>
<td>13-14 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV—positive</td>
<td>652</td>
<td>15.3</td>
<td>2.6</td>
<td>13.1</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis—negative</td>
<td>981</td>
<td>14.0</td>
<td>2.7</td>
<td>11.6</td>
<td></td>
</tr>
<tr>
<td>15-16 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV—positive</td>
<td>587</td>
<td>15.5</td>
<td>1.9</td>
<td>13.9</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis—negative</td>
<td>861</td>
<td>11.7</td>
<td>1.0</td>
<td>10.8</td>
<td></td>
</tr>
<tr>
<td>17-18 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV—positive</td>
<td>541</td>
<td>13.3</td>
<td>1.7</td>
<td>11.8</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis—negative</td>
<td>1196</td>
<td>9.8</td>
<td>0.7</td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>19-20 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV—positive</td>
<td>500</td>
<td>15.4</td>
<td>0.6</td>
<td>14.9</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis—negative</td>
<td>1284</td>
<td>10.0</td>
<td>0.6</td>
<td>9.4</td>
<td></td>
</tr>
<tr>
<td>21-22 wk</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>BV—positive</td>
<td>318</td>
<td>13.2</td>
<td>0.3</td>
<td>12.9</td>
<td></td>
</tr>
<tr>
<td>Bacterial vaginosis—negative</td>
<td>985</td>
<td>10.5</td>
<td>0.1</td>
<td>10.4</td>
<td></td>
</tr>
</tbody>
</table>

* Only patients who were still pregnant at 23 weeks of gestation are included in this column. Therefore, percentages for being delivered from screening to <23 weeks of gestation and 23 to <37 weeks of gestation do not sum to the percentage for being delivered from screening to <37 weeks of gestation.
increasing gestational age did not approach statistical significance for spontaneous preterm birth (P = .44) or for delivery at <23 weeks of gestation (P = .24).

The odds ratios for all preterm births among women with elevated pH and Gram stain scores of 7 to 8 or 9 to 10, compared with women without BV, are presented for total preterm birth in Figure 3. Although higher Gram stain scores were associated with an increased risk of preterm birth at most gestations, the odds ratio for all preterm births among women with scores of 9 to 10 was not higher when screening occurred earlier in gestation (probability value for interaction of score 9-10 with gestational age, .28). The odds ratio for all preterm birth among women with scores of 7 to 8 actually increased with increasing gestational age (P = .049), although this was no longer significant after adjustment was made for race and parity (P = .079). Results for spontaneous preterm birth were not substantially different from the corresponding values for all preterm births. In no case was there a trend for the odds ratio for preterm birth to be greater when BV was screened for and diagnosed earlier in gestation.

Information about pregnancy outcome was missing for 2927 screened women (18%), and women with BV were less likely to have missing pregnancy information (15%) than were women without BV (20%; P < .001). This was entirely due to participation in the clinical trial; among trial participants, 1.9% of women with BV and 2.0% of women without BV had missing pregnancy outcome information (P = .92) versus 22% of women with BV and 21% of women without BV among women not in the clinical trial (P = .17). When women enrolled in the clinical trial were excluded from this analysis, the results were not substantially changed (data not shown).

Comment

This study, which included more women who were screened at <20 weeks of gestation (n = 10,746) than all previous studies combined, did not find that BV, when diagnosed earlier in gestation, carried greater odds of pregnancy loss at <23 weeks or of preterm birth than when it was diagnosed later. This is in contrast to the results of a recent meta-analysis that reported an odds ratio of 7.55 for BV that was diagnosed at <16 weeks of gestation, 4.20 for BV that was diagnosed at <20 weeks of gestation, and 1.53 for BV that was diagnosed at ≥20 weeks of gestation.6

There are several possible explanations for our failure to confirm the results of the meta-analysis. First, our study was conducted in clinic populations in the United States, whereas most previous studies of women at <20 weeks of gestation were conducted outside of the United States. The rate of preterm birth among BV-negative women in these studies ranged from <2% to 11.8%.
most studies reported rates considerably lower than the preterm birth rate of 12.1% among BV-negative women in our population. This suggests that there are multiple other causes of preterm birth that are more common in the United States than in many other countries, which might obscure the relative increase in risk among BV-positive women here. Of note, our estimate of the odds ratio for preterm birth among BV-positive women was very similar to the odds ratio of 1.4 for the preterm low birth weight that is reported in the Vaginal Infections and Prematurity Study,16 which screened for BV at 23 to 26 weeks of gestation and was conducted in a population similar to ours.

Second, most previous studies of women at <20 weeks of gestation have been comparatively small, which results in statistically imprecise estimates of the association between BV and preterm birth. Even when these studies were combined in a meta-analysis,16 the odds ratio for preterm birth when BV was diagnosed at <20 weeks of gestation had a 95% CI of 2.11 to 8.39; the 95% CI for BV that was diagnosed at <16 weeks of gestation was 1.80 to 31.65.

Third, previous studies have used varying definitions of BV. These definitions include a clinical definition according to the Amsel criteria; Gram stains interpreted according to the Nugent or Spiegel method; Gram stain plus elevated pH, clue cells, or abnormal vaginal culture; or clue cells only. Cut-points for preterm delivery ranged from <32 to <37 weeks of gestation. Although unlikely, it is possible that different definitions of BV might account for our discordant results.

There are possible reasons that our study failed to find that BV that was diagnosed early in pregnancy carried a large relative risk of preterm birth. We did not use a standardized protocol for the assessment of gestational age among women who were ineligible for the clinical trial, and errors in gestational age measurement may have obscured our ability to observe an increased risk. However, we collected data on birth weight for these women, and when the analyses were repeated for low birth weight (<2500 g) and very low birth weight (<1500 g) the results were virtually identical to those for preterm birth, which suggests that differences in gestational age measurement did not account for our results.

We did not read the Gram stains of women who had vaginal pH values ≤4.4. Because the definition of BV for the clinical trial required both abnormal pH and abnormal Gram stain, screening for the trial was more efficient. However, it limits our ability to study the joint effects of pH and flora. In the Maternal-Fetal Medicine Unit Preterm Prediction Study,18 only 11.6% of women with normal pH had Gram stain scores of ≥7, versus 48.3% of women with pH >4.4 (unpublished data). There were 5194 women in the present study who had normal pH values and therefore did not have a Gram stain evaluated, so we estimate that we misclassified as normal 603 women who would have had Nugent scores of ≥7 had we read their Gram stains. Our current definition identified 4634 women as having BV and 8303 women as being normal, so we believe that the misclassification of 603 women, although not optimal, is acceptable.

We did not collect extensive data on antibiotic use after screening among women who were not in the clinical trial. Treatment of BV might have blunted an adverse effect on preterm birth. However, women who had received antibiotics recently were ineligible for screening, and we collected data on which of the women were ineligible for the trial because they received antibiotics after screening but before they would have been assigned randomly to therapy. We also collected extensive data on antibiotic use among women in the clinical trial. When we eliminated women who received these clinically indicated antibiotics and those who were assigned randomly to receive metronidazole in the trial, our results were not changed substantially. Therefore, we do not believe that unrecorded antibiotic use was responsible for the lack of an increased risk of preterm birth among women with BV compared with women without BV when they were screened early in pregnancy.

No outcome data were found for 18% of screened women. To determine the maximum effect that missing outcomes might have had on the results, we made the extreme worst-case assumption that all of the BV-positive and none of the BV-negative women with missing outcomes were delivered at <37 weeks of gestation. In this unlikely situation, the odds ratio for preterm birth among BV-positive women who were screened at <13 weeks of gestation would have been 3.7 (95% CI, 3.3 to 4.3). Although elevated, this is still considerably less than the value of 7.55 that was noted by Leitich et al6 for women with BV at ≤16 weeks of gestation.

Our study was not truly longitudinal—we screened each woman once, rather than multiple times in pregnancy so each week’s comparison was based on different women. However, of the 18 studies included in the meta-analysis of Leitich et al,6 only the studies by Meis et al19 and Riduan et al17 evaluated women more than once during pregnancy; the meta-analysis drew its conclusion by comparing results across the different studies.6 Although a single large study that would evaluate women at multiple times during pregnancy would be optimal, we believe that our study that evaluated women once according to a common protocol is an improvement over existing research. One way to assess the importance of this limitation is to determine whether, among women without BV (the control group), those women who were screened earlier in pregnancy had a different risk of preterm birth from 23 to 36 weeks of gestation than women who were screened later. As can be seen in Table II, there was a small, but statistically
It seems likely that, for many, if not most, of these women, BV did not differ by when in pregnancy they were screened, which suggests that the inherent risk of preterm birth among these women was not influenced by the timing of screening.

Perhaps a more important issue is that, like almost all previous research on this topic, we know the time in pregnancy when BV was first diagnosed, but we do not know the actual time when women initially acquired BV. It seems likely that, for many, if not most, of these women, BV was first diagnosed, but we do not know the actual time when women initially acquired BV.

In conclusion, we evaluated whether BV that was diagnosed earlier in the 8- to 22-week interval to have a higher risk of delivery from 23 to 36 weeks of gestation than women who were screened later. However, after adjustment for race and parity, this trend was no longer statistically significant (P = .11). Therefore, the baseline risk of preterm birth among women without BV did not differ by when in pregnancy they were screened, which suggests that the inherent risk of preterm birth among these women was not influenced by the timing of screening.

Acknowledgments


References

4. Lamont RF. Infection in the prediction and antibiotics in the prevention of spontaneous preterm labour and preterm birth. BJOG 2003;110(Suppl):70-5.


Antenatal maternal administration of phenobarbital for the prevention of exchange transfusion in neonates with hemolytic disease of the fetus and newborn

Thomas N. Trevett, Jr, MD,a,* Karen Dorman, RN, MS,a Georgine Lamvu, MD, MPH,b Kenneth J. Moise, Jr, MDa

Divisions of Maternal-Fetal Medicinea and Advanced Laparoscopy and Gynecologic Surgery,b Department of Obstetrics and Gynecology, University of North Carolina School of Medicine, Chapel Hill, NC

Received for publication May 5, 2004; revised July 22, 2004; accepted August 17, 2004

KEY WORDS
Red cell alloimmunization
Fetus
Hemolytic disease of the newborn infant
Phenobarbital
Bilirubin encephalopathy
Exchange transfusion

Objective: Hemolytic disease of the fetus and newborn infant (HDFN) can be associated with bilirubin encephalopathy, which is usually averted through neonatal exchange transfusions (EXT). However, EXT can be associated with significant procedure-related morbidity. We hypothesized that maternal oral administration of phenobarbital (PB) to women with HDFN would reduce the rate of EXT.

Study design: Cases of HDFN from January 1985 to June 2003 were reviewed. All patients who underwent serial intrauterine transfusions (IUTs) for red cell alloimmunization were included. Patients were offered oral phenobarbital (30 mg 3 times a day) after their last IUT in an effort to enhance fetal hepatic maturity. Variables studied included gestational age and hemoglobin multiples of the median at first transfusion and delivery, peak neonatal bilirubin, need for exchange transfusion, and maternal PB administration. Multivariate regression analysis was applied to determine relative risks and 95% CIs.

Results: Seventy-one patients met study criteria; 29% of the neonates underwent EXT. The use of antenatal PB was associated with a decreased incidence of EXT, 9% versus 52% ($P < .01$). After controlling for confounding variables, the relative risk for EXT after antenatal PB administration was 0.23 (95% CI: 0.06-0.76).

Conclusion: Maternal administration of PB reduces the need for neonatal EXTs in HDFN.

Hemolytic disease of the fetus and newborn infant (HDFN) can lead to significant perinatal morbidity, including encephalopathy secondary to hyperbilirubinemia. Bilirubin is liberated from the heme molecule as a result of immune-mediated hemolysis. It is transported to the hepatocyte via albumin where it then undergoes glucoronidation by a family of enzymes known as uridine-diphospho-glucuronosyltransferases (UGT). In humans, the major enzyme in this pathway is bilirubin-UDP-glucuronosyltransferase (UGT1A1). After conjugation, the bilirubin diglucuronide, which is highly water soluble, is then actively excreted into bile and
eliminated by way of the urinary and gastrointestinal tracts. The rate-limiting step in this process is the concentration of UGT1A1.1

Phenobarbital (PB) has been shown to enhance the capability of the neonatal liver to conjugate and eliminate bilirubin. Studies by Brown and Zeulzer2 and Gartner and Arias3 in guinea pigs, and by Catz and Yaffe4 in mice provide evidence that PB given to pregnant animals can stimulate the induction of the glucuronyl transerase enzymatic pathway in their offspring. The first human studies by Trolle5 indicated a similar reduction in neonatal jaundice in humans from 22% to 5%. PB has been shown to increase the expression of the UGT1A1, a mechanism that has only recently been elucidated. Sugatani et al6 discovered a 290 base pair (bp) enhancer sequence on the gene for UGT1A1 that binds PB, leading to increased production of the enzyme.

After birth, some infants affected by HDFN are unable to manage the increased production of bilirubin, leading to unconjugated hyperbilirubinemia. If untreated, this can lead to kernicterus with long-term neurologic consequences and even death. Phototherapy is usually the first line of treatment. However, if unsuccessful, neonates must undergo an exchange transfusion (EXT) to reduce the bilirubin load and the concentration of the maternal anti-red cell antibody. In a recent study, the incidence of major morbidities caused by this procedure, including transfusion reactions, line sepsis, and death after EXT, was 5%. Therefore, a reduction in the need for EXTs would further improve neonatal outcomes for HDFN.

We hypothesized that maternal administration of PB before delivery in women with red cell alloimmunization undergoing serial intrauterine transfusions (IUTs) before delivery would induce neonatal hepatic maturity and reduce the need for EXT.

Material and methods

Study design

This was a retrospective case-control study of all women from January 1985 to June 2003 with documented HDFN undergoing cordocentesis and IUTs at the University of North Carolina School of Medicine in Chapel Hill, NC, and the Baylor College of Medicine in Houston, Texas. Before initiating this study, we obtained consent from the University of North Carolina institutional review board to review the medical records of the patients and neonates of interest. Cases were identified through a computerized database maintained by 1 of the authors (K.M.). All women included in this sample had either the presence of an anti-red cell antibody associated with HDFN that titered above the critical threshold or a history of a previous pregnancy affected by HDFN.

| Table I Maternal and fetal characteristics |
|---------------|----------------------|
| Variable                  |                       |
| Maternal age (y)          | 29.1 ± 5.1           |
| Gravidity*               | 4 (1-8)              |
| Number of IUTs*          | 3 (1-6)              |
| Gestational age at first IUT (wks) | 25.9 ± 3.4         |
| Gestational age at delivery (wks) | 34.4 ± 4.2         |
| Hemoglobin at first IUT (MoMs) | 0.58 ± 0.24   |
| Hemoglobin at delivery (MoMs) | 0.97 ± 0.26   |
| Hydrops at first IUT      | 41%                  |
| Antibody type             | (N, %)               |
| group D                  | 49 (69)              |
| group Kell               | 7 (10)               |
| group D, C               | 10 (14)              |
| group D, C, E            | 2 (3)                |
| group D, C, M            | 1 (1)                |
| group D, E               | 1 (1)                |
| group c                  | 1 (1)                |

All parameters expressed as means ± SD unless otherwise indicated.
* Expressed as median and range.

Pregnancies at risk for HDFN were followed intensively with surveillance for the development of fetal anemia. Before the year 2000, patients underwent serial amniocenteses for determination of their ΔOD450, using graphs created by Liley et al5 to determine the severity of anemia. After 2000, patients were monitored with serial middle cerebral artery (MCA) peak systolic velocites (PSV) measured weekly. In the first affected pregnancy, weekly MCA PSV measurements were initiated when a critical titer for the putative anti-red cell antibody was detected; in previously affected pregnancies, the MCA PSV was measured starting at 18 weeks’ gestation. Fetuses with an MCA PSV greater than 1.5 multiples of the median (MoMs) underwent cordocentesis with combined intravascular and intraperitoneal transfusion if necessary. After documentation of subsequent bone marrow suppression through fetal cell stains at the time of IUT, the interval of transfusion was lengthened to 3 weeks. IUTs were repeated serially until the 35th week of gestation. After the last IUT, the patient was offered PB, 30 mg orally 3 times a day for 10 days. Delivery was planned after the treatment period. The neonate was monitored intensively in the nursery for signs of anemia and hyperbilirubinemia. Standard institutional-specific protocols that were based on the current guidelines at the time were used to determine when to initiate phototherapy or EXT.10-12

Medical records were extracted for the following parameters: estimated gestational age at the time of first IUT, fetal hematocrit at first IUT, total number of IUTs, gestational age at delivery, birth weight at delivery, Apgar scores, maternal PB use, levels of cord hematocrit and percentage of fetal cells at delivery, peak neonatal total and direct bilirubin, neonatal need for EXT.
blood transfusions or EXTs, and neonatal survival. Hematocrit was converted to approximate hemoglobin by dividing by a factor of 3. With the use of the equation developed by Mari et al, $e^{2.84-8.55/GA}$, median hemoglobin for each gestational age was calculated. Multiples of the median hemoglobin values were then calculated and used in the analyses.

A univariate analysis of the data was conducted to identify frequencies, means, standard deviations, ranges, missing data, and outliers. A bivariate analysis was then undertaken to look for crude associations between the prenatal PB exposure and the covariates and for associations between the outcome and the covariates. Lastly, binomial regression analysis was used to model the effect of PB administration on EXTs. Gestational age at delivery and neonatal peak total bilirubin among other variables were selected as possible confounders and introduced into the regression models to estimate the final effect of PB on transfusions. A $P$ value of < .05 was considered statistically significant. Where appropriate, 95% CIs for odds ratios were calculated.

**Results**

Seventy-one women and neonates who underwent serial IUTs over the study period were identified. The maternal and fetal characteristics of this cohort are shown in Table I. Thirty-three women received PB before delivery; all of their neonates survived. Thirty-eight women did not receive PB. The most common reason for not receiving PB was nonreassuring antenatal testing requiring delivery, followed by mature fetal lung profile at the last IUT. In this group, the overall perinatal survival was 76%; there were 9 neonatal deaths.

There were 21 (29%) neonates who required EXTs for treatment of severe hyperbilirubinemia (median 1, range 1-3). Of the 33 women who received PB before delivery, only 3 (9%) of their neonates required an exchange transfusion. Of the 31 surviving infants that were delivered of mothers who did not receive PB, 16 (52%) required an EXT ($P < .01$).

A bivariate analysis of the data was performed to demonstrate which variables other than maternal PB had an impact on the primary outcome, need for EXT. These results are shown in Table II. The only variables with a statistically significant difference between the groups of neonates were the neonatal peak bilirubin ($P < .01$) and the gestational age at delivery ($P < .01$).

A binomial regression analysis was undertaken to control for these variables. After adjusting for the gestational age at delivery and neonatal peak total bilirubin, administration of PB decreased the relative risk of the need for EXT to 0.23 (95% CI: 0.06-0.76). Table III represents the results of the multivariate regression analysis.

**Comment**

The cohort of women in the current study represents a population with severe HDFN as evidenced by the early mean gestational age and mean fetal hematocrit at the time of first transfusion, total number of IUTs, and
the percentage of fetuses with hydrops at the time of the first transfusion. Despite the severity of disease, the perinatal outcome was excellent with an overall survival of 87%. The majority of the neonates who did require an EXT only required 1 or 2 procedures. The 29% incidence of EXT in the current study is consistent with previous work by Filbey et al. who noted an incidence of 29% in neonates in Sweden who were affected by HDFN. Maternal PB late in gestation was, however, associated with more than a 75% reduction in the need for EXT.

The major limitation of our finding is the confounding influence of preterm delivery on the need for an EXT. Most of the preterm deliveries that occurred were undertaken on an emergent basis and thus the patients were unable to receive PB. Premature infants are at greater risk of requiring an EXT for hyperbilirubinemia because of their immaturity of the hepatic microsomal enzymatic conjugation pathways. Further, the level of unconjugated bilirubin at which the premature brain will be damaged is lower than the term brain because of the immaturity of the blood-brain barrier. Therefore, providers are more likely to initiate EXT when dealing with the premature infant. The mean gestational age at delivery of the pregnancies that underwent EXTs in our study was 2.6 weeks less than in pregnancies in which the infant did not require EXT, a statistically significant difference. For this reason, we controlled for gestational age in our multivariate analysis. After this analysis, the protective effect of maternal PB persisted.

The dosing regimen of 30 mg 3 times a day of oral PB used in our study was chosen on the basis of the work by Halpin et al. who performed a randomized controlled trial of providing PB or placebo to healthy pregnant women starting at 32 weeks of gestation in an effort to decrease hyperbilirubinemia. These authors showed a significant reduction in the mean neonatal unconjugated bilirubin from 8.5 to 6.4 mg/dL in the women treated with 1 g of PB before delivery. They also showed that the incidence of infants with levels exceeding 10 mg/dL at 96 hours of life was significantly reduced from 24.6% in controls to 7.3% in treated individuals. In our cohort, each woman received PB for a period of 7 to 10 days, with the maximum cumulative amount being 900 mg.

The decision to use any drug during pregnancy should include consideration of perinatal safety. Multiple investigators have evaluated fetal exposure to PB during the latter stages of pregnancy. PB is known to freely cross the placenta throughout pregnancy. Usage of PB has been investigated as a means to reduce the occurrence and severity of neonatal intracranial hemorrhage in preterm infants. These studies have not shown a significant decrease in rates of intraventricular hemorrhage, however, in follow-up studies at both 2 and 7 years of age, Thorp et al. found no difference in mean intelligence, achievement, and behavioral scores. Concerns have been raised over the administration of a barbiturate, a sedative, to the fetus. However, there have been no findings to date in multiple trials using antenatal PB regarding an increased incidence of neonatal depression. In conclusion, this retrospective study suggests that a brief course of maternal oral PB before delivery may reduce the need for neonatal EXT. Further study in a randomized controlled trial is necessary to confirm these results.

References

The cost-effectiveness of routine antenatal screening for maternal herpes simplex virus–1 and –2 antibodies

Stephen F. Thung, MD, William A. Grobman, MD, MBA

Department of Obstetrics and Gynecology, Division of Maternal-Fetal Medicine, Northwestern University Medical School, Chicago, Ill

Received for publication March 31, 2004; revised September 3, 2004; accepted September 3, 2004

Objective: The purpose of this study was to determine the cost-effectiveness of routine antenatal screening for herpes simplex virus 1 and 2 in women without a known history of genital herpes.

Study design: Decision analysis was used to compare 3 treatment strategies to prevent neonatal herpes infection in women without a known history of genital herpes simplex virus: (1) the current standard of care (no herpes simplex virus screening), (2) antepartum herpes simplex virus–1 and –2 antibody screening of the pregnant woman and her male partner with appropriate counseling, and (3) antepartum herpes simplex virus–1 and –2 antibody screening with appropriate counseling and acyclovir prophylaxis at 36 weeks of gestation in seropositive women.

Results: Our model predicts that using current guidelines, 1 of 5469 women will have a herpes-infected neonate. Strategy 2 and 3 cost $5,812,819 and $4,130,297, respectively, for every significant neurologic sequela or death prevented. The cost-effectiveness of these strategies, expressed as cost per quality life-year gained, was $219,513 and $155,988 respectively. These results were robust in the sensitivity analysis.

Conclusion: Routine herpes simplex virus screening in pregnancy is not cost-effective.

The incidence of neonatal herpes simplex virus (HSV) has been estimated to be between 1 in 3000 to 1 in 20,000 live births, with a case fatality rate that is estimated at 20%, even with appropriate treatment. Eight-five percent of these cases result from genital infections that occur at delivery; primary infections confer the greatest risk of perinatal infection. To minimize the chances of perinatal transmission in women with symptomatic genital infection, cesarean delivery is used commonly. Unfortunately, relatively few cases are likely to be prevented by this strategy because many cases of recently acquired or recurrent herpes infections remain unrecognized.

Recent advances have made it possible to identify HSV exposure with tests that are specific for HSV type 1 and 2 antibodies. Some authors have suggested that screening could reduce transmission if either counseling or pharmaceutical suppression, or both, were used appropriately in response to the screening results. It remains unknown, however, if routine screening of pregnant women for HSV-1 and -2 antibodies, along with suggested interventions in a pregnant population without a history of HSV, would be cost-effective. In an effort to evaluate whether HSV screening would indeed be cost-effective, we constructed a decision analysis model.
<table>
<thead>
<tr>
<th>Variable</th>
<th>Base case</th>
<th>Range</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal serostatus (HSV-1/-2) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative/negative</td>
<td>23</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Positive/negative</td>
<td>49</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Negative/positive</td>
<td>11</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Positive/positive</td>
<td>17</td>
<td>—</td>
<td>7</td>
</tr>
<tr>
<td>Paternal serostatus (HSV-1/-2) (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative/negative</td>
<td>31</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Positive/negative</td>
<td>51</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Negative/positive</td>
<td>05</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>Positive/positive</td>
<td>13</td>
<td>—</td>
<td>8</td>
</tr>
<tr>
<td>HSV test characteristic (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV 1 sensitivity</td>
<td>95</td>
<td>90-99</td>
<td>10</td>
</tr>
<tr>
<td>HSV 1 specificity</td>
<td>96</td>
<td>90-99</td>
<td>10</td>
</tr>
<tr>
<td>HSV 2 sensitivity</td>
<td>98</td>
<td>90-99</td>
<td>10</td>
</tr>
<tr>
<td>HSV 2 specificity</td>
<td>97</td>
<td>90-99</td>
<td>10</td>
</tr>
<tr>
<td>Seroconversion rate during pregnancy (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1 primary infection</td>
<td>0.67</td>
<td>0.50-2.50</td>
<td>6,9</td>
</tr>
<tr>
<td>HSV-1 nonprimary infection</td>
<td>0.07</td>
<td>0.00-0.50</td>
<td>6,9</td>
</tr>
<tr>
<td>HSV-2 primary infection</td>
<td>2.00</td>
<td>1.50-2.50</td>
<td>6</td>
</tr>
<tr>
<td>HSV-2 nonprimary infection</td>
<td>2.00</td>
<td>1.50-2.50</td>
<td>6</td>
</tr>
<tr>
<td>Reduction of seroconversion during pregnancy due to counseling (%)</td>
<td>0.75</td>
<td>0.90-0.50</td>
<td>11,12</td>
</tr>
<tr>
<td>Percentage of primary/nonprimary infection with viral shedding at delivery (%)</td>
<td>6</td>
<td>2-20</td>
<td>6</td>
</tr>
<tr>
<td>HSV-2</td>
<td>10</td>
<td>2-20</td>
<td>6</td>
</tr>
<tr>
<td>Recurrent viral shedding at delivery, seroconversion during pregnancy (%)</td>
<td>3.5</td>
<td>0-18</td>
<td>6,13,14</td>
</tr>
<tr>
<td>HSV-1</td>
<td>7</td>
<td>3-36</td>
<td>6,13</td>
</tr>
<tr>
<td>HSV-2</td>
<td>0.05</td>
<td>0.03-0.09</td>
<td>7</td>
</tr>
<tr>
<td>HSV-2</td>
<td>1.50</td>
<td>1.30-1.80</td>
<td>7</td>
</tr>
<tr>
<td>Reduction of recurrent viral shedding caused by acyclovir prophylaxis (%)</td>
<td>0.33</td>
<td>0.50-0.20</td>
<td>13,15,16</td>
</tr>
<tr>
<td>Symptomatic lesions identified during viral shedding (%)</td>
<td>10</td>
<td>1-40</td>
<td>17</td>
</tr>
<tr>
<td>Screening</td>
<td>40</td>
<td>10-75</td>
<td>6</td>
</tr>
<tr>
<td>Cesarean delivery with symptomatic or asymptomatic infection (%)</td>
<td>79</td>
<td>50-100</td>
<td>7</td>
</tr>
<tr>
<td>Symptomatic infection</td>
<td>79</td>
<td>50-100</td>
<td>7</td>
</tr>
<tr>
<td>Asymptomatic infection</td>
<td>23</td>
<td>10-30</td>
<td>18</td>
</tr>
<tr>
<td>Vertical transmission with viral shedding at delivery (%)</td>
<td>50</td>
<td>25-75</td>
<td>4</td>
</tr>
<tr>
<td>HSV-1 or -2 primary</td>
<td>33</td>
<td>25-75</td>
<td>4</td>
</tr>
<tr>
<td>HSV-1 recurrent</td>
<td>25</td>
<td>10-50</td>
<td>6,7</td>
</tr>
<tr>
<td>HSV-2 recurrent</td>
<td>0.5</td>
<td>0-5</td>
<td>4,6</td>
</tr>
<tr>
<td>Reduction of vertical transmission due to cesarean delivery (%)</td>
<td>0.2</td>
<td>0.0-0.5</td>
<td>7,19</td>
</tr>
<tr>
<td>Outcomes of infected neonates (QALY)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>51.5</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Mild neurologic deficits</td>
<td>6.0</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Moderate deficits</td>
<td>6.5</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>14.0</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Death</td>
<td>22.5</td>
<td>—</td>
<td>2</td>
</tr>
<tr>
<td>Utility of neonatal outcomes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal</td>
<td>1</td>
<td>—</td>
<td>20</td>
</tr>
<tr>
<td>Mild neurological deficit</td>
<td>1</td>
<td>0.8-1.0</td>
<td>20</td>
</tr>
<tr>
<td>Moderate</td>
<td>0.5</td>
<td>0.25-0.75</td>
<td>20</td>
</tr>
<tr>
<td>Severe</td>
<td>0.1</td>
<td>0.0-0.25</td>
<td>20</td>
</tr>
<tr>
<td>Discount rate (%)</td>
<td>3</td>
<td>1-5</td>
<td></td>
</tr>
</tbody>
</table>
Material and methods

Using a decision tree model, we compared 3 different strategic approaches to the prevention of neonatal HSV infection in women with no known history of genital HSV infections. The first strategy is the current standard of care, as outlined by the American College of Obstetricians and Gynecologists. In this strategy, serologic HSV screening is not performed, and women are offered cesarean delivery only for symptomatic genital lesions at delivery. In the second strategy, maternal serologic screening of type-specific antibodies for HSV-1 and -2 is performed and paternal screening of HSV-1, -2, or both is performed if the maternal serostatus demonstrates susceptibility to either HSV type. Women are informed of their serostatus and counseled about the diverse presentations of oral and genital infections. Discordant couples are counseled about methods to reduce the risk of genital transmission and informed to refrain from high-risk sexual activity during the third trimester. The third strategy is identical to the second with the addition of acyclovir prophylaxis at 36 weeks of gestation for women who are seropositive. Because some physicians may choose to forego paternal testing and only to intervene on the basis of maternal serologic status, this possibility was assessed in the sensitivity analysis.

In this model, neonatal HSV could be acquired from a primary, nonprimary, or recurrent maternal genital infection. A primary infection is defined as an infection with either HSV-1 or -2 in an individual who does not have circulating antibodies to either virus. A nonprimary infection occurs when an individual, in whom antibodies to one HSV serotype have developed previously, acquires the HSV infection to which she has no circulating antibodies. Recurrent infections result from reactivation of virus in an individual with antibodies to that viral type.

The estimates for primary and nonprimary seroconversion were obtained from Brown et al. The actual risk of HSV-1 seroconversion that is specifically due to genital disease rather than oral disease was unavailable in this data set. Although most HSV-1 infections result from oral infection before becoming sexually active, a greater proportion of new HSV-1 infections that are acquired in the reproductive age are a result of genital disease, and the probability of HSV-1 primary seroconversion because of genital infections is approximately one-third that of the HSV-2 seroconversion rate.

Recurrent viral shedding is more frequent in women who recently have acquired infection than those with more distant disease. Therefore, we used 2 different viral shedding rate estimates, depending on the temporal proximity of seroconversion to delivery. These estimates and other estimates that were used in the model, which were derived from the published literature, are presented in Table I.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Base case ($)</th>
<th>Range ($)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Screening cost</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HSV-1 or -2</td>
<td>37.50</td>
<td>19-56</td>
<td>12,27</td>
</tr>
<tr>
<td>HSV-1 and -2</td>
<td>75.00</td>
<td>38-122</td>
<td>12,27</td>
</tr>
<tr>
<td>Counseling cost</td>
<td>13.00</td>
<td>6-26</td>
<td>28</td>
</tr>
<tr>
<td>Acyclovir supplies</td>
<td>71.00</td>
<td>71-296</td>
<td>29,30</td>
</tr>
<tr>
<td>Delivery cost</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Elective cesarean delivery</td>
<td>7425</td>
<td>3,712-11,138</td>
<td>31</td>
</tr>
<tr>
<td>Labor cesarean delivery</td>
<td>9283</td>
<td>4,641-13,924</td>
<td>31</td>
</tr>
<tr>
<td>Vaginal delivery</td>
<td>4821</td>
<td>2410-7231</td>
<td>31</td>
</tr>
<tr>
<td>Infection costs</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(acute care + long-term care for neurologic deficits)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Normal/mild deficit</td>
<td>13,202</td>
<td>13,202-26,404</td>
<td>19,32</td>
</tr>
<tr>
<td>Moderate deficit</td>
<td>134,202</td>
<td>134,202-268,404</td>
<td>19,32</td>
</tr>
<tr>
<td>Severe deficit</td>
<td>325,602</td>
<td>325,602-651,204</td>
<td>19,32</td>
</tr>
<tr>
<td>Death</td>
<td>13,202</td>
<td>13,202-26,404</td>
<td>19,32</td>
</tr>
</tbody>
</table>

In both strategies 2 and 3, screening coupled with education and behavior modification was assumed to reduce the risk of acquiring a new HSV infection during pregnancy. In some trials, counseling has failed to result in reductions in sexually transmitted disease. Therefore, more modest reductions in risk were examined in the sensitivity analysis. Screening and education also could impact neonatal infections by increasing patient awareness of the signs and symptoms of genital HSV. Approximately 10% of women who are seropositive for HSV-2 admit to having symptoms in the past, but the likelihood of recognizing symptoms has been demonstrated to increase to approximately 40% when women know their serostatus. Improved detection would enable women to benefit from cesarean delivery at the time of a symptomatic genital infection, which is a strategy that has been thought to decrease transmission by 80%.

Acyclovir prophylaxis at 36 weeks of gestation decreases the risk of viral shedding at delivery in patients who are experiencing their first symptomatic episode and in patients with known recurrent infections. We chose 400 mg of acyclovir 3 times per day for this analysis and estimated that, with prophylaxis, the relative risk of viral shedding at delivery would be 0.33.

We assigned a utility value of 1 for normal health and mild neurologic deficits, 0.5 for moderate neurologic deficits, 0.1 for severe neurologic deficits, and 0 for death. Although the life span of infected neonates is not well described, in order not to bias the model against screening, we assigned a life expectancy of 76 years for both healthy individuals and affected neonates but examined a lifespan range of 50 to 76 years for affected neonates in the sensitivity analysis.
Cost data are presented in Table II and are presented in 2003 US dollars. Costs in the literature before 2003 were adjusted to 2003 dollars with the medical care component of the Consumer Price Index.

For a cohort of 100,000 women, we calculated, for each strategy, the incidence of neonatal HSV infection, the associated long-term neurologic deficits (mild, moderate, or severe) and death, and the direct costs to the health care system. The primary outcome of the study was the cost-effectiveness that was measured as the marginal cost per quality life-year (QALY) gained. Costs and QALYs were discounted at 3%. Cost-effectiveness was defined as a marginal cost per QALY ratio of <$50,000.

**Results**

The results for the base-case model are presented in Table III. Our model predicts that the current standard of care would result in a neonatal HSV infection incidence of 1 per 5469 deliveries and cost $558,103,353 per 100,000 women. Screening and counseling, as appropriate, of the pregnant woman and her male partner increased the cost by $11,685,150 and prevented 4.7 neonatal infections and 2.01 associated significant neurologic deficits or deaths. Correspondingly, this strategy would cost $219,513 per QALY gained. The addition of acyclovir prophylaxis increased the cost by $16,586,931 and prevented 9.39 neonatal infections and 3.8 associated significant neurologic deficits or deaths, when compared with the standard of care. The cost-effectiveness of this strategy, compared with the base case, would be $155,988 per QALY gained.

**Table III**  Summary of results (per 100,000 women)

<table>
<thead>
<tr>
<th>Variable</th>
<th>Standard</th>
<th>Screening</th>
<th>Screening/prophylaxis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total infections*</td>
<td>18.28</td>
<td>13.58</td>
<td>8.89</td>
</tr>
<tr>
<td>Infections averted*</td>
<td>—</td>
<td>4.70</td>
<td>9.39</td>
</tr>
<tr>
<td>Total neurologic deficits/death*</td>
<td>7.82</td>
<td>5.81</td>
<td>4.02</td>
</tr>
<tr>
<td>Neurologic deficits/death averted*</td>
<td>—</td>
<td>2.01</td>
<td>3.8</td>
</tr>
<tr>
<td>Total cost ($)</td>
<td>558,103,353</td>
<td>569,788,503</td>
<td>574,690,284</td>
</tr>
<tr>
<td>Marginal cost ($)</td>
<td>—</td>
<td>11,685,150</td>
<td>16,586,931</td>
</tr>
<tr>
<td>Marginal cost/infections averted ($)</td>
<td>—</td>
<td>2,484,980</td>
<td>1,765,852</td>
</tr>
<tr>
<td>Marginal cost/neurologic or deaths averted ($)</td>
<td>—</td>
<td>$5,812,819</td>
<td>4,130,297</td>
</tr>
<tr>
<td>Total QALY*</td>
<td>2,969,793.01</td>
<td>2,969,846.25</td>
<td>2,969,899.35</td>
</tr>
<tr>
<td>QALY gained*</td>
<td>—</td>
<td>53.24</td>
<td>106.34</td>
</tr>
<tr>
<td>Marginal cost/QALY gained ($)</td>
<td>—</td>
<td>219,513</td>
<td>155,988</td>
</tr>
</tbody>
</table>

* Per 100,000 women.

**Comment**

Although rare, neonatal HSV infection has a high rate of morbidity and mortality. Because of this problem, some experts have suggested that routine antenatal screening for HSV antibodies could reduce the neonatal infection rate. However, before any major public health initiative, a better understanding of the costs of the program and the potential benefits must be understood, and we undertook this decision analysis to estimate the additional costs of a comprehensive program using herpes screening and interventions to reduce the incidence of neonatal infection.

Our analysis demonstrates that the cost of a screening program with appropriate interventions to reduce neonatal infection at delivery would not be cost-effective. This finding is in agreement with Rouse and Stringer, who assessed the cost-effectiveness of routine HSV-2 screening alone to reduce new maternal HSV infections that were acquired proximate to delivery. That study estimated the cost to prevent 1 significant neurologic deficit or death would be $891,000. Despite incorporating testing for HSV-1 in addition to HSV-2, our strategy still did not reach the cost-effectiveness threshold.
Although no strategy in our analysis appeared cost-effective, the addition of acyclovir prophylaxis was more cost-effective than screening with counseling alone. Our data are consistent with studies that demonstrated that acyclovir prophylaxis, in women who are known to have recurrent HSV infection, is cost-saving when compared with the current standard.\textsuperscript{30,33} Yet, the economic benefits of acyclovir for women with known infection are lost when a program of routine screening is instituted.

As with any decision analysis, our data are limited by the quality of the estimates of neonatal infection rates and medical costs. To ensure that our conclusions were not primarily dependent on the base estimates that we chose, we used a wide range in our sensitivity analysis and found that the model was robust. Not a single variation, even those variations that should bias the model heavily toward screening, caused the cost per QALY to be $50,000.

Our analysis was from the perspective of the health care system. As such, we did not capture the indirect or social costs of routine HSV screening and intervention. The impact psychologically or financially on a family

\textbf{Figure}  Tornado diagram summary of the 1-way sensitivity analysis for antenatal screening with counseling and acyclovir prophylaxis.
that cares for a neurologically impaired child is difficult to assess and was not included in our analysis. The social costs of informing a couple that they have been exposed to HSV-2, a known sexually transmitted disease, and the anxiety that could result during the pregnancy are also unknown.

As far as we are aware, this is the first analysis to evaluate a comprehensive protocol that involves screening and plausible interventions to reduce the incidence of neonatal HSV. Although screening with counseling and screening with counseling and acyclovir prophylaxis may not be cost-effective for the population at large, we cannot claim that these strategies should never be offered. Every woman must be considered individually. However, we believe that this analysis demonstrates that the incorporation of routine HSV-1 and -2 antibody tests during antepartum care for women without a history of genital HSV infection is not a cost-effective intervention and remains unwarranted.

References

Combination of vaginal pH with vaginal sialidase and prolidase activities for prediction of low birth weight and preterm birth

Sabina Cauci, PhD,a,* James McGregor, MD,b Poul Thorsen, MD, PhD,c Jakob Grove, PhD,c Secondo Guaschino, MDd

Department of Biomedical Sciences and Technologies, School of Medicine, University of Udine, Udine, Italy,a Department of Obstetrics and Gynecology, Cedars-Sinai Medical Center, Los Angeles, Calif, and Obstetrix Medical Group of Arizona, Tucson, Ariz,b NANEAN, Department of Epidemiology and Social Medicine, Aarhus University, Aarhus, Denmark,c and Obstetric and Gynecologic Unit, Department of Reproductive and Development Sciences, IRCCS Burlo Garofolo Hospital, School of Medicine, University of Trieste, Trieste, Italyd

Objectives: The purpose of this study was to assess if easy to measure vaginal fluid biomarkers are predictive for low birth weight (LBW, <2500 g), very LBW (VLBW, <1500 g), spontaneous preterm at <37 weeks’ gestation, and total preterm deliveries (at <37, <35, <32 weeks’ gestation).

Study design: Low and high cutoffs for vaginal fluid pH, sialidase, and prolidase activities were examined in a nested case-control study of 579 Danish women (from a study population of 2846 women) with samples collected at mean 17 weeks’ gestation. One hundred sixteen LBW (17 VLBW), 117 preterm deliveries (85 spontaneous), and 418 normal term deliveries were analyzed.

Results: Vaginal pH ≥4.7 or pH ≥5 by itself was not associated with LBW or prematurity. Conversely, combination of pH ≥5 and high sialidase activity demonstrated OR 17 (CI 1.8-150) for LBW; OR 31 (CI 1.8-516) for VLBW; along with OR 18 (CI 1.6-204) for preterm at <35 weeks’ gestation; and OR 31 (CI 1.9-542) for preterm at <32 weeks’ gestation. The combination of pH ≥5 and high prolidase activity demonstrated OR 13 (CI 1.3-122) for LBW; OR 33 (CI 2.0-553) for VLBW, as well as OR 9.2 (CI 0.6-150) for preterm at <35 weeks’ gestation; and OR 35 (CI 2.0-586) for preterm at <32 weeks’ gestation. In this population, no woman having high sialidase and high prolidase activity had a term birth, or a baby weighting ≥2500 g at birth.
Low birth weight (LBW, <2500 g), including very LBW (VLBW, <1500 g), resulting primarily from short gestation and preterm delivery remains the most common determinant for subsequent neonatal mortality, morbidity, and long-term neurodevelopmental disorders, including cerebral palsy, in industrialized countries. Many cases of LBW have no readily recognizable etiologically linked risk factors identifiable in asymptomatic mothers before mid gestation. Among the multiple pathophysiologic pathways to LBW and prematurity, several investigations have shown that abnormal vaginal flora, including bacterial vaginosis (BV), is associated with preterm delivery (PTD), LBW, early and late miscarriage, and maternal complications. However, only limited proportions of women with BV actually have adverse pregnancy outcomes. In one study, only 6.3% had a LBW preterm infant, and 3.4% had a LBW full-term infant. Thus, more specific predictive markers for LBW and preterm delivery are needed among women with BV and other cases of LBW/PTD-associated altered vaginal flora.

BV is clinically characterized by a vaginal pH ≥4.5, amine odor, adherent white discharge, clue cells (Amsel's criteria), and decrease in lactobacilli. Thorsen et al described a microbial pathologic “core” for BV, resulting from synergistic relations between Gardnerella vaginalis and anaerobic bacteria, and this observation has been supported by further studies. BV-associated anaerobic microorganisms produce hydrolytic enzyme activities detectable in vaginal fluid, including sialidase and prolidase. Sialidase (neuraminidase) and prolidase (proline aminopeptidase) activities in pregnant women with BV have been previously correlated with increased risks of adverse pregnancy outcomes.

Some, but not all, US studies demonstrated that an elevated vaginal pH (≥4.5 or ≥5.0) by itself, or combined with an elevated Nugent Gram stain score, or with elevated neutrophils was associated with preterm delivery and LBW. In a previous nested case-control study performed in the same European population, we found that sialidase or prolidase activity as single vaginal markers were associated with subsequent LBW, but not with spontaneous preterm delivery at <37 weeks’ gestation.

The intent of the present analysis was to determine whether combinations of readily measured objective parameters of vaginal fluid, ie, pH, sialidase, and prolidase activities, can detect and quantify increased risks for LBW or VLBW, spontaneous preterm at <37 weeks, and total preterm deliveries at <37 or <35 or <32 weeks’ gestation among European women evaluated in the second trimester of gestation.

Material and methods

Study population

We selected 579 women from a cohort totaling 2846 singleton pregnant women at their first prenatal visit (before the 24th week of gestation) enrolled prospectively from November 1992 to February 1994 at Odense Hospital (Denmark). The purpose of the original study was to evaluate associations between maternal infections and adverse pregnancy outcomes. Inclusion and exclusion criteria were previously described (appropriate informed consent was obtained, and clinical research human experimentation of the authors’ institutions). LBW and VLBW were defined as birth weight <2500 g and <1500 g, respectively. PTD was defined as spontaneous delivery (non-medically indicated), including spontaneous rupture of membranes or labor before 37 weeks’ gestation. In addition, total deliveries (spontaneous and induced) that occurred at <37 weeks’ or <35 weeks’, or <32 weeks’ gestation were examined.

Nested case-control study sample

A total of 117 prematurity (85 were spontaneous, ie, PTD) cases and 116 LBW cases were obtained. Among LBW cases, 72 were from preterm deliveries at <37 weeks’ gestation, and 44 were from term deliveries. A randomly selected group of 418 women delivering normal birth weight babies at term (NTD) was used as control group. The rationale for a number of 418 controls was to get at least 3 controls per case in any circumstance and included was also an oversampling of 20%. None of the deliveries among NTD were medically induced. Among participants in the nested case-control study, mean age at predicted date of delivery was 29 years, (18-42 years); mean gestational age at enrollment was 16w + 5d (16 full weeks’ gestation plus 5 days) (range, 7w + 4d to 24w + 0d); mean gestational age of 16l cases and 418 controls were 17w + 2d and 16w + 1d, respectively. Among the 579 women enrolled, 43 had preeclampsia, 11 had pregnancy-induced hypertension, and 5 had essential hypertension.
Table I  Characteristics of women included in the nested-case control study

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>NBW</th>
<th>LBW</th>
<th>VLBW</th>
<th>NTD</th>
<th>PTD</th>
<th>&lt; 37 wk</th>
<th>&lt; 35 wk</th>
<th>&lt; 32 wk</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of women</td>
<td>463</td>
<td>116</td>
<td>17</td>
<td>418</td>
<td>85</td>
<td>117</td>
<td>49</td>
<td>15</td>
</tr>
<tr>
<td>Age (y)</td>
<td>28.8</td>
<td>(18.4-41.9)*</td>
<td>28.1</td>
<td>(20.9-40.9)*</td>
<td>29.9</td>
<td>(23.8-38.4)*</td>
<td>28.9</td>
<td>(18.5-40.9)*</td>
</tr>
<tr>
<td>Gestational weeks at enrollment</td>
<td>16.3</td>
<td>(7.6-23.4)*</td>
<td>18.0</td>
<td>(9.6-24.3)*</td>
<td>18.6</td>
<td>(9.6-20.1)*</td>
<td>16.1</td>
<td>(7.6-23.4)*</td>
</tr>
<tr>
<td>Gestational weeks at delivery</td>
<td>40.1</td>
<td>(32-43.3)*</td>
<td>35.8</td>
<td>(26.3-42.3)*</td>
<td>30.1</td>
<td>(26.3-35.3)*</td>
<td>40.3</td>
<td>(37.0-43.3)*</td>
</tr>
<tr>
<td>Infant weight at birth (g)</td>
<td>3490</td>
<td>(516)</td>
<td>2011</td>
<td>(449)</td>
<td>1136</td>
<td>(252)</td>
<td>3554</td>
<td>(494)</td>
</tr>
<tr>
<td>BV positive</td>
<td>14.5</td>
<td>10.7</td>
<td>17.6</td>
<td>14.6</td>
<td>12.9</td>
<td>13.7</td>
<td>16.3</td>
<td>13.3</td>
</tr>
<tr>
<td>Sialidase &gt; + 1 positive</td>
<td>14.9</td>
<td>28.7</td>
<td>25.0</td>
<td>15.3</td>
<td>20.2</td>
<td>19.0</td>
<td>27.1</td>
<td>21.4</td>
</tr>
<tr>
<td>Sialidase &gt; + 2 positive</td>
<td>0.86</td>
<td>3.48</td>
<td>6.3</td>
<td>0.96</td>
<td>2.38</td>
<td>1.72</td>
<td>4.2</td>
<td>7.1</td>
</tr>
<tr>
<td>Prolidase &gt; + 1 positive</td>
<td>33.2</td>
<td>42.5</td>
<td>33.3</td>
<td>33.9</td>
<td>35.4</td>
<td>33.3</td>
<td>45.7</td>
<td>38.5</td>
</tr>
<tr>
<td>Prolidase &gt; + 2 positive</td>
<td>1.09</td>
<td>4.43</td>
<td>6.7</td>
<td>0.96</td>
<td>3.66</td>
<td>3.51</td>
<td>4.3</td>
<td>7.7</td>
</tr>
<tr>
<td>Vaginal pH &gt; 5</td>
<td>12.3</td>
<td>19.0</td>
<td>11.8</td>
<td>12.0</td>
<td>14.1</td>
<td>15.4</td>
<td>18.4</td>
<td>13.3</td>
</tr>
<tr>
<td>Previous PTD</td>
<td>4.97</td>
<td>15.5</td>
<td>11.8</td>
<td>3.35</td>
<td>15.3</td>
<td>17.1</td>
<td>10.2</td>
<td>6.7</td>
</tr>
<tr>
<td>Previous spontaneous abortion</td>
<td>19.7</td>
<td>22.4</td>
<td>5.9</td>
<td>19.6</td>
<td>23.5</td>
<td>23.1</td>
<td>22.4</td>
<td>13.3</td>
</tr>
<tr>
<td>Previous induced abortion</td>
<td>18.8</td>
<td>15.5</td>
<td>17.6</td>
<td>18.7</td>
<td>22.4</td>
<td>20.5</td>
<td>24.5</td>
<td>13.3</td>
</tr>
<tr>
<td>LBW in last pregnancy</td>
<td>3.02</td>
<td>13.8</td>
<td>11.8</td>
<td>2.63</td>
<td>7.06</td>
<td>9.40</td>
<td>6.1</td>
<td>6.7</td>
</tr>
<tr>
<td>Serious bleeding in pregnancy</td>
<td>1.51</td>
<td>2.59</td>
<td>0.0</td>
<td>1.44</td>
<td>2.35</td>
<td>2.56</td>
<td>2.0</td>
<td>0.0</td>
</tr>
<tr>
<td>≥ 6 h work walking daily</td>
<td>42.8</td>
<td>50.0</td>
<td>47.1</td>
<td>42.1</td>
<td>55.3</td>
<td>52.1</td>
<td>55.1</td>
<td>46.7</td>
</tr>
<tr>
<td>≥ 6 h work standing daily</td>
<td>46.9</td>
<td>46.6</td>
<td>41.2</td>
<td>45.7</td>
<td>56.5</td>
<td>52.1</td>
<td>49.0</td>
<td>40.0</td>
</tr>
<tr>
<td>≥ 4 alcoholic drinks weekly</td>
<td>1.95</td>
<td>4.35</td>
<td>5.9</td>
<td>1.92</td>
<td>4.71</td>
<td>4.27</td>
<td>4.1</td>
<td>0.0</td>
</tr>
<tr>
<td>≥ 10 cigarettes daily at enrollment</td>
<td>6.97</td>
<td>13.0</td>
<td>0.0</td>
<td>7.23</td>
<td>7.14</td>
<td>9.57</td>
<td>8.3</td>
<td>0.0</td>
</tr>
<tr>
<td>Body mass index</td>
<td>21.8</td>
<td>(15.8-40.9)*</td>
<td>21.3</td>
<td>(17.2-39.7)*</td>
<td>22.6</td>
<td>(17.8-28.1)*</td>
<td>21.8</td>
<td>(15.8-40.9)*</td>
</tr>
<tr>
<td>* Median (range).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 Mean (SD).</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

The following subsets of pregnant women were examined: NBW (≥ 2500 g at birth); LBW (< 2500 g at birth); VLBW (< 1500 g at birth); NTD (control women who delivered an infant weighing ≥ 2500 g at birth at ≥ 37 weeks’ gestation); PTD (spontaneous delivery at < 37 weeks’ gestation); < 37 weeks (total deliveries at < 37 weeks’ gestation); < 35 weeks (total deliveries at < 35 weeks’ gestation); < 32 weeks (total deliveries at < 32 weeks’ gestation).

Vaginal sample collection

Samples were collected by a physician at enrollment visit. BV was clinically diagnosed by Amsel’s criteria. Samples of vaginal fluid were collected as previously published.

Sialidase activity

Sialidase activity was determined by incubation of 50 μL of the vaginal sample with 50 μL of the substrate at pH 5.0. Specific activity was expressed as nanomoles of methoxyphenol produced by comparison with
a standard curve of pure methoxyphenol. Sialidase levels were defined as: no activity, $0.19$ nmol; $+1$ cutoff, $0.19$ nmol; $+2$ cutoff, $5.00$ nmol of methoxyphenol.\textsuperscript{13,16}

Statistical analysis

Odds ratios (ORs) and 95% CIs were calculated using logistic regression to estimate the relative risk for each adverse pregnancy outcome. Various factors were examined as potential confounders, and those that qualified were included in the model to adjust the ORs. Stata (StataCorp, College Station, Tex) and SPSS (Statistical Package for Social Sciences; Chicago, Ill) were used for data analyses.

Results

Of the 2846 eligible women, 579 were examined in this nested case-control study of LBW, VLBW, and prematurity. Characteristics of study subsets of women are described in Table I.

Figure 1 illustrates findings associated with vaginal pH. Study women at approximately 17 weeks’ gestation with vaginal pH $\geq 4.7$ or $\geq 5.0$ did not demonstrate a significantly increased incidence of subsequent LBW, VLBW, spontaneous, or total preterm deliveries.

Prolidase activity

Prolidase activity was determined as described.\textsuperscript{7} Absorbance (mOD) was read at 405 nm. Prolidase levels were defined as: no activity, $<22$ mOD; $+1$ cutoff, $\geq 22$ mOD; $+2$ cutoff, $\geq 2000$ mOD.\textsuperscript{13,16}

Statistical analysis

Odds ratios (ORs) and 95% CIs were calculated using logistic regression to estimate the relative risk for each adverse pregnancy outcome. Various factors were examined as potential confounders, and those that qualified were included in the model to adjust the ORs. Stata (StataCorp, College Station, Tex) and SPSS (Statistical Package for Social Sciences; Chicago, Ill) were used for data analyses.

Results

Of the 2846 eligible women, 579 were examined in this nested case-control study of LBW, VLBW, and prematurity. Characteristics of study subsets of women are described in Table I.

Figure 1 illustrates findings associated with vaginal pH. Study women at approximately 17 weeks’ gestation with vaginal pH $\geq 4.7$ or $\geq 5.0$ did not demonstrate a significantly increased incidence of subsequent LBW, VLBW, spontaneous, or total preterm deliveries.

Figure 2 illustrates findings associated with progressive levels of sialidase activity alone or in combination

Figure 3 illustrates findings associated with progressive prolidase activity levels $+1$ and $+2$ combined with vaginal pH $\geq 5.0$ for prediction of LBW ($<2500$ g), and VLBW ($<1500$ g) vs newborn infant weighting $\geq 2500$ g at birth (NBW). ORs for spontaneous delivery at $<37$ weeks’ gestation (PTD), and all preterm deliveries at $<37$, $<35$, and $<32$ weeks’ gestation were evaluated vs NTD (delivery at $\geq 37$ weeks of gestation of an infant with $\geq 2500$ g weight at birth). Prolidase and vaginal pH were measured on a total of 578 women: 463 women who had NBW, 115 LBW, 16 VLBW, 418 NTD, 84 PTD, 114 women who had a delivery at $<37$ weeks’, 48 weeks who had a delivery at $<35$ weeks’, and 13 women who had a delivery at $<32$ weeks’ gestation.
with pH ≥5.0. A positive +1 sialidase activity was associated with significantly increased (OR 2.3) risk of LBW, irrespective of pH. Sialidase values of +2 demonstrated crude OR 4.1 (CI 1.0-17) for LBW. The crude OR for LBW increased 4-fold with a concomitant vaginal pH ≥5, crude OR 17 (CI 1.8-150). An even larger crude OR 31 (CI 1.8-516) was observed for VLBW.

Results for prematurity differed somewhat from LBW: Figure 2 shows that an elevated sialidase and vaginal pH ≥5.0 was not significantly associated with spontaneous PTD, as well as total preterm deliveries at <37 weeks’ gestation (vs NTD). However, a positive +1 sialidase activity demonstrated OR 2.1 (CI 1.0-4.1) for preterm at <35 weeks’ gestation. Dramatically, the combination of pH ≥5 with high sialidase (+2) had large statistically significant ORs for early preterm delivery: OR 18 (CI 1.6-204) for preterm delivery at <35 weeks’, and OR 32 (CI 1.9-542) for preterm delivery at <32 weeks’ gestation.

Figure 3 illustrates results for prolidase activities. A positive prolidase activity (+1) did not demonstrate a statistically significant OR for LBW, whether or not combined with pH ≥5. However, high levels of prolidase activity (+2) demonstrated statistically significant associations (OR 4.2, CI 1.2-15) for LBW. The OR was dramatically increased 3-fold by combination with pH ≥5, OR 13 (CI 1.3-122). Consistently large ORs for VLBW were observed in women with high prolidase (+2) and pH ≥5 (OR 33, CI 2.0-553, Figure 3). High prolidase (+2) in combination with pH ≥5 demonstrated OR 35 (CI 2.0-586, Figure 3) for prematurity at <32 weeks’ gestation.

A number of potential confounders were examined. Those included previous PTD, previous spontaneous abortion, previous induced abortion, serious bleeding in

---

### Table II

<table>
<thead>
<tr>
<th>Combination of 2 enzyme markers and pH</th>
<th>&gt;2500 g</th>
<th>&gt;2500 g</th>
<th>LBW</th>
<th>LBW</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>VLBW</th>
<th>VLBW</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialidase &gt; +1 and prolidase &gt; +1</td>
<td>52</td>
<td>11</td>
<td>22</td>
<td>19</td>
<td>1.8†</td>
<td>1.0-3.2</td>
<td>3</td>
<td>20</td>
<td>2.0</td>
<td>0.5-7.2</td>
</tr>
<tr>
<td>Sialidase &gt; +1 and prolidase &gt; +2</td>
<td>1</td>
<td>0.2</td>
<td>3</td>
<td>2.7</td>
<td>10‡</td>
<td>1.0-100</td>
<td>1</td>
<td>6.7</td>
<td>3.3</td>
<td>2.0-553</td>
</tr>
<tr>
<td>Sialidase &gt; +2 and prolidase &gt; +1</td>
<td>4</td>
<td>0.9</td>
<td>4</td>
<td>3.5</td>
<td>3.5†</td>
<td>0.8-15</td>
<td>1</td>
<td>6.7</td>
<td>8.2</td>
<td>0.9-78</td>
</tr>
<tr>
<td>pH ≥5.0, sialidase &gt; +1 and prolidase &gt; +1</td>
<td>0</td>
<td>2</td>
<td>1.8</td>
<td>*</td>
<td>1</td>
<td>6.7</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH ≥5.0, sialidase &gt; +2 and prolidase &gt; +1</td>
<td>30</td>
<td>6.5</td>
<td>15</td>
<td>13</td>
<td>2.0†</td>
<td>1.0-4.0</td>
<td>1</td>
<td>6.7</td>
<td>1.0</td>
<td>0.1-8.1</td>
</tr>
<tr>
<td>pH ≥5.0, sialidase &gt; +1 and prolidase &gt; +2</td>
<td>1</td>
<td>0.2</td>
<td>3</td>
<td>2.7</td>
<td>10‡</td>
<td>1.0-100</td>
<td>1</td>
<td>6.7</td>
<td>3.3</td>
<td>2.0-553</td>
</tr>
<tr>
<td>pH ≥5.0, sialidase &gt; +2 and prolidase &gt; +1</td>
<td>1</td>
<td>0.2</td>
<td>4</td>
<td>3.5</td>
<td>13‡</td>
<td>1.4-121</td>
<td>1</td>
<td>6.7</td>
<td>3.3</td>
<td>2.0-553</td>
</tr>
<tr>
<td>pH ≥5.0, sialidase &gt; +2 and prolidase &gt; +2</td>
<td>0</td>
<td>2</td>
<td>1.8</td>
<td>*</td>
<td>1</td>
<td>6.7</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sialidase and prolidase activity was measured in a total of 461 women who had no LBW, 113 women who had LBW, and 15 women who had VLBW.

* No woman with sialidase > +2 and prolidase > +2 had an infant having ≥2500 g weight at birth; thus, ORs could not be calculated.

† In these cases, there was confounding by smoking for which the ORs was consequently adjusted.

---

### Table III

<table>
<thead>
<tr>
<th>Combination of 2 enzyme markers and pH</th>
<th>NTD</th>
<th>NTD</th>
<th>PTD</th>
<th>PTD</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>&lt;37 wk</th>
<th>&lt;37 wk</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialidase &gt; +1 and prolidase &gt; +1</td>
<td>48</td>
<td>12</td>
<td>11</td>
<td>13</td>
<td>1.2</td>
<td>0.6-2.4</td>
<td>14</td>
<td>12</td>
<td>1.1</td>
<td>0.6-2.0</td>
</tr>
<tr>
<td>Sialidase &gt; +1 and prolidase &gt; +2</td>
<td>1</td>
<td>0.2</td>
<td>1</td>
<td>2.4</td>
<td>5.1</td>
<td>0.3-83</td>
<td>2</td>
<td>1.8</td>
<td>7.4</td>
<td>0.7-82</td>
</tr>
<tr>
<td>Sialidase &gt; +2 and prolidase &gt; +1</td>
<td>4</td>
<td>1.0</td>
<td>2</td>
<td>2.4</td>
<td>2.6</td>
<td>0.5-14</td>
<td>2</td>
<td>1.8</td>
<td>18</td>
<td>0.3-10</td>
</tr>
<tr>
<td>Sialidase &gt; +2 and prolidase &gt; +2</td>
<td>0</td>
<td>1</td>
<td>1.2</td>
<td>*</td>
<td>1</td>
<td>0.9</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>PH ≥5.0, sialidase &gt; +1 and prolidase &gt; +1</td>
<td>28</td>
<td>6.7</td>
<td>5</td>
<td>6.1</td>
<td>0.9</td>
<td>0.3-2.4</td>
<td>8</td>
<td>7.0</td>
<td>1.0</td>
<td>0.5-2.4</td>
</tr>
<tr>
<td>PH ≥5.0, sialidase &gt; +1 and prolidase &gt; +2</td>
<td>1</td>
<td>0.2</td>
<td>1</td>
<td>2.4</td>
<td>10</td>
<td>0.9-116</td>
<td>2</td>
<td>1.8</td>
<td>7.4</td>
<td>0.7-82</td>
</tr>
<tr>
<td>PH ≥5.0, sialidase &gt; +2 and prolidase &gt; +1</td>
<td>1</td>
<td>0.2</td>
<td>2</td>
<td>2.4</td>
<td>10</td>
<td>0.9-116</td>
<td>2</td>
<td>1.8</td>
<td>7.4</td>
<td>0.7-82</td>
</tr>
<tr>
<td>PH ≥5.0, sialidase &gt; +2 and prolidase &gt; +2</td>
<td>0</td>
<td>1</td>
<td>1.2</td>
<td>*</td>
<td>1</td>
<td>0.9</td>
<td>*</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Sialidase and prolidase activity was measured on a total of 416 women who had NTD (delivery of an infant with ≥2500 g weight at birth at >37 weeks’ gestation), 82 women who had a PTD, and 114 women who had a delivery at <37 weeks’ gestation.

* No woman with sialidase > +2 and prolidase > +2 had a NTD.
pregnancy, 6 or more work hours walking daily, 6 or more work hours standing daily, more than 4 alcoholic drinks per week, smoking more than 10 cigarettes a day at enrollment, LBW in the last delivery, BMI, and age. In most cases, no association with both outcome and marker variables were found. Only for LBW as outcome was there confounding by smoking. In those cases, the estimates were controlled for that.

Table II details combinations of positive sialidase and prolidase activities that demonstrated statistically significant associations with LBW. In particular, the combination of high prolidase (+2) with a positive sialidase (+1) had crude OR 13 (CI 1.3-122) for LBW (adjusted for smoking the OR was 10 [CI 1.0-100]). This combination demonstrated an OR 33 (CI 2.0-553) for VLBW. No woman having the combination of high prolidase (+2) with high sialidase (+2) had an infant weighting $\geq 2500$ g at birth (thus, ORs for this profile could not be calculated).

Table III shows that all combinations of sialidase and prolidase activities were not statistically significant for PTD or total preterm deliveries at $< 37$ weeks' gestation (versus NTD). No woman having the combination of the highest values of both sialidase (+2) and prolidase (+2) activity had a NTD.

Table IV shows that the combination of high prolidase (+2) with a positive sialidase (+1) demonstrated an OR 35 (CI 2.0-586) for prematurity at $< 32$ weeks' gestation. The determination of a pH $\geq 4.7$ in addition to all combinations of positive and/or elevated sialidase and prolidase activities as reported in Tables II, III, and IV did not substantially increase the ORs for study outcomes (data not shown). Determination of a vaginal pH $\geq 5$ in women with sialidase +2 and prolidase +1 increased 4-fold the risk for LBW (OR 17, CI 1.9-153, Table II); VLBW (OR 33, CI 2.0-553, Table II); delivery at $< 35$ weeks’ (OR 19, CI 1.7-212, Table IV), and at $< 32$ weeks’ gestation (OR 35, CI 2.0-586, Table IV).

In this study, 91 women were BV positive. No statistically significant findings were obtained for BV association with LBW (OR 1.5, CI 0.9-2.6), nor with any other study adverse pregnancy outcomes.

**Comment**

Much attention has recently focused on use of vaginal pH and presence of BV as a tool to identify women at risk for LBW/PTD caused by reproductive tract altered microflora.\(^2,4,14,15\) Abnormal vaginal flora disorders appear heterogeneous with respect to clinical presentation and response to therapy. It is increasingly recognized that many instances of altered vaginal microflora do not fulfill criteria for inclusion in the BV group. On the other hand, BV-positive women appear to be highly heterogeneous when evaluated by presence of vaginal biomarkers. Specific BV subgroups have been recently described based on host immune responses and levels of microbial enzymes.\(^12,13,16\) Thus, it appears that in some BV-positive women the alteration of vaginal ecology is a harmless condition.

Vaginal biomarkers have been suggested to play an important role in risk stratification in women with abnormal microflora. We applied a multimarker strategy that incorporates pH and microbial enzyme activities in a nested case-control study in a low-risk population of Danish pregnant women. We observed that evaluation of vaginal pH in combination with other readily measurable markers in vaginal fluid (sialidase and prolidase activities) is associated with increased risks of LBW, VLBW, and total early prematurity at $< 35$ or $< 32$ weeks’ gestation.

Previous studies on this cohort demonstrated that the clinical diagnosis of BV was not significantly associated with increased risk for LBW nor PTD.\(^5,13\) These previous studies focus attention on how to best identify women at risk of subsequent adverse outcome by

<table>
<thead>
<tr>
<th>Combination of 2 vaginal markers and pH</th>
<th>$&lt; 35$ wks n</th>
<th>$&lt; 35$ wks %</th>
<th>Odds ratio</th>
<th>95% CI</th>
<th>$&lt; 32$ wks n</th>
<th>$&lt; 32$ wks %</th>
<th>Odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sialidase $&gt; +1$ and prolidase $&gt; +1$</td>
<td>8</td>
<td>17</td>
<td>1.6</td>
<td>0.7-3.7</td>
<td>2</td>
<td>15</td>
<td>1.4</td>
<td>0.3-6.5</td>
</tr>
<tr>
<td>Sialidase $&gt; +1$ and prolidase $&gt; +2$</td>
<td>1</td>
<td>2.2</td>
<td>9.2</td>
<td>0.6-150</td>
<td>1</td>
<td>7.7</td>
<td>35</td>
<td>2.0-586</td>
</tr>
<tr>
<td>Sialidase $&gt; +2$ and prolidase $&gt; +1$</td>
<td>2</td>
<td>4.3</td>
<td>4.7</td>
<td>0.8-26</td>
<td>1</td>
<td>7.7</td>
<td>8.6</td>
<td>0.9-83</td>
</tr>
<tr>
<td>Sialidase $&gt; +2$ and prolidase $&gt; +2$</td>
<td>1</td>
<td>2.2</td>
<td>*</td>
<td></td>
<td>1</td>
<td>7.7</td>
<td>*</td>
<td></td>
</tr>
<tr>
<td>pH $\geq 5.0$, sialidase $&gt; +1$ and prolidase $&gt; +1$</td>
<td>5</td>
<td>11</td>
<td>1.7</td>
<td>0.6-4.6</td>
<td>1</td>
<td>7.7</td>
<td>1.2</td>
<td>0.2-9.2</td>
</tr>
<tr>
<td>pH $\geq 5.0$, sialidase $&gt; +1$ and prolidase $&gt; +2$</td>
<td>1</td>
<td>2.2</td>
<td>9.2</td>
<td>0.6-150</td>
<td>1</td>
<td>7.7</td>
<td>35</td>
<td>2.0-586</td>
</tr>
<tr>
<td>pH $\geq 5.0$, sialidase $&gt; +2$ and prolidase $&gt; +1$</td>
<td>2</td>
<td>4.3</td>
<td>19</td>
<td>1.7-212</td>
<td>1</td>
<td>7.7</td>
<td>35</td>
<td>2.0-586</td>
</tr>
<tr>
<td>pH $\geq 5.0$, sialidase $&gt; +2$ and prolidase $&gt; +2$</td>
<td>1</td>
<td>2.2</td>
<td>*</td>
<td></td>
<td>1</td>
<td>7.7</td>
<td>*</td>
<td></td>
</tr>
</tbody>
</table>

Sialidase and prolidase activities were measured on a total of 416 women who had NTD, 46 women who had a delivery at $< 35$ weeks’, and 13 women who had a delivery at $< 32$ weeks’ gestation.

* No woman with sialidase $> +2$ and prolidase $> +2$ had a NTD.
evaluation of microbial flora and/or a single biochemical parameter. Our present results highlight that use of combined objective and easy to use quantifiable biochemical markers in the vaginal fluid may identify women who are at significant risk of LBW, VLBW, and prematurity.

A recent study performed on US women reported an increased incidence of preterm birth, LBW, and VLBW in women with vaginal pH ≥ 4.5 and Nugent Gram score of 9 to 10 early in pregnancy. Another study showed that among US pregnant women with vaginal pH ≥ 5 had OR 1.3 and OR 2.0 for preterm at <37 weeks and <32 weeks’ gestation, respectively. We were unable to confirm these findings regarding vaginal pH ≥ 5, although we found similar trends especially for LBW (OR 1.7, not statistically significant), and total deliveries at <35 weeks’ gestation (OR 1.7, not statistically significant). These differing results may derive in part from the size and study methods of our and the US trial. Discrepancies could possibly be due to population determined differences in vaginal pH values among European vs US pregnant women.

Sialidases are enzymes involved in the pathogenesis of several diseases. Sialidases cleave sialic acid off from various glycoproteins such as IgA, innate immune factors, mucins and cellular receptors, thus altering both innate and acquired local immunity mechanisms. Some bacteria typically present in the BV milieu, particularly Bacteroides and Prevotella produce sialidases. It is of note that sialidase in vaginal fluid has a maximal hydrolytic activity at pH 5, suggesting possible synergism between sialidase and elevated pH.

Previous studies of vaginal sialidase activity on US pregnant women have not yielded consistent results. In a previous study performed on the same Danish population, sialidase activity, per se, was a better predictive marker than BV and was associated with LBW. In the present study, we found that sialidase (+1, +2) activity itself may be a better marker for LBW than an elevated vaginal pH ≥ 4.7 or ≥ 5. However, by combining biochemical parameters we found that the determination of a vaginal pH ≥ 5 increases 4-fold the risk for LBW of women at the highest level of sialidase activity (from OR 4.1 to 17). Confirmingly, the combination of high sialidase and pH ≥ 5 demonstrated a statistically significant OR 31 for VLBW. We observed that high sialidase and pH ≥ 5 had significant consistently elevated ORs for total early preterm deliveries: OR 18 for delivery at <35 weeks’, and OR 32 for delivery at <32 weeks’ gestation, and was associated with a trend for elevated risk for spontaneous PTD (OR 10).

Prolidases are proteolytic enzymes that facilitate matrix remodeling and cellular infiltration, and can modulate cytokines and other immune mediators. Increased prolidase hydrolysis in the vagina and cervix could result in the breakdown of the protective mucosal barriers.

G. vaginalis is a strong in vitro producer of prolidase activity. Multiple other BV-associated bacteria including: Mobiluncus spp., Peptostreptococcus spp., Streptococcus intermedius, Bifidobacterium spp. can produce prolidases.

In a previous study, presence of prolidase activity constituted a single marker for LBW, and high prolidase values were more predictive for LBW than BV. In the present study we demonstrate that high prolidase activity is a marker for both LBW and prematurity when combined with an elevated vaginal pH. The concomitant finding of vaginal pH ≥ 5 and high prolidase activity was associated with elevated risk for LBW (OR 13), for VLBW (OR 33), and total deliveries at <32 weeks’ gestation (OR 35).

Our present findings support the notion of synergistic inter-relationships among virulence factors produced by bacteria present in altered vaginal microflora. Combinations of microbial enzyme activities were associated with higher risk of adverse outcome than a single biochemical marker positivity. Importantly, among the few subjects with the highest levels of both microbial enzyme activities, each (2/2) of the infants were born preterm and weighting less than 2500 g.

Potential strengths of the present study include: (1) study population is homogenous with universal access to health care, and relatively stable; (2) the biomarkers are easily and objectively measurable on both fresh and frozen samples; and (3) neither clinical evaluation of Amsel’s criteria nor interpretation of Gram slides is necessary. It is of note that diagnosis of BV by Amsel’s criteria in this population was not statistically associated with any of the study adverse outcomes. Differentiated misclassification among outcome variables was minimal, as information on outcomes was collected after exposure variables, and laboratory analyses were blinded to all other study variables.

Our findings are consistent with the hypothesis that only a subgroup of women with abnormal vaginal flora are really at risk of adverse pregnancy outcome, and identification of women colonized by bacteria producing sialidases and/or prolidases is a more selective tool than BV.

Weakness of our study include the limited number of pregnancy adverse outcomes obtained from the original cohort of 3596 women, which yielded wide ranges in the observed CI, and prevented further stratification of subsets of women. Other weaknesses include the homogeneity of the Danish study population.

We speculate that sialidase and prolidase microbial activities, perhaps increased by elevated pH, in vaginal fluid may synergistically contribute to lower and impair defenses and cause tissue damage. Studies by us and others demonstrate that high enzymatic activity...
levels in vaginal fluid suppress factors of the adaptive and innate vaginal immune response.7,16 Enzymatic perturbations of lower genital tract defenses may correlate with capability of microbes and/or virulence factors to penetrate into the upper genital tract, causing intrauterine infection and inflammation that can impair fetal growth as well as cause prematurity.1-5,15

In the present study we found a stronger association of combinations of biomarkers with total early preterm deliveries (at 35 or 32 weeks’ gestation) than with spontaneous or total preterm delivery at <37 weeks’ gestation. These findings support observations of other authors that the relationship of lower tract infection markers to preterm birth is strongest at the earliest gestational ages, and that infection is estimated to be more likely the pathogenesis of early preterm births (around 60%) than that preterm at <37 weeks’ gestation (around 20%).2-15

Our results suggest that combinations of easily measured vaginal biomarkers (pH, sialidase, and prolidase activities) are associated with clinically important risks of LBW and prematurity when measured in the second trimester.

Future controlled trials are needed to replicate our findings, and to evaluate strategies of early pregnancy screening and selection of patients to possibly abrogate hydrolytic enzyme activities and restore “healthy” vaginal flora. If effective, this strategy of early identification and treatment (antimicrobial, and/or probiotic, and/or antiproteolytic) could reduce burdens of disease associated with prematurity and LBW caused by abnormal reproductive tract microflora.

References


The relationship between an aortic isthmus blood flow velocity index and the postnatal neurodevelopmental status of fetuses with placental circulatory insufficiency

Jean-Claude Fouron, MD, a,* Julie Gosselin, PhD, b Marie-Josée Raboisson, MD, a Julie Lamoureux, MSc, a Claudine-Amiel Tison, MD, d Catherine Fouron, MA, b Linda Hudon, MD c

Fetal Cardiology Unit, Pediatric Cardiology Division, a Departments of Pediatrics, Rehabilitation, b and Obstetrics, c Ste-Justine Hospital, University of Montreal, Montreal, Quebec, Canada, Department of Pediatrics, Paris V School of Medicine, Paris, France d

Objective: The purpose of this study was to evaluate the association between an abnormal aortic isthmus blood flow index and postnatal neurodevelopmental outcome in fetuses with placental circulatory insufficiency.

Study design: Forty-eight children who were born between 1991 and 1999 were included in this study on the basis of abnormal umbilical artery Doppler velocimetry. Prenatal isthmus blood flow index was obtained by dividing the sum of the systolic and diastolic Doppler blood flow velocity integrals by the systolic blood flow integrals.

Neurodevelopmental outcome between 2 and 5 years was classified as optimal, when neurologic assessment and developmental quotient were within normal limits and as nonoptimal when abnormal neurologic findings and/or a nonoptimal developmental quotient was present. Neurodevelopmental outcome was analyzed in relation to isthmus flow index and pulsatility indices in the umbilical artery.

Results: The mean gestational age at delivery was 33.0 ± 2 weeks. Nonoptimal neurodevelopmental outcome was found in 60.4% of the children (29/48). An inverse correlation was found between the isthmus blood flow index and postnatal neurodevelopmental outcome. All 13 children with an isthmus blood flow index of <0.5 were in the nonoptimal group. All 19 children with an optimal outcome had an isthmus blood flow index of >0.5, but this was also the case for 16 other children with nonoptimal neurodevelopmental outcome. An isthmus blood flow index cut-off value of 0.70 was associated with the highest overall positive and negative predictive

Supported by a grant from the Quebec Heart Foundation.

* Reprint requests: Jean-Claude Fouron, MD, Fetal Cardiology Unit, Sainte-Justine Hospital, 3175, Côte Ste-Catherine, Montreal, Quebec H3T 1C5, Canada.

E-mail: fouron@sympatico.ca
Both experimental\textsuperscript{1,2} and clinical\textsuperscript{3-5} investigations have demonstrated that placental circulatory insufficiency causes fetal hypoxemia by reducing umbilical blood flow. In these circumstances, the fetus can still maintain adequate cerebral oxygenation, because of many adaptive mechanisms among them, blood flow redistribution towards essential fetal organs (such as the brain, heart, and adrenal glands).\textsuperscript{6} In severe cases, however, this defense system is overwhelmed, and decompensation occurs that results in metabolic acidemia and cerebral hypoxia.\textsuperscript{5-8} In clinical practice, growth-restricted fetuses with placental circulatory insufficiency are being delivered on the basis of signs that reflect neurocirculatory decompensation.\textsuperscript{9} Not surprisingly, the incidence of neurodevelopmental disorders among intrauterine growth restriction (IUGR) survivors has been found to be as high as 50%.\textsuperscript{10}

Ideally, to prevent postnatal sequellae of fetal cerebral hypoxia, delivery should be induced just before decompensation. Abnormal ductus venosus Doppler waveforms have been suggested as a useful parameter to identify such fetuses.\textsuperscript{11} However, these venous changes are also often late signs of fetal compromise and frequently are associated with metabolic acidemia, myocardial cell destruction,\textsuperscript{12} and higher likelihood of perinatal death.\textsuperscript{5,13} Acute experiments in the ovine fetus have shown that a stepwise increase in resistance to placental blood flow causes a fall in oxygen delivery to the brain when a predominant reverse diastolic flow is observed through the aortic isthmus.\textsuperscript{1} In the human fetus, predominant diastolic reverse blood flow through the aortic isthmus is associated with nonoptimal postnatal neurodevelopmental outcome\textsuperscript{14} and impairment of the venous Doppler waveforms.\textsuperscript{15} However, although a predominant diastolic reverse blood flow in the fetal aortic isthmus appears to be a highly specific sign of decompensation, it has a relatively poor sensitivity. Indeed, close to 50% of previous IUGR children with nonoptimal neurodevelopmental outcome did not have a predominant reverse ischemic blood flow in their prenatal life.\textsuperscript{14} To better correlate the blood flow pattern through the fetal aortic isthmus with impending decompensation and acidemia, we developed an index that takes into account the amount and the direction of the isthmus blood flow on a continuous scale.\textsuperscript{16} The objective of this study was to investigate the association between changes in this aortic isthmus blood flow index (IFI) and the postnatal neurodevelopmental outcome of fetuses with placental circulatory insufficiency.

**Material and methods**

The protocol of the investigation was approved by the ethics committee on human research of our institution, and informed consent was signed by all participants.

**Studied population**

This study was part of a larger study that attempted to document the usefulness of different pre- and perinatal parameters to predict neurodevelopmental outcome. To be eligible, all fetuses had to show an abnormal umbilical Doppler velocity waveform (pulsatility index at the fetal end of the cord $>95$th percentile),\textsuperscript{17} and delivery had to occur after 28 weeks of pregnancy to avoid the potential confounding effects of extreme prematurity. Exclusion criteria included residence outside the Montreal metropolitan area, families who did not speak French at home (to exclude bias in the developmental assessments that were carried out in French), chromosome abnormalities, congenital malformations, and evidence of sociofamilial problems (such as drugs addiction, alcoholism, mental illness, consanguinity, welfare beneficiary, and history of battered children). The Doppler findings at the level of the isthmus were never disclosed to the attending staff. The timing of delivery was decided on the basis of conventional criteria that were classified in 2 categories: fetal (absence of weight gain, abnormal biophysical profile score,\textsuperscript{18} reverse diastolic blood flow in the umbilical artery) or maternal (severe preeclampsia). The initial cohort included 124 subjects, among which 55 subjects were twins. For the purpose of the current study, however, only the singleton infants with an adequate Doppler tracing of the aortic isthmus blood flow, which was recorded no $>7$ days before delivery, and with a neurodevelopmental assessment that was completed between the age of 2 and 5 years were retained. IUGR was defined as birth weight below the third percentile for gestational age.\textsuperscript{19}

**Doppler investigation**

The technique of Doppler investigation of the fetal aortic isthmus has been described previously.\textsuperscript{14} Briefly,
from a horizontal 4-chamber view of the fetal heart, a 90-degree rotation of the transducer provides a sagittal view of the fetus on which the aortic arch can be observed. The isthmus is then identified between the origin of the left subclavian artery and the aortic end of the ductus arteriosus. Only recordings obtained during nonbreathing and a quiet fetal state were kept for further calculations. The proposed index was obtained by dividing the sum of the systolic (S) and diastolic (D) Doppler blood flow velocity integrals by the systolic blood flow integral (IFI = S + D/S).16

Positive and negative signs are assigned to antegrade and retrograde velocity values, respectively. Figure 1 shows a real-time ultrasound picture of the aortic arch and the location of the Doppler sample volume in the isthmus and shows the types of isthmic Doppler patterns that can be observed in fetuses with abnormal increase in placental vascular resistance. The actual IFI values that correspond to these 5 types are also shown.

**Neurodevelopmental assessment**

Neurodevelopmental outcome was established according to the results obtained from 2 assessments that were administered by independent examiners who were unaware of the Doppler results. The first assessment was a standardized neurologic evaluation that evaluated passive and active tone, deep tendon reflexes, primitive reflexes, head circumference measurement, and cranial suture status.20 Results of this assessment were classified

---

**Figure 1**  **Top.** Real-time echographic imaging of the aortic arch. The asterisk identifies the localization of the sample volume for Doppler interrogation in the isthmus. **Bottom.** Examples of isthmic Doppler blood flow velocity waveforms that were recorded in fetuses with various degrees of placental circulatory insufficiency. Above these tracings, 5 types (in roman numbers) with their corresponding IFI values (in Arabic numbers) relate to the increasing severity of the condition. **Type I.** Forward blood flow is observed in diastole; the IFI is >1 but lower than normal. **Type II.** No isthmic diastolic velocities are recorded. **Type III.** Reverse diastolic blood flow is present, but with predominant antegrade blood flow in systole. **Type IV.** Antegrade systolic are equal to the retrograde diastolic velocities. **Type V.** The reverse diastolic blood flow is dominant and net blood flow through the isthmus is retrograde. Values of IFI are then negative, <0.
in 2 categories: (1) optimal neurologic function, when either 1 isolated abnormal sign was found or none; (2) nonoptimal neurologic function, when severe or moderate neuromotor impairment was present that resulted in the diagnosis of cerebral palsy or milder signs that were compatible with independent walk by the corrected age of 2 years.

Concomitantly, the Griffiths Mental Developmental Scales were used to assess developmental performances. The general developmental quotient (DQ) was obtained by the averaging of scores from 6 different subscales. This DQ was initially defined on a continuous scale (100 ± 12.67) and subsequently dichotomized into 2 categories according to the work of Bowen et al and Ley et al: optimal when the score was ≥87; nonoptimal when the score was <87.

The neurodevelopmental outcome was then defined as a combination of the results of both assessments: optimal, when neurologic assessment and DQ were within normal limits; nonoptimal, when abnormal neurologic findings and/or nonoptimal DQ were present.

### Statistical analysis

Descriptive statistics were computed for all the variables that were considered in this study. The comparability of the 2 outcome groups (optimal and nonoptimal) was investigated through *t*-tests (numeric variables) and chi-squared tests (categoric variables) on various perinatal and sociodemographic variables. To compare the criterion that had predictive validity of the umbilical artery pulsatility index with that of the IFI, the eta-squared coefficient was computed. Eta coefficient is a directional correlation that measures the association between the numeric indices (independent) and the dichotomic outcome (dependent). When eta is squared, the result is a correlation ratio that can be interpreted as a percentage of explained variation. Then, the predictive validity of IFI was assessed through the estimation of sensitivity, specificity, predictive values, and likelihood ratios at 4 different cut-off points. All hypotheses testing was done at the .05 level of significance. Finally, a forced entry logistic regression analysis was carried out with the score of the IFI index as the independent predictor and neurodevelopmental outcome as a binary outcome variable. Odds ratios and 95% confidence intervals were estimated. Two models were tested: a simple model that included only the index; the other model adjusted for birth weight, gestational age, and maternal level of schooling. Predicted probabilities were computed from the former model to create an expectancy table. A third model, a backward stepwise (P < .05 to enter; P < .06 to remove any variable) logistic regression model, was tested to determine the relationship between umbilical artery velocimetric indices and the outcome.

### Results

Of the 69 singleton fetuses who were part of the initial cohort, 56 fetuses fulfilled the selection criteria. Eight families could not be located. The final study group

<table>
<thead>
<tr>
<th>Table I</th>
<th>Perinatal and sociodemographic characteristics of the subjects, according to neurodevelopmental outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Optimal (n = 19)</td>
</tr>
<tr>
<td>Interval between last Doppler and delivery (d)*</td>
<td>1.7 ± 1.6</td>
</tr>
<tr>
<td>Gestation (wk)*</td>
<td>32.7 ± 2.3</td>
</tr>
<tr>
<td>Delivery indications: Fetal (n)</td>
<td>13 (68.4%)</td>
</tr>
<tr>
<td>Birth weight (g)*</td>
<td>1484 ± 398</td>
</tr>
<tr>
<td>Days in neonatal intensive care unit*</td>
<td>1.3 ± 1.9</td>
</tr>
<tr>
<td>Male (n)</td>
<td>11 (57.9 %)</td>
</tr>
<tr>
<td>IUGR (n)</td>
<td>10 (52.6%)</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)</td>
<td>3 (15.8%)</td>
</tr>
<tr>
<td>Assisted ventilation (n)</td>
<td>4 (21.1%)</td>
</tr>
<tr>
<td>Maternal education (high school level) (n)</td>
<td>8 (42.1%)</td>
</tr>
<tr>
<td>Family income &lt; $30000 (n)</td>
<td>1 (5.2%)</td>
</tr>
<tr>
<td>Age at neurodevelopmental assessment (y)*</td>
<td>4.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.

<table>
<thead>
<tr>
<th>Table II</th>
<th>Comparison of Doppler velocimetric indices and their eta-squared coefficients in relation to neurodevelopmental outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indices</td>
<td>Sample size</td>
</tr>
<tr>
<td>Umbilical artery pulsatility index</td>
<td>48</td>
</tr>
<tr>
<td>IFI</td>
<td>48</td>
</tr>
</tbody>
</table>
included 48 children who were born between 1991 and 1999. The perinatal and sociodemographic characteristics of the subjects according to their neurodevelopmental outcome are presented in Table I. No significant difference was found between the 2 groups. No grade III or IV intraventricular hemorrhage has been observed on ultrasound or computed tomography scan during the neonatal period. Steroids were administered to only 1 subject, which prevented any between group comparisons.

The Doppler velocimetric indices are presented in Table II. A significant difference between the 2 outcome groups was observed for the IFI values, which were lower for children in the nonoptimal group. On the other hand, no significant difference was found for the umbilical artery pulsatility indices. Table II gives the eta-squared coefficients for velocimetric indices in relation to the neurodevelopmental outcome. As evident in this table, eta-squared coefficients are high.

The distribution of the fetuses according to their IFI and their neurodevelopmental outcome are presented in Figure 2. A nonoptimal development was found in 29 children, which represented 60% of the population. It is apparent from Figure 2 that all 13 fetuses with an IFI of <0.5 were in this nonoptimal group. On the other hand, all 19 cases with an optimal outcome had an IFI of >0.5, but this was also the case for 16 other children with nonoptimal development. Table III gives predictive validity indices for 4 cut-off values of the IFI. With respect to the IFI value below which a fetus was at a greater risk of presenting with abnormal neurodevelopment, a cut-off value of 0.70 was associated with the highest overall positive and negative predictive values. It was also associated with a sensitivity of 0.55 and a specificity of 0.89.

In the simple logistic regression model that used the IFI as the independent variable, an inverse correlation was found between the isthmic blood flow velocity index and the postnatal neurodevelopmental outcome. Clinically speaking, as the IFI diminishes, the probability of having a nonoptimal outcome increases. The estimate for the regression coefficient and its standard error was −3.12 ± 1.15 (P = .007). The 95% confidence interval for this coefficient indicates that, for each unit decrease in IFI, the probability of a nonoptimal outcome would be between 2.4 and 200 times greater. The average probabilities of nonoptimal outcome, as predicted by the logistic regression model for 5 subgroups of IFI, are as follows: IFI <0: 0.985 (95% CI, 0.96-1.00); from 0 to 0.49: 0.902 (95% CI, 0.86-0.95); from 0.5 to 0.69: 0.765 (95% CI, 0.73-0.80); from 0.7 to 0.99: 0.597 (95% CI, 0.53-0.66); and >1: 0.385 (95% CI, 0.35-0.42).

The backward stepwise model indicated that umbilical pulsatility index did not show any significant contribution in the explanation of the outcome.

**Comment**

In this study, a significant negative correlation was found between the isthmic blood flow velocity index and postnatal neurodevelopmental outcome, which added to the demonstration of predictive validity for the IFI. This observation can be explained by the unique position of the isthmus between the 2 fetal arterial systems disposed in parallel. In the presence of a placental circulatory insufficiency, the increase in placental vascular resistance, coupled with the hypoxemic cerebral vasodilation, cause a decrease in the forward diastolic blood flow through the isthmus; with further deterioration, the diastolic isthmic blood flow disappears and, in very severe cases, could become markedly retrograde. When reverse blood flow occurs in the aortic isthmus, blood coming from the
pulmonary artery and descending aorta is being diverted from its normal destination, mainly the placenta; the brain then is perfused partly by blood that is deprived of substrates, placental or maternal, that are essential for fetal development and, at the same time, by red cells very poorly saturated in oxygen. The greater the reverse ischemic blood flow, the lower the IFI and the higher should be the risk of prenatal cerebral damage. The results of the logistic regression model point in that conceptual direction.

According to this concept, all fetuses with a forward systolic and diastolic ischemic flow (IFI, >1) would be in a “safe zone,” protected from cerebral hypoxia. However, approximately 40% of the 23 fetuses who were in this “safe zone” in our sample had a neurodevelopmental impairment. This observation confirms that other factors besides the degree of prenatal hypoxia participate in the development of unfavorable postnatal outcome. Perinatal events and manipulations in the neonatal intensive care unit could be responsible for this finding. However, the review of the charts did not disclose any adverse major postnatal events (such as ventricular hemorrhage or periventricular leukomalacia). Furthermore, the demographic variables that are presented in Table I were comparable in both groups. The evidence of different neurodevelopmental outcomes in children who had presumably the same level of hypoxic stress in utero could also suggest individual differences in cerebral sensitivity. The mechanisms underlying brain injury that is associated with uteroplacental insufficiency are, for the most part, unknown. Proteomic and genomic profiling of cells and tissues only recently has begun to be explored in the mature subject after focal cerebral ischemia. Individual genetic polymorphism or differences in placental and cerebral gene expression in the presence of hypoxemia are speculative explanations that are worth investigating.

Neurodevelopmental status was chosen as the outcome measure in this study because contemporary improvement in postnatal care allows survival of most IUGR fetuses who are delivered at >29 weeks of pregnancy. Rate of survival alone is therefore an insufficient criterion for the assessment of prenatal management in this context. Furthermore, the search for a marker that would allow clinicians to identify IUGR fetuses who must be delivered before evidence of cerebral hypoxia must fulfill at least 2 major requirements: first, ensure the highest possible rate of detection of fetuses at risk; second, avoid any significant increase of unnecessary preterm deliveries. Our results reveal that the IFI values that come closer to these criteria are in the type III range, more precisely between 0.5 and 1. In this sample, the sensitivity of the cut-off at 0.7 is approximately 3.5 times that of a cut-off at 0 (0.17).

In conclusion, this preliminary report concerning an ongoing investigation demonstrates that traditional criteria that are used for timing delivery in cases of placental circulatory insufficiency are associated with an elevated risk of nonoptimal postnatal neurodevelopment. It also shows a good negative relationship between fetal ischemic blood flow velocity index and the incidence of postnatal neurodevelopmental outcome. A greater number of patients who come from >1 center need to be investigated to establish with confidence the IFI level at which delivery of an IUGR fetus would be indicated rationally. It will then become necessary to position this “cut-off level” in relation to other more readily available testing modalities and to set up randomized studies to verify that intervention at the “impending-decompensation” stage produces any benefit.

References


Maternal nonpregnant vascular function correlates with subsequent fetal growth

Marc E. A. Spaanderman, MD, PhD,a,* Christine Willekes, MD, PhD,
Arnold P. G. Hoeks, PhD,b Timo H. A. Ekhart, Robert Aardenburg, MD,
Dorette A. Courtar, MD, Hugo W. F. van Eijndhoven, MD,
Louis L. H. Peeters, MD, PhD

Departments of Obstetrics and Gynecologya and Biophysics, b University Hospital Maastricht and University Hospital Nijmegen, Nijmegen, The Netherlands

Received for publication May 9, 2004; revised August 5, 2004; accepted August 31, 2004

KEY WORDS
Preeclampsia
Pulsatility index
Pregnancy
Vascular compliance
Distensibility
Plasma volume
Fetal growth

Objective: Evidence is accumulating that fetal growth is influenced by preexisting maternal disorder(s) hampering endothelial function. We tested the hypothesis that in nonpregnant normotensive, formerly preeclamptic women, vascular function predicts the development of fetal growth restriction.

Methods: In 60 formerly preeclamptic women, we measured central hemodynamic and vascular and clotting function mid follicular phase during the menstrual cycle. Inclusion for final analysis required besides normotension, a subsequent singleton pregnancy, established within 1 year after the prepregnant evaluation and ongoing beyond 16 weeks' gestation. In the ongoing pregnancy we determined birth weight and birth weight percentile.

Results: Among 60 formerly preeclamptic women, 45 (75%) were normotensive. Thirty-one (69%) participants succeeded in establishing an ongoing pregnancy within 1 year and were included for final analysis. Of the 31 subsequent pregnancies, 8 (26%) were complicated by fetal growth restriction. Prepregnant left and right uterine artery pulsatility index (PI) correlated inversely with carotid artery compliance (r = 0.57, P = .005, r = 0.62, P = .002) and venous compliance (r = 0.49, P = .02 and r = 0.45, P = .04, respectively). The latter, in turn, correlates with plasma volume (r = 0.63, P = .001) and total peripheral vascular resistance index (r = −0.45, P = .02). Finally, prepregnant left and right uterine artery PI correlated inversely with subsequent achieved fetal growth (r = −0.68, P < .0001 and r = −0.58, P = .001, respectively).

Conclusion: In nonpregnant normotensive, formerly preeclamptic women, an elevated uterine artery PI predisposes to subsequent restriction in fetal growth.

* Reprint requests: Marc Spaanderman, MD, PhD, University Medical Center Nijmegen St Radboud, Department Obstetrics and Gynecology, PO Box 9101, Nijegen, Gelderland 6500 HB, The Netherlands.
E-mail: m.spaanderman@obgyn.umcn.nl

0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.
doi:10.1016/j.ajog.2004.08.035
The current concept about the gestational circulatory adaptation is that systemic arterial vasodilation represents the trigger for a cascade of compensatory changes such as a rise in cardiac output, vascular compliance, and activation of the renin-angiotensin-aldosterone system.\textsuperscript{1-7} Pregancies complicated by vascular complications such as preeclampsia and intrauterine growth restriction have been associated with altered early pregnancy circulatory adaptation.\textsuperscript{2,3,8} In addition to biochemical markers, pregnancies characterized by an elevated first- or second-trimester uterine artery pulsatility index (PI) appeared to be prone for subsequent vascular complications.\textsuperscript{9,12} Both low-dose aspirin and vitamins C and E have been proven to reduce the incidence of forthcoming vascular complications in those women with an elevated uterine artery PI.\textsuperscript{13-15}

Although most studies use predictive markers after the institution of pregnancy, evidence has been brought up in favor of preexisting factors, giving rise to endothelial and trophoblastic dysfunction as risk indicators for subsequent pregnancy-related vascular complications.\textsuperscript{16-21} First, these observations raise the question whether alterations in vascular function, as observed during pregnancy, already exist before pregnancy. Second, if so, do they have clinically relevant predictive value with respect to vascular complications in a subsequent pregnancy? Third, are these circulatory factors interrelated? Exploration of these potential differences in the nonpregnant vascular function between women with or without the development of subsequent vascular complications would not only improve our understanding of these problems, but may also contribute to the development of predictive models and possible preventive strategies.

The objective of this study was to evaluate whether the nonpregnant vascular compliance and resistance of the large arteries and veins in early human pregnancy relate to forthcoming vascular complications. To this end, we determined in the follicular phase of the menstrual cycle the cardiac output, arterial blood pressure, plasma volume, and dynamic function of the uterine, carotid and femoral artery, and forearm veins, in conjunction with subsequent maternal and fetal pregnancy outcome variables in formerly preeclamptic women.

**Methods**

**Patient selection and characteristics**

Sixty formerly preeclamptic, nondiabetic women were enrolled in this study. Data acquisition was initiated at least 5 months postpartum. Inclusion for further analysis required a pregnancy occurring within 1 year after the prepregnant measurement session. Furthermore, only ongoing singleton pregnancies (gestational age >16 weeks) in women without preexisting hypertension were included. As a consequence, from the group of 60 formerly preeclamptic participants having completed the nonpregnant measurement sessions, only 31 women could be included for final analysis (Figure 1). All subjects were white, and none of them smoked. Formerly preeclamptic women were recruited from our outpatient clinic at the time of postpartum follow-up. A part of the included women comprised the same study cohort as reported previously.\textsuperscript{22} Preeclampsia and (gestational) hypertension were defined according to the criteria of the National High Blood Pressure Education Program Working Group Report on High Blood Pressure in Pregnancy.\textsuperscript{23} A small-for-gestational age (SGA) infant was defined as a child whose birth weight was below the 10th percentile according to Dutch percentile set by Kloosterman.\textsuperscript{24} All experiments were preceded by 1 week of standardized sodium intake (100 mmol/d sodium), representing the mean sodium intake in the Dutch population. To this end, all participants consulted the hospital’s dietitian before the study. The women’s compliance with the diet was assessed by measuring 24-hour sodium output on the day before the experiment. On separate days and after an overnight fast, participants were tested for thrombophilia and hemodynamic and vascular function. Thrombophilia was defined as the condition characterized by the presence of at least 1 clotting disorder known to be associated with an increased risk to develop venous thromboembolism. All formerly preeclamptic participants received aspirin throughout pregnancy. In addition, those with thrombophilia or hyperhomocysteinemia were treated with low-molecular-weight heparin and with pyridoxine and folic acid supplementation, respectively. Participants gave written informed consent. The medical ethical committee approved the study.

**Experimental procedure**

The methodology of the measurements and calculations with respect to the thrombophilic screening and hemodynamic and vascular function have been detailed elsewhere.\textsuperscript{2,4,8,22}

*Thrombophilic screening* consisted of screening for the presence of the antiphospholipid antibodies (anticardiolipin antibodies and lupus anticoagulant), the measurement of protein C activity, total and free protein S antigen, activated protein C resistance, and factor V Leiden mutation and antithrombin activity. A methionine-loading test was used to diagnose hyperhomocysteinemia.

**Measurement of hemodynamic and vascular function**

In the follicular phase (day 5 ± 2) of the menstrual cycle, measurements were performed under standardized environmental conditions in a temperature-controlled
room (25°C-26°C) and with as little as possible external disturbances. Participants were requested to refrain from using caffeine- or alcohol-containing beverages for at least 10 hours before the experiment. The measurement session started at 8:00 AM. Throughout each measurement session, subjects were in the supine position on a comfortable bed. Arterial blood pressure and heart rate (HR, beats/min⁻¹) were recorded at 2 minutes interval by a semiautomatic oscillometric device (Dinamap Vital Signs Monitor 1846, Critikon, Tampa, Fla) throughout the measurement session. At 8:15 AM, after a 15-minute rest, carotid and femoral artery
compliance and uterine artery PIs were assessed non-invasively. Arterial wall displacement was visualized by a 2-dimensional real-time B-mode imager attached to a vessel wall moving detector system and analyzed offline (Pie Medical, Maastricht, The Netherlands). Three recordings of 5 consecutive heart beats were used to establish the changes in arterial diameter during the cardiac cycle as a function of time. From the recorded data, the mean increment above basal diastolic diameter (absolute distension \( \Delta d \)) from diastole to systole and relative distension \( \Delta d/d \) were calculated. From these data, the distensibility coefficient \( DC = 2 \times \frac{\Delta d/d}{d_2} \), kPa\(^{-1}\) and compliance coefficient \( CC = \pi \cdot d_2 \cdot \frac{\Delta d/2}{\Delta p}, \text{mm}^2\cdot\text{kPa}^{-1} \) were calculated. The brachial pulse pressure (systolic minus diastolic pressure) is taken as \( \Delta p \) and was calculated by taking the mean of 3 sets of Dinamap recordings measured during establishment of the vascular images.

After the assessment of femoral and carotid artery dynamics, the PI of both uterine arteries was measured. Transvaginal ultrasound was used to obtain a sagittal section of the uterus and the cervical canal. The internal cervical os was first identified. Subsequently, the transducer was gently tilted from side to side, and color flow mapping was used to identify the uterine arteries. Pulsed-wave Doppler was used to obtain flow velocity waveforms from the ascending branch of the uterine artery at the point closest to the internal os. When 3 similar consecutive waveforms were obtained, the PI was measured.

Thereafter, at 9:30 AM, the measurement of the venous compliance \( \text{VC} = \frac{\Delta V}{\Delta p} \text{DL forearm volume mm Hg}^{-1} \), defined as the plethysmographically determined slope of the relationship of intravascular volume and pressure changes of the left forearm (Periflow, JSI, Beerse, Belgium), was performed.

### Measurement of plasma volume and cardiac function

After the procedure to quantify vascular function, plasma volume was measured. Plasma volume was determined with the iodine 125-albumin \( ^{125}\text{I-HSA} \) indicator dilution method and expressed in milliliters per kilogram (mL/kg) lean body mass (LBM). The LBM was calculated, not measured.

**Echocardiography** to assess cardiac function was performed in semileft lateral position, after completion of the plasma volume measurement and after 5 minutes of rest, using a cross-sectional, phased array echocardiographic Doppler system (Hewlett-Packard Sonos 2000 and 2500, Andover, Mass). The HR, necessary to calculate cardiac output (stroke volume \( \times \text{HR} \)) and cardiac index (cardiac output/body surface area), was obtained by taking the mean of the reciprocal of 5 consecutive R-R intervals on the electrocardiograph. Stroke volume (SV, mL) was calculated by multiplying the aortic velocity integral and the aortic valve area. Aortic flow was measured across the aortic valves from an apical approach. The average area under the aortic velocity curve (aortic velocity integral) of 5 consecutive ejections was used to calculate SV. Aortic valve diameter necessary for the calculation of the aortic area was measured offline at the orifice during systole by using M-mode. The value used for mean arterial pressure (MAP, mm Hg) was obtained during the carbon monoxide measurement by using the Dinamap apparatus and was calculated as the mean of 3 consecutive recordings, which in turn was used to calculate total peripheral vascular resistance index \( (80 \times \text{MAP/CI}, \text{dyne s}^{-1}\cdot\text{cm}^{-5}) \).

### Statistical analysis

Data are presented as medians with ranges unless stated otherwise. Differences between groups were tested with the Mann-Whitney \( U \) test. Correlations, when calculated between concomitantly measured potentially related variables, were tested by Spearman’s rank correlation analysis. A \( P \)-value less than .05 was considered statistically significant. From the possible predictors, we constructed a receiver operating characteristic (ROC) curve to explore the predictive potential. At the calculated cutoff points, we calculated the Mantel-Haenszel common odds ratio and 95% CI. We subsequently performed a multivariate backward stepwise logistic regression analysis that included as dependent variables only those that were found to be correlated between the occurrence of growth restriction and maternal circulatory variables. This analysis allowed us to identify actual independent maternal predictors of fetal growth restriction.

### Results

Of the 60 eligible formerly preeclamptic participants, 31 normotensive, formerly preeclamptic women were available for final analysis. Eight women (26%) gave birth to a SGA infant. The demographic characteristics of included participants are listed in Table I. The 2 subgroups were comparable with respect to age, height, body mass index, parity, urinary sodium output, incidence of thrombophilia, and interval between pregnancies. In the subsequent pregnancy, women in both groups delivered at a comparable gestational age. Moreover, the incidence gestational hypertension and preeclampsia was similar. Nevertheless, women in the appropriate-for-gestational age (AGA) group gave birth more prematurely but to infants of higher birth weights.
Table I Demographic data from the 2 subgroups of formerly preeclamptic women

<table>
<thead>
<tr>
<th></th>
<th>AGA (n = 23)</th>
<th>SGA (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>29 (22-34)</td>
<td>28 (24-32)</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>170 (160-176)</td>
<td>168 (154-180)</td>
</tr>
<tr>
<td>Body mass index (kg/m²)</td>
<td>24 (19-34)</td>
<td>25 (19-33)</td>
</tr>
<tr>
<td>Parity</td>
<td>1 (1-2)</td>
<td>1 (1-2)</td>
</tr>
<tr>
<td>Sodium excretion (mmol/24h⁻¹)</td>
<td>98 (39-166)</td>
<td>83 (43-122)</td>
</tr>
<tr>
<td>Thrombophilia</td>
<td>12 (52%)</td>
<td>5 (63%)</td>
</tr>
<tr>
<td>Between-pregnancy interval (y)</td>
<td>2.2 (1.0-4.1)</td>
<td>2.4 (1.2-4.9)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>12 (52%)</td>
<td>5 (63%)</td>
</tr>
<tr>
<td>Preeclampsia</td>
<td>3 (13%)</td>
<td>1 (13%)</td>
</tr>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>39 (34-41)</td>
<td>38 (26-40)</td>
</tr>
<tr>
<td>Prematurity</td>
<td>5 (22%)</td>
<td>1 (13%)*</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3440 (1690-4240)</td>
<td>2500 (480-2780)*</td>
</tr>
</tbody>
</table>

* P < .05.

Table II Hemodynamic data

<table>
<thead>
<tr>
<th></th>
<th>AGA (n = 23)</th>
<th>SGA (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Systolic blood pressure (mm Hg)</td>
<td>118 (102-133)</td>
<td>113 (106-124)</td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)</td>
<td>72 (60-85)</td>
<td>69 (60-85)</td>
</tr>
<tr>
<td>MAP (mm Hg)</td>
<td>88 (73-101)</td>
<td>85 (75-97)</td>
</tr>
<tr>
<td>HR (beats/min)</td>
<td>62 (44-86)</td>
<td>61 (55-80)</td>
</tr>
<tr>
<td>Cardiac index (L.min⁻¹.m⁻²)</td>
<td>3.0 (2.3-3.6)</td>
<td>2.9 (2.4-3.4)</td>
</tr>
<tr>
<td>TPVR index (×10²dyne.s.cm⁻¹.m⁻²)</td>
<td>21 (13-27)</td>
<td>24 (19-29)</td>
</tr>
<tr>
<td>Plasma volume (mL/kgLBM⁻¹)</td>
<td>49 (41-55)</td>
<td>48 (42-56)</td>
</tr>
</tbody>
</table>

No differences were observed between both subgroups. TPVR, Total peripheral vascular resistance.

Table III Vascular data

<table>
<thead>
<tr>
<th></th>
<th>AGA (n = 23)</th>
<th>SGA (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Carotid diameter (mm)</td>
<td>6.6 (6.2-7.1)</td>
<td>6.6 (6.3-7.2)</td>
</tr>
<tr>
<td>Carotid compliance coefficient (mm²/kPa⁻¹)</td>
<td>0.95 (0.64-1.19)</td>
<td>1.23 (0.73-1.58)</td>
</tr>
<tr>
<td>Carotid distensibility coefficient (×kPa⁻¹)</td>
<td>28.4 (16.5-32.3)</td>
<td>35.1 (22.4-43.0)</td>
</tr>
<tr>
<td>Brachial pulse pressure (mm Hg)</td>
<td>47 (38-62)</td>
<td>47 (39-51)</td>
</tr>
<tr>
<td>Femoral diameter (mm)</td>
<td>8.5 (7.1-9.3)</td>
<td>7.8 (7.5-8.9)</td>
</tr>
<tr>
<td>Femoral compliance coefficient (mm²/kPa⁻¹)</td>
<td>0.36 (0.17-0.63)</td>
<td>0.42 (0.30-0.79)</td>
</tr>
<tr>
<td>Femoral distensibility coefficient (×kPa⁻¹)</td>
<td>6.4 (2.7-11.1)</td>
<td>8.8 (5.1-17.0)</td>
</tr>
<tr>
<td>Venous compliance (mL.dL⁻¹.mm Hg⁻¹)</td>
<td>5.7 (3.1-8.2)</td>
<td>5.1 (3.5-6.9)</td>
</tr>
<tr>
<td>PI LUA</td>
<td>1.9 (1.2-3.1)</td>
<td>3.0 (1.9-4.3)</td>
</tr>
<tr>
<td>PI right uterine artery</td>
<td>1.9 (0.9-3.1)</td>
<td>2.7 (2.0-4.0)</td>
</tr>
</tbody>
</table>

* Brachial pulse pressure (mm Hg) measured simultaneously with the assessment of the carotid artery variables.
† P < .05.
‡ Brachial pulse pressure (mm Hg) measured simultaneously with the assessment of the femoral artery variables.

Table IV

<table>
<thead>
<tr>
<th></th>
<th>AGA (n = 23)</th>
<th>SGA (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Concurrent femoral artery compliance coefficient</td>
<td>1.11 mm²/kPa</td>
<td></td>
</tr>
</tbody>
</table>

Analogy to the uterine artery PI, the compliance of the carotid and femoral artery were also submitted to ROC curve plotting to assess cutoff points. A nonpregnant carotid artery compliance coefficient ≥1.11 mm²/kPa was associated with a sensitivity of 71% and a specificity of 75% in predicting subsequent SGA, whereas the concurrent femoral artery compliance coefficient ≥0.36 mm²/kPa yielded a sensitivity of 86% and a specificity of...
50% (Figure 5). Above these cutoff points, the calculated common OR reached 7.5 (95% CI 1.02-55) for the carotid artery, whereas the femoral artery did not reach statistical significance in predicting subsequent development of fetal growth restriction (Table IV). In addition, we performed a multivariate backward logistic regression analysis in which the carotid and femoral artery compliance and the PI of the LUA were included. After analysis, only the PI of the LUA remained as an independent variable ($P < .0001$). No correlation could be observed between the uterine PI on the one hand and thrombophilia on the other.

Finally, we performed Spearman’s rank correlation analyses on possible related variables to gain more insight in the position of the uterine circulation as part of the general hemodynamic function. A high PI of the LUA correlates with a low venous compliance ($r = -0.49$, $P = .02$), a high carotid artery distensibility coefficient ($r = 0.54$, $P = .007$), but not with the femoral artery distensibility coefficient ($r = 0.25$, $P = .25$).
In addition, a high venous compliance is associated with a large plasma volume compartment ($r = 0.63$, $P = .001$), whereas the latter is associated with a low carotid artery distensibility coefficient ($r = -0.49$, $P = .02$) and a low total vascular resistance index ($r = -0.46$, $P = .02$).

**Comment**

Although the underlying pathogenetic mechanisms of vascular complications in pregnancy are still unclear, there is general agreement about defective spiral artery development and subsequent abnormal Doppler wave forms of the uterine artery as indicators of increased likelihood to develop these complications. However, also outside of pregnancy, circulatory abnormalities have been associated with forthcoming vascular complications. In this prospective study, preexistent maternal vascular variables such as an elevated uterine artery PI and carotid artery compliance predisposed for restricted fetal growth.

To evaluate whether certain prepregnant vascular abnormalities preceded an abnormal course of the subsequent pregnancy, we determined in the prepregnant period, the venous forearm compliance along with the compliance and distensibility of capacitive (carotid artery) and conductive arteries (femoral and uterine arteries). In line with our previous observation of a reduced adaptive capacity of these arteries and veins in early pregnancy in formerly preeclamptic women, we expected a reduced compliance of the arterial system predisposing to recurrent pregnancy-related vascular complications. To our surprise, the opposite proved to be the case. Both demographic characteristics and general central hemodynamic function did not differ appreciably between the 2 formerly preeclamptic subgroups. However, the observed elevated uterine artery PI predisposed for giving birth to an SGA infant. It also correlated with higher carotid artery elasticity and reduced venous compliance, which, in turn, correlated with a reduced plasma volume compartment. The latter is accompanied by an elevated total vascular resistance. These observations seem to be consistent with an enhanced sympathetic drive over the vasculature and with it, redistribution of the cardiac output at the expense of nonvital tissues.

A high vascular compliance enhances the systolic flow velocity and reduces the vascular impedance. Consequently, the impedance mismatch with a high-resistance peripheral bed will increase, augmenting pulse

| **Table IV** Univariate analysis on fetal growth restriction |
|----------------------|------|-----------|
| PI uterine artery    |     |           |
| PI LUA ≤ 2.66        | 1   | reference |
| PI LUA ≥ 2.66        | 42  | 3.2-556   |
| PI right uterine artery ≤ 2.33 | 1   | reference |
| PI right uterine artery ≥ 2.33 | 6   | 0.8-46    |
| Carotid artery compliance coefficient (CaCC) |           |
| CaCC <1.11 mm²/kPa⁻¹ | 1   | reference |
| CaCC ≥1.11 mm²/kPa⁻¹ | 7.5 | 1.02-55   |
| Femoral artery compliance coefficient (FeCC) |           |
| FeCC <0.36 mm²/kPa⁻¹ | 1   | reference |
| FeCC ≥0.36 mm²/kPa⁻¹ | 6   | 0.6-62    |

Figure 5 Birth weight and percentile as a function of prepregnant right uterine artery PI. The straight line represents the mean linear regression in the population studied; the curved lines represent the 95% CI for the mean predicted line.
wave reflection. The combination of a higher systolic peak and enhanced reflection may explain the high PI in the subject group with a higher risk on fetal growth restriction. The backward linear regression analysis shows that only the (left) uterine artery PI remained as independent variable on fetal growth restriction, indicating that the carotid and femoral artery compliance are related to the PI via the uterine artery compliance. This view is further supported by the observed correlations between these concomitant variables. Hence, the higher compliance we observed in the subgroup with an enhanced risk on restricted fetal growth expresses a general vascular condition.

The current study was not designed to prove the efficacy of vascular characteristics in relation to forthcoming vascular complications, rather than providing the position of all these variables in relation to each other. As far as we know, this is the first study providing evidence for a general alteration in hemodynamic function in association with abnormalities in the uterine circulation even before pregnancy. Although the number of patients included in the study was modest, the extensiveness of simultaneously measured hemodynamic variables improves our insight in the reported first- and second-trimester alterations in the uterine circulation in relation to the general hemodynamic capacity to meet the increasing demands of maternal uterine perfusion. A large prospective study in high-risk patients is needed to assess the true value of these variables to predict the occurrence of SGA infants in a subsequent pregnancy. If so, women with an elevated prepregnant uterine artery PI may, in analogy to pregnant women with first- and second-trimester abnormalities on the uterine Doppler signals, benefit from low-dose aspirin and/or vitamins C and E supplementation.13-15

In conclusion, in nonpregnant normotensive, formerly preeclamptic women, a low venous compliance and plasma volume coincide with a high carotid compliance, total peripheral vascular resistance index, and uterine artery PI, the latter giving rise to the incidence in fetal growth restriction.

References

Markers of periodontal infection and preterm birth

Karim Jarjoura, DMD, MS, Patricia C. Devine, MD, Annette Perez-Delboy, MD, Miriam Herrera-Abreu, MS, Mary D’Alton, MD, Panos N. Papapanou, DDS, PhD*

Division of Periodontics, Section of Oral and Diagnostic Sciences, Columbia University School of Dental and Oral Surgery and Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Columbia University Medical Center, New York, NY

Received for publication April 16, 2004; revised June 18, 2004

KEY WORDS
Periodontal disease
Preterm birth
Low birth weight
Risk factor

Objective: This study was undertaken to explore the relationship between clinical, microbiologic, and serologic markers of periodontitis and preterm birth (PTB).

Study design: We compared women with a singleton gestation giving birth before the 37th week (cases, n = 83) with term delivery controls (n = 120). Periodontal examination and collection of dental plaque and blood samples were performed within 48 hours after delivery. Microbial levels and maternal immunoglobulin G titers to oral bacteria were analyzed. Multivariate regression models were fitted controlling for common covariates.

Results: Cases showed greater mean attachment loss (1.7 vs 1.5 mm, \(P = .003\)) and higher prevalence of periodontitis (30.1% vs 17.5%, \(P = .027\)). No differences in microbial or serum antibody levels were detected between the groups. Logistic regression revealed that PTB was associated with attachment loss (adjusted odds ratio: 2.75, 95% CI: 1.01-7.54). Linear regression indicated a significant (\(P = .04\)) association between attachment loss and low birth weight (LBW).

Conclusion: The data support the notion that periodontitis is independently associated with PTB and LBW.

© 2005 Elsevier Inc. All rights reserved.

The role of infection in the pathogenesis of preterm birth (PTB) has been well documented.\(^1\) Evidence of intrauterine infection (IUI), defined as the positive recovery of microorganisms from the amniotic cavity or the chorioamniotic space, has been reported in approximately 25% of women with singleton gestation and PTB.\(^2\) Women admitted with preterm labor and positive amniotic fluid cultures were shown to be approximately 2.5 times more likely to deliver prematurely than noninfected women.\(^3\) Similarly, the incidence of neonatal complications was found to increase in women with evidence of IUI.\(^4\) Although the pathophysiology of infection-induced preterm birth is not fully understood, evidence suggests that the process is mediated by the local production of inflammatory cytokines and prostaglandins by cells of the fetal membranes and decidua upon exposure to bacteria or bacterial byproducts.\(^3\) Indeed, concentrations of interleukin-1\(\beta\) (IL-1\(\beta\)), interleukin-6 (IL-6), and tumor necrosis factor-\(\alpha\) (TNF-\(\alpha\)) were found to be elevated in

---

Supported by the Academic Advancement Fund, Columbia University School of Dental and Oral Surgery, and an unrestricted gift from Colgate-Palmolive, Inc.


E-mail: pp192@columbia.edu
patients with IUI and shown to be inversely correlated to gestational age. Collectively, these findings indicate that inflammation of the uterofetal unit in response to a local microbial insult can trigger the onset of parturition.

Periodontal disease, a chronic, low-grade infection characterized by inflammation and eventual loss of tooth-supporting tissues, was recently shown to be associated with pregnancy complications. Multivariate logistic regression models, controlling for the effect of common demographic, socioeconomic, and pregnancy-related risk factors, revealed a significant association between periodontitis and preterm/low birth weight (LBW) with reported odds ratios (ORs) between 3.5 and 7.9. Similarly to IUI, the strongest association was shown between severity of periodontitis and decreasing gestational age at birth. In addition, evidence of “active” periodontitis during pregnancy, defined as incident attachment loss between 2 consecutive visits, was found to increase the risk of preterm birth and intrauterine growth restriction (IUGR). Microbiologic and immunologic markers of periodontal infection were also reported to be associated with adverse pregnancy outcomes. Finally, early findings from intervention studies indicate that periodontal therapy during pregnancy may result in a reduction in the incidence of PTB.

The significant socioeconomic impact of prematurity, coupled with the fact that the PTB rate has remained virtually unchanged in the United States during the past 2 decades underscores the importance of identifying new risk factors amenable to early intervention. The proposed association between periodontitis, which is both preventable and treatable, and PTB is likely complex and probably nonuniform in different populations. This study was therefore undertaken to further explore this relationship by concomitantly examining clinical, microbiologic, and immunologic markers of periodontal infection in a predominantly Hispanic cohort of women.

Material and methods

Study sample

The study protocol was approved by the Columbia University Institutional Review Board. The study sample consisted of women with a singleton gestation who gave birth at the Sloan Hospital for Women, Columbia University Medical Center, between February 2001 and April 2003. Approximately 3500 deliveries per year took place at this unit during the study period. Potential enrollees were made aware of the aims and procedures of the study by means of posted flyers, and women interested in participating notified their care-taking physician. Cases, ie, women who gave birth before the 37th week of gestation, were compared with term delivery controls with respect to markers of periodontal infection. Pregnancy dating was confirmed for all patients with an ultrasound examination before 20 weeks’ gestation. Women with fetal or uterine anomalies, cervical incompetence, and/or labor induction were excluded, as were women who required antibiotic prophylaxis before dental procedures. All subjects signed an informed consent before enrollment.

Sociodemographic, behavioral, and pregnancy-related variables

Maternal demographic and obstetric data were obtained from the patient’s medical record. This information was then confirmed by patient interview after completion of the consent process. Drug use was defined as any drug taken during the antenatal period and identified either at patient admission or by urine toxicology. Tobacco use was defined as any regular tobacco consumption during pregnancy, as disclosed at patient interview. A urine specimen was routinely sent for analysis on all patients at the time of initial prenatal evaluation and whenever a patient presented signs or symptoms suggestive of a urinary tract infection, including dysuria, increased frequency, proteinuria or hematuria, or preterm labor. Urinary tract infection (UTI) was identified by using a standard laboratory technique and was defined as presence of more than 10^5 colonies of any recognized pathogenic organism. Antibiotic coverage was selected on the basis of the organism(s) identified. Chorioamnionitis was defined as the presence of a fever (100.4°F or higher) in a patient with ruptured membranes and no other identifiable source of infection, and was treated with the appropriate antibiotics. History of previous preterm delivery (gestational age at birth before 37 weeks) was recorded.

Periodontal examination

The periodontal examination was performed chairside at the postpartum unit with an external light source within 48 hours after delivery. Assessments of dental plaque (Pl), bleeding on probing (BoP), probing depth (PD, the distance between the gingival margin and the base of the periodontal pocket), and clinical attachment loss (CAL, the distance between the cementoenamel junction and the base of the pocket) were performed at 3 sites per tooth (mesiobuccal, midbuccal, and distobuccal) in 2 randomly selected, diametrically opposed, quadrants. All teeth present in a selected quadrant were examined, except for third molars. All clinical assessments were performed by a single, calibrated examiner, who was blinded to case status. Pl and BoP were recorded dichotomously as either present or absent. PD and CAL were recorded to the nearest whole millimeter.
Dental plaque collection and microbiologic analysis

Subgingival plaque samples were collected from the mesiobuccal aspect of 1 molar, 1 premolar, 1 canine, and 1 incisor tooth per quadrant examined, for a maximum of 8 samples per patient. All samples were collected by a single scaling stroke, by using a sterile Gracey curette inserted at the bottom of the pocket, as previously described. Analysis of the plaque samples was performed by means of the checkerboard DNA-DNA hybridization method with respect to the following 12 periodontal species: Porphyromonas gingivalis, Prevotella intermedia, Prevotella nigrescens, Tannerella forsythensis, Actinobacillus actinomycetemcomitans, Fusobacterium nucleatum, Treponema denticola, Micromonas micros, Campylobacter rectus, Eikenella corrodens, Eubacterium nodatum, and Streptococcus intermedius.

Serologic analysis

Five milliliters of blood were collected through venipuncture by an experienced phlebotomist, and were centrifuged at 2000 revolutions/min for 15 minutes to collect serum. Serum immunoglobulin G (IgG) antibodies to the previously listed species were assessed with the use of the checkerboard immunoassay as previously described.

Data analysis

A dichotomous definition of periodontitis was used, based on the presence of 5 or more sites per subject with CAL of 3 mm or greater. This threshold corresponds to approximately 12% of periodontal sites affected, consistent with the definition of localized moderate chronic periodontitis.

The χ² test and a 2-tailed Student t test were used to test for differences between the groups in categorical and numerical variables, respectively. Nonparametric comparisons were performed for noncontinuous variables. To further investigate the relationship between periodontitis and gestational age, cases were subdivided into 2 groups: premature (gestational age < 37 but ≥32 weeks) and very premature (gestational age < 32 weeks). A post hoc multiple comparison test (Student–Newman–Keuls test) was used to compare attachment loss and prevalence of periodontitis in the above groups. Multivariate logistic regression and linear regression analyses were performed using PTB and LBW as outcome variables. All analyses were performed by using the SAS software, version 9.0 (SAS Institute, Cary, NC).

Results

A total of 203 subjects, 83 cases and 120 controls enrolled in the study, and their sociodemographic characteristics are shown in Table I. The prevalence of periodontitis among controls and cases is shown in Table II. The clinical periodontal characteristics are shown in Table III.
variables are described in Table I. Mean age and age range, and race/ethnicity distribution were similar between the 2 groups. The percentage of patients with private medical insurance, used as a measure of socioeconomic status, was slightly lower in cases, although the difference was not statistically significant. Tobacco use during gestation was significantly greater in cases ($P = .011$).

Comparisons of pregnancy-related variables are shown in Table II. Mothers giving birth prematurely had higher incidence of chorioamnionitis (19.3% vs 2.5%, $P < .0001$), significantly higher frequency of positive history of previous preterm delivery (8.4% vs 0.8%, $P < .0001$), preterm premature rupture of membranes (55.4% vs 19.2%, $P < .0001$), but lower mean body mass index (BMI) (27.7 vs 30.0, $P = .019$) than term delivery controls. Parity, prevalence of fetal growth restriction, and UTI were not significantly different between the groups.

Periodontal characteristics are presented in Table III. Number of teeth present, the percentage of tooth sites with visible plaque, or BoP was similar in cases and

**Table IV** Periodontal disease indicators in relation to gestational age at birth and birth weight

<table>
<thead>
<tr>
<th>Gestational age</th>
<th>Birth weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>≤37 wk (n=115)</td>
<td>≥2500 g (n=136)</td>
</tr>
<tr>
<td>&lt;37 wk but ≥32 wk (n=59)</td>
<td>&lt;2500 g but ≥1500 g (n=39)</td>
</tr>
<tr>
<td>&lt;32 wk (n=22)</td>
<td>&lt;1500 g (n=17)</td>
</tr>
<tr>
<td>CAL (mm)</td>
<td>1.5 (0.3)</td>
</tr>
<tr>
<td>Periodontitis</td>
<td>17.5%</td>
</tr>
</tbody>
</table>

Mean values (SD).

* Significantly different from ≤37 wk, $P = .003$.
† Significantly different from ≥2500 g, $P = .004$.
‡ Significantly different from ≥37 wk, $P = .014$.

Figure 1 Log transformed mean bacterial counts in the dental plaque of women in the control, premature, and very premature groups. Species abbreviations as follows: Pg: P gingivalis; Pi: P intermedia; Pn: P nigrescens; Tf: T forsythenis; Aa: A actinomycetemcomitans; Fn: F nucleatum; Td: T denticola; Mn: M micros; Cr: C rectus; Ec: E corrodens; En: E nodatum; and Si: S intermedius.

Figure 2 Log transformed maternal serum IgG titers (ng/mL) in the control, premature, and very premature groups. Species abbreviations are shown in Figure 1 legend.
controls. No differences were detected in mean PD (2.4 vs 2.5, \(P = .135\)) or the percentage of sites per subject with PD 5 mm or greater between the groups. In contrast, statistically significant differences between the groups were observed for mean attachment loss (1.5 vs 1.7, \(P = .003\)) as well as for the average percentage of sites per subject with CAL of 3 mm or greater (\(P = .013\)). A significantly higher percentage of cases were found to have periodontitis when compared with controls (30.1% vs 17.5%, \(P = .027\)). Comparison between case subgroups and term delivery controls revealed an inverse relationship between gestational age at birth and both mean CAL and prevalence of periodontitis (Table IV). Similar findings were observed when groups were subdivided on the basis of birth weight.

Figure 1 illustrates the mean microbial levels in each group for each of the investigated periodontal species. High levels of \(P\) gingivalis, \(T\) forsythensis, and \(T\) denticola, common periodontal pathogens, were found in both case subgroups and controls. With the exception of \(M\) micros, the levels of which were found to be significantly lower in cases, no statistically significant differences were detected between the groups. Figure 2 describes serum IgG levels against these periodontal species. A trend for higher antibody titers was observed in the 2 case subgroups when compared with controls but did not reach statistical significance.

Tables V and VI present the findings from the logistic and the linear multiple regression analyses, respectively. Both tables present final models after elimination of weakly associated variables. Chorioamnionitis was the strongest independent variable for either outcome in both models. In contrast, when controlling for the effect of other confounders, smoking was not found to be significantly associated with either PTB or LBW in either model. In the logistic model (Table V), attachment loss was significantly associated with PTB (adjusted OR: 2.75, 95% CI 1.01-7.54). A positive history of PTB was strongly associated with PTB and LBW (adjusted OR: 8.6 and 15.7, respectively), whereas BMI was found to be inversely associated with both outcome variables. The multivariate linear model (Table VI) corroborated the associations shown in the logistic model, with attachment loss being negatively associated with gestational age at birth (\(P = .06\)) and birth weight (\(P = .04\)).

**Comment**

In a predominantly Hispanic sample of postpartum women with relatively low levels of periodontal disease, our study demonstrated a significantly increased loss of periodontal tissue support in women who were delivered preterm, and indicates an independent effect of periodontitis on adverse pregnancy outcomes after adjustment for important risk factors.

Earlier case-control studies available in the literature have provided inconsistent data on the issue of periodontitis and PTB. In a study of 124 predominantly white women with substantially higher prevalence and severity of periodontitis, Offenbacher et al\(^\text{7}\) reported statistically significant differences in mean attachment loss between women who gave birth to preterm and/or LBW infants compared with term delivery controls (3.10 vs 2.80 mm). In contrast, in a case-control study of 164 black women 12 to 19 years old, Mitchell-Lewis et al\(^\text{13}\) failed to detect any differences in clinical periodontal conditions between women who were delivered preterm, LBW infants and control women. Similarly, a large case-control study\(^\text{21}\) of an ethnically mixed population in the United Kingdom that included 236 women who were delivered preterm LBW infants and 507 controls with normal birth outcomes, also failed to detect differences in periodontal status between the cases and control. However, it is important to emphasize that none of the latter 2 studies included assessments of CAL, but solely analyzed periodontal data on gingival inflammation and PD.

On the other hand, several cohort studies have revealed a positive association between the severity of

<table>
<thead>
<tr>
<th>Variable</th>
<th>PTB</th>
<th></th>
<th>LBW</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Adjusted OR</td>
<td>95% CI</td>
<td>Adjusted OR</td>
<td>95% CI</td>
</tr>
<tr>
<td>Smoking</td>
<td>2.55</td>
<td>0.72-8.95</td>
<td>3.38</td>
<td>0.92-12.36</td>
</tr>
<tr>
<td>Previous preterm delivery</td>
<td>8.60</td>
<td>0.87-85.41</td>
<td>15.68</td>
<td>1.50-163.67</td>
</tr>
<tr>
<td>BMI</td>
<td>0.92</td>
<td>0.87-0.98</td>
<td>0.93</td>
<td>0.87-0.99</td>
</tr>
<tr>
<td>Clinical chorioamnionitis</td>
<td>17.33</td>
<td>2.92-102.82</td>
<td>10.99</td>
<td>2.79-43.35</td>
</tr>
<tr>
<td>CAL</td>
<td>2.75</td>
<td>1.01-7.54</td>
<td>1.99</td>
<td>0.73-5.45</td>
</tr>
</tbody>
</table>

| Table V  Multivariate logistic regression for PTB (<37 wk) and LBW (<2500 g) |
|--------------------------------|-----------------------------------|-----------------------------------|
| Variable                        | PTB                               | LBW                               |
|                                | Adjusted OR| 95% CI       | Adjusted OR| 95% CI      |
| Smoking                        | 2.55         | 0.72-8.95 | 3.38         | 0.92-12.36 |
| Previous preterm delivery      | 8.60         | 0.87-85.41 | 15.68         | 1.50-163.67 |
| BMI                            | 0.92         | 0.87-0.98 | 0.93         | 0.87-0.99  |
| Clinical chorioamnionitis      | 17.33        | 2.92-102.82 | 10.99        | 2.79-43.35 |
| CAL                            | 2.75         | 1.01-7.54 | 1.99         | 0.73-5.45  |

<table>
<thead>
<tr>
<th>Variable</th>
<th>PTB</th>
<th>LBW</th>
</tr>
</thead>
<tbody>
<tr>
<td>smoking</td>
<td>2.55</td>
<td></td>
</tr>
<tr>
<td>previous preterm delivery</td>
<td>8.60</td>
<td></td>
</tr>
<tr>
<td>BMI</td>
<td>0.92</td>
<td></td>
</tr>
<tr>
<td>clinical chorioamnionitis</td>
<td>17.33</td>
<td></td>
</tr>
<tr>
<td>CAL</td>
<td>2.75</td>
<td></td>
</tr>
</tbody>
</table>

| Table VI Multivariate linear regression for gestational age at birth (d) and birth weight (g) |
|---------------------------------|----------------------------------|----------------------------------|
| Variable                        | Gestational age                  | Birth weight                     |
|                                | Parameter estimate | P value | Parameter estimate | P value |
| Smoking                        | -8.79                            | .15 | -249.36            | .24 |
| Previous preterm delivery      | -12.26                           | .04 | -370.70            | .07 |
| BMI                            | 0.42                             | .12 | -28.46             | .02 |
| Clinical chorioamnionitis      | -38.20                           | .0001 | -1118.75        | .0001 |
| CAL                            | -7.91                            | .06 | -281               | .04 |

Coefficient of determination, model for gestational age: 0.274. Coefficient of determination, model for gestational age: 0.263.
periodontal disease and the incidence of PTB. In a study of 639 pregnant women in Chile, Lopez et al\textsuperscript{12} reported that the incidence of delivery of a preterm, LBW infant increased from 2.5\% in periodontally healthy women to 8.6\% in women with periodontal disease. Jeffcoat et al\textsuperscript{11} studied prospectively a sample of 1313 primarily black pregnant women and reported an adjusted OR of 4.4 for preterm delivery and generalized periodontitis. This OR increased to 5.3 for delivery before 36 weeks of gestation, and to 7.1 for delivery before the 32nd week. The data of the current study also indicate an inverse, dose-response association of periodontitis and both gestational age at birth and birth weight. As shown in Table IV, the prevalence of periodontitis increased from 17.5\% in women delivered at term to 25.4\% in women delivered between 37 and 32 weeks, and peaked at 45.4\% in the less than 32-week group. A very similar trend was observed between prevalence of periodontitis and birth weight.

Results from the multivariate regression analyses revealed that attachment loss remained significantly linked to both PTB and LBW, after controlling for the effect of known covariates. However, the strength of the association between periodontal disease and either adverse pregnancy outcome was lower in this study than previously reported.\textsuperscript{7,11,12} This could, in part, be attributed to (1) the inclusion of chorioamnionitis in the regression models, and (2) the conceivable improvement of clinical periodontal conditions as a result of the antibiotic therapy prescribed for the treatment of chorioamnionitis. Another interesting observation was the lack of any significant association between smoking and either adverse pregnancy outcome, despite the established association between tobacco and PTB.\textsuperscript{22} However, smoking is strongly associated with periodontitis as well,\textsuperscript{23} which may explain its lack of significant effect in the regression models. Consistent with earlier reports, previous preterm delivery was found to be positively associated with both PTB and LBW,\textsuperscript{24} whereas maternal BMI was associated with a prolonged gestation and increased birth weight.\textsuperscript{25}

Comparison of mean microbial levels between the groups did not reveal any significant difference for the majority of microorganisms tested. This finding is in contrast to our earlier observations from a case-control study of parenting teenagers, in which we observed higher levels of \textit{T forsythensis}, and \textit{C rectus} in women with PTB.\textsuperscript{13} However, it is conceivable that the administration of ampicillin for the treatment of chorioamnionitis in the immediate antenatal period, which was approximately 8 times more frequent in women in the case group (Table II), has had an impact on the composition of the subgingival microbiota\textsuperscript{26} as well as on the levels of gingival inflammation. Thus, this fact may have resulted in an attenuation of the differences between cases and controls.

Similarly, no statistically significant differences were detected in serum antibody levels between the groups, although a trend for higher serum IgG levels was observed in women in the very premature group. Studies investigating the relationship between maternal antibody titers against periodontal pathogens and PTB have reported conflicting results. Dasanayake et al\textsuperscript{14} found that women with elevated second-trimester serum IgG levels against \textit{P gingivalis} were more likely to give birth to a LBW infant compared with those with lower serum values. On the other hand, Madianos et al\textsuperscript{27} reported that the greatest odds of prematurity were observed in the absence of a maternal IgG response to microorganisms associated with gingivitis coupled with a fetal IgM response to \textit{P gingivalis, T denticola}, and \textit{T forsythensis}. These authors suggested that a lack of a proper maternal immune response to infecting periodontal bacteria may have resulted in fetal exposure, ultimately leading to premature onset of labor. Given that a broad range of antibody responses to periodontal pathogens has been observed in patients with various forms of periodontal disease,\textsuperscript{17,28} the association between such titers and pregnancy complications remains similarly speculative at the current time.

In conclusion, our data indicate that periodontitis is independently associated with both PTB and LBW. Early results from intervention trials\textsuperscript{13,15,16} have shown that basic periodontal therapy significantly reduces the incidence of PTB and LBW. For example, in a non-randomized study, our group\textsuperscript{13} reported an approximately 30\% reduction in the incidence of preterm, LBW infants in a group of women who received simple periodontal prophylaxis during pregnancy, when compared with a group of women who did not receive such treatment before delivery (13.5\% vs 18.9\%). A randomized clinical trial from Chile\textsuperscript{15} showed an incidence of preterm LBW of 1.8\% in pregnant women with periodontitis who were treated before 28 weeks of gestation compared with 10.1\% in a control group of untreated women. These studies suggest that periodontitis may be regarded as a true risk factor for prematurity. However, more definitive large scale randomized controlled trials in racially and ethnically diverse populations are necessary to provide unequivocal evidence.

References


Vitamin C and E supplementation in women at high risk for preeclampsia: A double-blind, placebo-controlled trial

Dorothy Beazley, a Robert Ahokas, b Jeffrey Livingston, c Mary Griggs, b Baha M. Sibai, MD d,*

Department of Obstetrics and Gynecology, Tufts-NEMC, Boston, Mass; Department of Obstetrics and Gynecology, University of Tennessee, Memphis, Tenn; Department of Obstetrics and Gynecology, Carilion Hospital, Roanoke, Va; Department of Obstetrics and Gynecology, University of Cincinnati, Cincinnati, Ohio

Received for publication May 7, 2004; revised September 2, 2004; accepted September 2, 2004

Objective: We sought to determine the effect of supplemental antioxidant vitamins C and E on the rate of preeclampsia in high-risk pregnant women.

Study design: Women at risk for preeclampsia (previous preeclampsia, chronic hypertension, pregestational diabetes, or multifetal gestation) were recruited at 14 to 20 weeks’ gestation and randomly assigned to receive either 1000 mg of vitamin C and 400 IU of vitamin E or placebo daily in addition to their regular prenatal vitamins. The primary outcome was the occurrence of preeclampsia. An estimated sample size of 220 women in each arm was determined to be necessary to demonstrate a 50% reduction in the rate of preeclampsia.

Results: Funding was terminated after 109 women had been recruited; 9 were lost to follow-up or withdrew. We analyzed data from the remaining 100 women to look for differences in outcome and to estimate the required sample size for future studies. The rate of preeclampsia was not different: 17.3% in women who received supplemental vitamins C and E, versus 18.8% in the placebo group. Assuming a baseline rate of preeclampsia in the placebo group between 15% and 20%, we can estimate that 500 to 950 women in each arm will be required to show a clinically important reduction in the rate of preeclampsia.

Conclusion: The potential benefit of vitamin C and E supplementation to prevent preeclampsia in women with clinical risk factors is smaller than we estimated. Future studies of antioxidant vitamin supplementation in this population will require more than 500 women in each arm.

Preeclampsia is a multisystem disorder of pregnancy that is seen more frequently in women with prior preeclampsia, chronic hypertension, renal disease, insulin-dependent diabetes mellitus, and multiple gestation, and in women with abnormal uterine artery Doppler scans.1 Despite numerous attempts at early diagnosis and treatment, efforts to prevent preeclampsia

Key Words: Preeclampsia; Pregnancy; Vitamin C; Vitamin E

0022-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.

have been disappointing. Recently, it has been proposed that preeclampsia is a disorder of increased oxidative stress, offering the possibility of targeted therapy aimed at oxidative stress reduction with antioxidants.2 There is only 1 randomized trial evaluating the effects of vitamin C plus E supplementation on the rate of preeclampsia.3 This trial was conducted in women identified to be at risk for preeclampsia because of abnormal uterine artery Doppler scan at 18 to 20 weeks’ and 24 weeks’ gestation. In this study, the rate of preeclampsia was reduced from 17% to 8% (54% reduction; \( P = .02 \)) in the intent-to-treat cohort. Our objectives were first to evaluate the effects of antioxidant vitamin supplementation with vitamin C 1000 mg and vitamin E 400 IU for the prevention of preeclampsia in an inner city population at high risk of preeclampsia.

### Materials and methods

This is a double-blind, randomized clinical trial of vitamin C 1000 mg and vitamin E 400 IU supplementation versus placebo in pregnant women at high risk of preeclampsia. The primary outcome was development of preeclampsia.

Inclusion criteria were pregnancy at 14 weeks 0 days to 20 weeks 6 days with a history of prior preeclampsia, chronic hypertension, insulin-requiring diabetes mellitus, or multiple gestation. To determine sample size, we assumed a rate of preeclampsia of 20% in this population.3 On the basis of a proposed reduction in preeclampsia of 50% with vitamin C and E supplementation,3 we calculated that a total of 220 women need to be randomly assigned in each group to have a power of 80% with an alpha of .05. Because of cessation of funding, the trial was terminated early after 109 women were randomly assigned.

### Results

Pregnancy outcomes are listed in the Table. There were no pregnancy losses before 20 weeks’ gestation in either group. Overall, 18 women had preeclampsia develop, 9 in each group. Of these, 6 women had severe preeclampsia develop (3 in each group), 5 had mild preeclampsia, and 7 had superimposed preeclampsia develop. The rate of preeclampsia was 17.3% in the vitamin supplement group and 18.8% in the placebo group (relative risk = 0.92; 95% CI, 0.4-2.13).

### Comments

The findings of our study reveal that antioxidant supplementation with vitamin C 1000 mg and vitamin E 400 IU had no effect on the rate of preeclampsia in a high-risk patient population. However, we must caution that our study was terminated prematurely and the sample size was inadequate to answer this question. Nevertheless, considering the marginal differences in the rate of preeclampsia found between the 2 groups (17.3% vs 18.8%) even if the study was continued, it would have been unlikely to demonstrate a 50% reduction in rates of preeclampsia with vitamins C and E.

Our randomized trial has limitations and may be improved in 2 ways. First, we terminated the trial prematurely because of inadequate funding. Second, the most serious limitations of this study is what we have learned from this trial. On the basis of the results of only 1 previous trial3 on this subject, we calculated our sample size on the basis of a 50% reduction in rate of preeclampsia with vitamin supplementation (from 20% in placebo to 10% in vitamin group). Therefore, a 50% reduction is unrealistic with vitamin supplements. A more realistic expectation should have been a 33% or even 25% reduction in rates of preeclampsia. This would have required at least 500 to 950 women in each arm to show a meaningful reduction in preeclampsia. Thus, we recommend that future trials base their calculated sample size on these realistic expectations. In addition, adequate funding should be available before initiation of any randomized clinical trial.

### References


### Table

<table>
<thead>
<tr>
<th>Pregnancy outcome</th>
<th>Placebo* (n = 48)</th>
<th>Vitamins C and E* (n = 52)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Delivery age (wk)</td>
<td>37.2 ± 3.9</td>
<td>36.8 ± 3.6</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3050 ± 1021</td>
<td>2911 ± 901</td>
</tr>
<tr>
<td>&lt;2500 (%)</td>
<td>12 (25)</td>
<td>13 (25)</td>
</tr>
<tr>
<td>&lt;10 (%)</td>
<td>4 (8.3)</td>
<td>2 (3.8)</td>
</tr>
<tr>
<td>Delivery &lt;37 wk (%)</td>
<td>14 (30)</td>
<td>20 (38.4)</td>
</tr>
</tbody>
</table>

Mean ± SD.

* No significant differences between groups.
The onset of postpartum depression: Implications for clinical screening in obstetrical and primary care

Zachary N. Stowe, MD,a,b,* Amy L. Hostetter, BA,a D. Jeffrey Newport, MD, MS, MDiva

Departments of Psychiatry and Behavioral Sciencesa and Gynecology and Obstetrics,b Emory University School of Medicine, Atlanta, Ga

Received for publication March 2, 2004; revised June 30, 2004; accepted July 23, 2004

Objective: Inconsistent diagnostic criteria fail to delineate guidelines for postpartum depression surveillance. This study evaluates the validity of commonly accepted postpartum onset criteria.

Study design: Consecutive referrals to the Emory Women’s Mental Health Program for evaluation of postpartum depression fulfilling criteria for major depression and taking no psychotropic medication were included. Diagnostic interview, demographics, depression scales, and the time of illness onset were obtained. Descriptive analysis was conducted for 3 participant groups: pregnancy onset, early postpartum onset within 6 weeks of delivery, and late postpartum onset.

Results: Among participants, 11.5% reported prenatal onset, 22.0% late postpartum onset, and 66.5% early postpartum symptom onset. Those reporting pregnancy onset were more likely to be unmarried, and those with a late postpartum onset were less likely to report a past history of postpartum depression.

Conclusion: The perinatal vulnerability to depression begins before delivery and extends beyond 6 weeks postpartum. Depression surveillance is therefore warranted during prenatal visits, at the postnatal check up, and at pediatric visits during the initial 6 months of the first postnatal year.

The puerperium is recognized as a time of increased vulnerability to depression, but the diagnostic criteria for postpartum depression (PDD), particularly time of onset, has been frequently debated. The DSM-IV applies the “postpartum onset” specifier when an episode begins within the first 4 weeks postpartum, whereas ICD-10 criteria extend this window to 6 weeks after delivery. The diagnostic criteria for PPD onset emphasize the importance of depression screening at postnatal follow-up visits, but these criteria were largely derived from postpartum psychosis data and may not accurately reflect the clinical course of the more common nonpsychotic perinatal depressive illness.

Previous investigations utilized a variety of postpartum time criteria, often up to 6 months after delivery. The inconsistency in defining time of onset across studies has hindered efforts at meta-analysis of the PPD data.

Supported in part by an unrestricted grant from Pfizer, Inc., and a National Institutes of Health K23 Patient-Oriented Research Career Development Award (D.J.N.).

Dr Stowe is a member of the GlaxoSmithKline Advisory Board.

* Reprint requests: Zachary N. Stowe, MD, Emory University School of Medicine, Women’s Mental Health Program, 1365 Clifton Road NE, Suite B6100, Atlanta, GA 30322.

E-mail: zstowe@emory.edu

0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.
Despite these limitations, preliminary evidence indicates that women are vulnerable to depression prior to delivery and remain so later in the postpartum period. For example, several investigations demonstrated higher rates of depressive symptoms during pregnancy among women with PPD. O’Hara’s report that depressive symptoms during pregnancy were associated with continued complaints of depression in the postpartum and that 17% (2/12) of women fulfilling criteria for major or minor depression during the first 9 weeks postpartum were depressed during pregnancy. Similarly, Buesching et al reported that 17.5% (10/57) of postpartum women with Zung Depression Scale scores indicative of mild to moderate depression at 6 weeks postpartum had higher depression scores during gestation. Watson et al found that 12% (15/128) of women met DSM-IV criteria for depressive neurosis at 6 weeks postpartum, but an additional 10% (13/128) became depressed later in the postpartum.

These studies raise important questions germane to clinical surveillance for maternal depression during pregnancy and after the initial postnatal checkup. Equally, these investigations raise important questions with respect to the DSM-IV (within 4 weeks) and ICD-10 (within 6 weeks) onset criteria for PPD. The current study evaluated the timing of depression onset in a large sample of women referred with a presumptive diagnosis of PPD.

Methods

Subjects

Three hundred fifteen consecutive referrals to the Emory Women’s Mental Health Program for evaluation of PPD were screened for study inclusion. Women were included if they presented during the first postpartum year, fulfilled DSM-IV criteria for major depression, had received no psychotropic medication during the current episode, and were able to describe a clear point of the onset of illness.

Procedures

At initial presentation, participants completed the Edinburgh Postnatal Depression Scale, Beck Depression Inventory (BDI), and an intake questionnaire reporting the time of illness onset, personal and family psychiatric history, and demographic information. The time of illness onset was defined as the beginning of the current major depressive episode. Transient nonsyndromal mood disturbances, such as the postpartum blues, which had resolved before the depressive episode, were not identified as the time of illness onset unless such symptoms were continuous with the depressive episode itself. The Emory University School of Medicine Institutional Review Board approved the study protocol.

Data analysis

Participants were divided into 3 groups based on reported time of illness onset: pregnancy onset, illness onset during pregnancy; early postpartum onset, illness onset within the first 6 weeks postpartum; and late postpartum onset, illness onset after 6 weeks postpartum. Frequencies and percentages of the number of participants in each group were tabulated. Descriptive analyses of demographic and clinical data were conducted using frequency tests for categorical data and analysis of variance with post hoc Tukey-Kramer multiple pairwise comparison tests for continuous data.

Results

Of 315 women screened for participation, 209 fulfilled inclusion criteria. Prospective participants were excluded for taking psychotropic medication (n = 49), primary diagnosis other than major depressive disorder (n = 29), or inability to recall with specificity the time of the onset of this episode of illness (n = 28). The Table illustrates that of the 209 women included in the study, 24 (11.5%) reported pregnancy onset (mean onset 21.8 ± 12.7 weeks’ gestation), 46 (22.0%) reported late postpartum onset (13.3 ± 6.7 weeks), and 139 (66.5%) reported early postpartum onset (2.2 ± 1.7 weeks).

Statistical analyses of clinical depression rating scale scores and demographic data are summarized in the Table. Significant differences between the onset groups were limited. Women with depression onset during pregnancy were more likely to be unmarried at time of conception and had higher BDI scores at initial presentation than those with late postpartum onset. Those with a late postpartum onset were less likely to report a past history of depression. A stratified analysis demonstrated that those in the late onset group were less likely to have reported a history of postpartum depression but were not less likely to have reported a history of nonpuerperal depression.
Comment

All of the participants in this study fulfilled diagnostic criteria for major depression during the first postpartum year; however, one-third did not experience the onset of illness within the first 6 postpartum weeks. The results of the current study are consistent with previous reports of perinatal depression beginning during pregnancy or later than 6 weeks post partum. The statistically higher BDI scores in this group may also reflect such stressors, although the small absolute difference in scores is unlikely to be clinically meaningful.

The study’s data regarding past psychiatric history include two important findings. First, although nearly 90% of the pregnancy onset group had a past history of depression with over half reporting a history of PPD, these women were not referred for psychiatric evaluation until after delivery. Potential explanations for this delay from illness onset to treatment include: (1) women and their clinicians mistake the symptoms of antenatal depression for those of pregnancy; (2) they recognize the depression but purposely postpone treatment to avoid fetal antidepressant exposure; or (3) antenatal screening for maternal depression, even in a high-risk group with previous PPD, is not routinely conducted. The absence of a comparator group comprised of women referred antenatally for treatment of depression precludes any definitive conclusion. Second, the late postpartum onset group was less likely to report a past history of PPD, although not nonpuerperal depression, than those with an earlier onset. This may reflect that perinatal depression arising in pregnancy and the early postpartum is a distinct syndrome from that occurring in the late

Figure  Results of a scatterplot of the frequency of the onset of illness for women presenting for evaluation of postpartum depression across pregnancy and the first postpartum year.
postpartum or outside the puerperium altogether, although there is little evidence to support such a distinction, or those with a history of PPD are particularly vigilant for signs and symptoms indicative of relapse and thus recognize illness onset earlier.

The importance of clarifying the onset of an illness generally heralded as a postpartum event is not without clinical precedent. For example, recent data regarding postpartum autoimmune thyroiditis, historically considered a strictly postpartum phenomenon, have demonstrated that up to 50% have positive titers for thyroperoxidase autoantibodies during pregnancy. The ready identification of thyroperoxidase antibodies during pregnancy coupled with increasing evidence of their adverse impact upon obstetrical outcome and fetal neurodevelopment has led to new recommendations to conduct “prenatal” screening for “postpartum” thyroiditis.

Substantial clinical and preclinical data indicate that maternal depression and stress during the peripartum also have an untoward impact on obstetrical outcome and infant development. Furthermore, puerperal depression is one of the most commonly cited complications of childbirth and represents an eminently treatable condition that is readily identifiable in the primary care setting with minimal time investment. The mounting data that maternal depression and stress during the peripartum may adversely affect both obstetrical outcome and infant well-being underscore the need to clarify the windows for heightened monitoring of maternal mood, but the onus of identification cannot lie solely in postnatal obstetrical clinics because 11.5% of the current subjects had onset prior to delivery, and 22.0% experienced the onset of illness after the conventional 6-week postpartum follow-up visit. These findings indicate that clinical guidelines regarding the monitoring of puerperal depressive illness should be revised to include prenatal obstetrical visits and pediatric visits during the initial 6 months of the first postnatal year.

From a research perspective, investigations of obstetrical outcome and the impact of maternal depression on infant well-being must consider maternal mood prior to delivery as a potentially important variable. The limited success of previous studies endeavoring to clarify the role of gonadal steroids, psychosocial stressors, and other variables in the pathogenesis of PPD or to resolve the controversy as to whether PPD is neurobiologically distinct from nonpuerperal depression may in part be a consequence of examining heterogeneous

<table>
<thead>
<tr>
<th>Table</th>
<th>Demographic and clinical characteristics of study groups</th>
</tr>
</thead>
<tbody>
<tr>
<td>Variables</td>
<td>Study groups</td>
</tr>
<tr>
<td>Number</td>
<td>24 (11.5%)</td>
</tr>
<tr>
<td>Demographic variables</td>
<td></td>
</tr>
<tr>
<td>Age, y (mean ± SD)</td>
<td>30.8 ± 5.8</td>
</tr>
<tr>
<td>Race (n, %)</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>22 (91.7%)</td>
</tr>
<tr>
<td>Black</td>
<td>2 (8.3%)</td>
</tr>
<tr>
<td>Other</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Marital status (n,%),</td>
<td></td>
</tr>
<tr>
<td>Never married</td>
<td>3 (12.5%)</td>
</tr>
<tr>
<td>Married</td>
<td>17 (70.8%)</td>
</tr>
<tr>
<td>No longer married</td>
<td>4 (16.7%)</td>
</tr>
<tr>
<td>Education, y (mean ± SD)</td>
<td>14.8 ± 2.3</td>
</tr>
<tr>
<td>Clinical variables</td>
<td></td>
</tr>
<tr>
<td>Past history of major depression (n,%),</td>
<td></td>
</tr>
<tr>
<td>Any past depression</td>
<td>21 (87.5%)</td>
</tr>
<tr>
<td>Past postpartum depression</td>
<td>14 (58.3%)</td>
</tr>
<tr>
<td>Past nonpuerperal depression</td>
<td>14 (58.3%)</td>
</tr>
<tr>
<td>Family history of major depression (n,%),</td>
<td></td>
</tr>
<tr>
<td>Beck depression inventory (mean ± SD)</td>
<td>31.3 ± 9.0</td>
</tr>
<tr>
<td>Edinburgh postnatal depression scale (mean ± SD)</td>
<td>19.8 ± 5.0</td>
</tr>
</tbody>
</table>

Stowe, Hostetter, and Newport 525
samples of women with the onset of illness over a broad time period.

References


5. Purdy D, Frank E. Should post-partum mood disorders be given a more prominent or distinct place in the DSM-IV? Depress Anxiety 1993;1:59-79.


Implementing prenatal screening for cystic fibrosis in routine obstetric practice

Melissa H. Fries, Col, USAF MC,a,* Michael Bashford, Lt Col, USAF MC,b
Mark Nunes, MDc

Uniformed Services University of the Health Sciences, Bethesda, Md,a Air Force Medical Genetics Center, Keesler AFB, Miss,b and INOVA Medical Center, Fairfax, Va c

Received for publication April 9, 2004; revised June 29, 2004; accepted July 15, 2004

Objective: The purpose of this study was to assess the outcome of the type of prescreening counseling on choices for prenatal cystic fibrosis screening.

Study design: From October 2001 to November 2002, regardless of ethnicity, all prenatal patients (n = 855) at the Air Force Medical Genetics Center, Biloxi, Miss, received education on prenatal screening for cystic fibrosis by group genetic counseling either by a presentation by a genetics professional (430 patients) or by a similar audiovisual presentation only (425 patients). A combination pretest/postest document was used to evaluate learning and served as the consent. Partner testing was recommended for mutation-positive patients.

Results: Fifty-eight percent patients requested screening, of whom 68% were white. Regardless of the type of counseling, patients showed an improvement in knowledge based on pre- and posttest scores. There was no significant difference in choices to undergo screening on the basis of counseling method. Fifteen mutation carriers were identified. Only 6 partners of mutation-positive patients were available and consented to be tested. To date, no infants have been born with cystic fibrosis.

Conclusion: Audio-visual counseling is an effective means to educate patients about genetic screening and does not require a trained genetics professional to administer. Partner testing in mobile populations may prove problematic.

Cystic fibrosis (CF) is the most common life-shortening autosomal recessive disease among the European white population, with an incidence of 1 in 3300 live births. The condition is caused by mutations in the CF transmembrane conductance regulator gene on chromosome 7, which encodes a cyclic adenosine monophosphate—regulated chloride channel in epithelial cells. Patients with CF have chronic pulmonary disease and infections, gastrointestinal and nutritional abnormalities, salt loss, and male infertility related to obstructive
azoospermia. In the past, the condition was largely fatal. Improvements in care, particularly antibiotic therapy, have resulted in substantially increased survival; recent analyses have shown that the probability of a child born after 1989 living >45 years was 80.4%.

To date, >1200 mutations in the gene have been identified, although a single mutation, ΔF508, accounts for approximately 70% of the mutations in European white patients. The disease incidence varies with ethnic group. In populations of European white patients or Ashkenazi Jewish patients, the incidence is 1 in 3000 live births, with a carrier rate of 1 in 29 live births. The incidence is less in the Hispanic population in the United States (1/8500 live births; carrier rate, 1/46 live births); the black population in the United States (1/17,000 live births; carrier rate, 1/65 live births); and the Asian population in the United States (1/32,400 live births; carrier rate, 1/90 live births). The probability of the detection of mutations also varies with ethnicity. Using the standard recommended mutation panel, 97% of the mutations can be identified in the Ashkenazi Jewish population, 92% of which are in the European white population. Only 81% of the mutations can be identified in the black population, and 72% of the mutation scan be identified in the Hispanic population.

Because of the high CF carrier frequency and mutation detection rate in certain ethnic groups, carrier screening by genetic testing is now considered a reasonable option to provide information to at-risk couples. In April 1997, a consensus statement that was issued by the National Institutes of Health recommended CF carrier screening be offered to adults with a family history of CF, partners of individuals with CF, couples planning a pregnancy, and couples seeking prenatal testing. A preliminary economic evaluation of such a screening program indicated that the averted medical-care cost for affected infants would offset approximately 74% to 78% of the costs of the screening. However, before implementation of these recommendations in the obstetrics and gynecology community, practice guidelines, educational materials, informed consent protocols, and laboratory standards for testing were needed.

In October 2001, the American College of Obstetricians and Gynecologists (ACOG), in support of recommendations from the American College of Medical Genetics, advised CF carrier screening to be offered to individuals with a family history of CF, reproductive partners of individuals with CF, or couples in whom 1 or both partners were Ashkenazi Jewish or white and were planning a pregnancy or seeking prenatal care and be made available to all patients as desired. This statement was accompanied by patient education pamphlets and general information for providers. A specific implementation strategy was not detailed, although sequential testing (testing the female patient first, followed by her partner if she were found to be a carrier) was suggested. Our study details the outcome of a prenatal screening program for CF that was initiated at a large military medical facility with an ethnically diverse population. We studied the benefits of differing patient education protocols and identified a significant problem that arose while we were implementing our CF screening program.

**Methods**

The Air Force Medical Genetics Center at Keesler Air Force Base, Miss, initiated a pilot program for prenatal CF screening in antepartum patients on October 17, 2001, in response to the ACOG recommendations. Because this was a clinical program to implement a new recommendation, institutional review board approval was not indicated. A sequential testing strategy was implemented, with screening offered to pregnant women at their new mothers’ orientation class. This class provided information about the process of antepartum care and general health during pregnancy and was held at the first obstetrics visit. All patients, regardless of ethnicity, received group genetic counseling about CF carrier screening and its implications and were given a combined demographic form and pretest on CF knowledge, which they completed before counseling (Figure 1). Questions regarding the demographics on the pre/posttest were answered before the patients received the group counseling. During the first 7 months of the project, the counseling was accomplished by a slide presentation that was given by a genetics professional (M.H.F.), followed by a posttest (on the reverse of their pretest) that then became part of their formal consent. Although the posttest questions were slightly different in their wording from the pretest questions, their content was largely matched question for question. The genetics professional explained the slides and answered questions. After May 2002, group counseling was provided only by the slide show made by the Air Force Medical Genetics Center, with cued narration for each slide, which was administered by a Women’s Health nurse practitioner. The practitioner did not provide any supplemental counseling regarding the testing nor answer any questions; patients completed the posttest and signed their consent forms as previously accomplished. Patients were given the ACOG pamphlet regarding prenatal screening for CF to take home for further review, although their decision for or against testing was made at the time of the group counseling.

The decision to counsel all patients regardless of ethnicity was made to provide the greatest possibility for patient education and nondirective decision-making. The group format also enhanced educational efficiency. Although this approach differs somewhat from the recommendations, we felt it was most appropriate in
Figure 1: Pre/post test consent form for CF screening

Date________________ Name_______________________
Sponsor’s SSN_________________________ Age____________
Phone number___________________________
Are you pregnant? Yes No If yes, will this be your first baby? Yes No
Last Menstrual Period:________________________

What are your parents’ ethnic (racial) backgrounds?
White Nonhispanic Hispanic American African American Asian American
Ashkenazi Jewish Other:__________
Using the above list, what is your partner’s ethnic background?__________

Is there anyone in your or your partner’s family with cystic fibrosis? Yes No
If yes, who?________________________

Have you ever been tested for cystic fibrosis before? Yes No (if yes, when and where?________________________)

Cystic fibrosis is a severe illness which begins in early childhood and causes problems with the digestion and breathing. Testing is available to identify couples who may be at high risk for having a baby with cystic fibrosis. Please take this short quiz to see how much you know about this potential problem for your baby.

1. True or false (please circle): Cystic fibrosis is an inherited disease.

2. If a person has cystic fibrosis, he or she has inherited two abnormal genes for this condition,
   a. 1 from the mother and 1 from the father
   b. Both from the mother
   c. Both from the father
   d. From neither parent

3. True or false: You can carry one abnormal gene for cystic fibrosis and not have any health problems from it.

4. In which ethnic group is cystic fibrosis most common?
   a. African Americans
   b. Asian Americans
   c. Hispanic Americans
   d. European Caucasians

5. True or false: Genetic testing for cystic fibrosis is usually a blood test.

6. True or false: Genetic testing for cystic fibrosis can tell 100% that a person does not carry an abnormal gene for the condition.

7. True or false: A baby can have cystic fibrosis if only one of its parents is a carrier.

8. True or false: If both parents are found to be carriers for cystic fibrosis, they must have a test done on their baby before it’s born.

9. True or false: A person’s ethnic (racial) background can change how likely a genetic test for cystic fibrosis will come back abnormal.
Genetic testing for Cystic Fibrosis Consent Form

Now that you have learned about cystic fibrosis, please answer the following questions to help you decide if you want to have genetic testing performed for this condition.

1. True or false: You can be a carrier of cystic fibrosis and not have any medical problems or family history of the condition.

2. True or false: A baby can have cystic fibrosis if only one of its parents is a carrier.

3. True or false: You are more likely to be a carrier of cystic fibrosis if you are of European Caucasian background.

4. True or false: If a genetic test shows that the mother is a carrier of cystic fibrosis, the next step is to test the baby’s father.

5. True or false: A genetic test for cystic fibrosis can detect 100% of all carriers.

6. True or false: If the genetic test for cystic fibrosis is normal, the chance that a person could be a carrier is reduced but is never zero.

7. True or false: Parents who are both carriers of cystic fibrosis must have testing done on their baby before it’s born.

8. True or false: Mothers must be tested again in each pregnancy to see if they are still a carrier of cystic fibrosis.

9. True or false: The accuracy of genetic testing for cystic fibrosis depends on accurate information about race and family history.

After learning about cystic fibrosis, you have the choice to decide if you want to be tested to determine if you are a carrier or not. Please check the appropriate box and sign below.

_______ Yes, I have understood the answers to the above questions and the material presented to me, and I want to be tested to see if I carry a gene for cystic fibrosis. I understand that the results of the test may recommend further testing from my baby’s father. If both of us are found to be carriers, I will need genetic counseling and may be offered prenatal testing of my baby. I may also decide to have testing done on my baby after it’s born. I also understand that if my test is negative, there is still a very small chance that I could be a carrier.

_______ No, I do not wish to be tested for cystic fibrosis carrier status

__________________________________________    ____________________________
Signature                                          Date

Figure 1 (Continued).
meeting the needs of the particular ethnic mix of obstetric patients in our community.

All testing was accomplished at the Air Force Medical Genetics Center at Keesler AFB. DNA was extracted from specimens in a standard fashion, and screening was accomplished with multiplex polymerase chain reaction and allele-specific oligonucleotide reverse dot blot scored by chemiluminescence. Twenty mutations were tested initially. In September 2002, the panel was expanded to include the recommended American College of Medical Genetics 25 mutation panel, which added I148T, 711 C>T, G85E, 3120 C>G>A, 1898 C>G>A, 3659delC, 2184delA, and 1078delT to the group of other mutations that were tested. Test completion time was approximately 2 weeks.

If no mutations were identified, a report was generated by the genetics center and sent to the computer laboratory data system and to the patients’ obstetric files. The report indicated the patients’ postgenetic testing risk based on their ethnic background and was discussed by their obstetrics provider at the patients’ next obstetric visit. If a mutation were identified, the patient was contacted by telephone by a provider from the Air Force Medical Genetics Center laboratory, and testing from the father of the pregnancy was recommended. Patients had been informed of this recommendation during their initial counseling process. Patients did not receive formal genetic counseling at this point, with the plan to offer formal genetic counseling and prenatal diagnosis if both parents were identified as carriers.

Statistical comparison of groups on the basis of acceptance or declination of CF screening was performed using chi-squared evaluation of 2 by 2 contingency tables and 2-tailed Z test.

### Results

In the time period of October 2001 to November 2002, a total of 855 patients were counseled, of whom 827 completed pre/posttests and 489 requested CF screening (58.2%). Mean age of those patients who requested screening was 24.6 years and of those patients who declined screening was 25.2 years. Sixty-nine percent of the counseled patients were white, of whom 68% requested testing. Table I details the ethnic background of the total number of patients, those accepting testing, and their decision to accept testing on the basis of the type of counseling and ethnicity. There was no significant difference in overall acceptance of screening when those who were counseled by audiovisual means were compared with professional counseling, which was reconfirmed when the group was stratified by ethnicity.

Forty percent of the counseled patients (289/731 patients who indicated pregnancy status) were in their first pregnancy, with 60.5% of this group electing to have testing regardless of ethnic background (Table II). This was a significant increase (\( P < .03 \)) in the choice for testing among those who were in their first pregnancy when compared with those patients who were not in their first pregnancy.

In the group of 506 tested patients, based on their ethnic mix, a total of 15.23 mutations would have been
expected. Fifteen mutations were identified, of which 13 were ΔF508 mutations with 1 G5551D mutation and 1 ΔI507 mutation.

All patients, regardless of their choice for genetic testing or the method of counseling, showed a significant improvement in knowledge on the basis of the results of the 9-question pre- and posttests ($P < .01$; Table III). Pre- and posttest scores were not significantly different between the groups who accepted and declined screening. In the group who accepted screening, there was a significant difference ($P < .05$) between the pre- and posttest scores of those who were counseled by genetics professional versus audio-visual counseling groups, with the audio-visual group having a slightly higher mean pretest score and a slightly lower posttest score. In the group who declined testing, there was no significant difference in mean pre- or posttest scores on the basis of the type of counseling. Both groups, regardless of counseling method, however, did have a significant improvement in posttest scores compared with pretest scores.

The 15 patients with mutations that were identified by genetic screening were contacted by telephone by a genetics professional who discussed the significance of the test result and the importance of obtaining a blood specimen from the father of the pregnancy. Although all 15 patients were contacted multiple times, only 6 fathers ultimately consented to testing. No mutations were found in this group. The principal predictor of the success of obtaining paternal testing was marital status. Of the 9 married patients, 5 fathers submitted specimens. Of the 6 unmarried patients, only 1 father submitted a specimen. No prenatal CF diagnosis procedures were performed, because no maternal/paternal mutation couples were identified, and no carrier mothers elected to have amniocentesis in the absence of paternal testing. No infants who were born to the group of mutation-positive mothers have been diagnosed with CF either on newborn testing or by disease symptoms in the first 6 months of their lives.

**Table III**  
Pre- and postcounseling test scores

<table>
<thead>
<tr>
<th>Patients</th>
<th>Genetics professional counseling (%)</th>
<th>Audio visual counseling (%)</th>
<th>Pretest</th>
<th>Posttest</th>
<th>$P$ value</th>
<th>Pretest</th>
<th>Posttest</th>
<th>$P$ value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Acceptors (n = 481)</td>
<td>68.3 ± 17.5</td>
<td></td>
<td>64.5 ± 20</td>
<td>86.5 ± 14.8</td>
<td>.01</td>
<td>72.0 ± 20</td>
<td>80.2 ± 16.0</td>
<td>.01</td>
</tr>
<tr>
<td>Decliners (n = 336)</td>
<td>64.5 ± 20</td>
<td></td>
<td>14.9 ± 12.1</td>
<td>84.4 ± 14.9</td>
<td>.01</td>
<td>68.7 ± 22.1</td>
<td>81.8 ± 14.0</td>
<td>.01</td>
</tr>
</tbody>
</table>

Data are given as mean percentage of correct responses to questions ± SD.

* $N = 418$.

† $N = 399$.

Comment

This pilot program was well received by patients who, from their improved posttest scores, appeared to understand the concepts and materials that were presented. The acceptance rate for testing (58%) was in alignment with other genetic screening programs for CF, which had reported a 57% to 60% test uptake rate. Our data show a significant increase in choice for screening in primigravid women, which indicates that populations who have a higher number of primigravid women may have a higher screen uptake rate. In addition, because of the greater likelihood that patients in the decliner group may have had other children, it is possible that the previous birth of a healthy child may alter patients’ perceptions of their risk.

Pretest counseling was provided for all patients regardless of ethnicity. This was in accord with the ACOG recommendations that the test be made available to all populations. Not all patients in the high-risk population wished to have screening (only 68% of white patients chose screening) and some patients in lower-risk populations (approximately 35%-55% in black and Hispanic populations) desired screening. A higher proportion of Hispanic patients than was anticipated requested screening; the reasons for this are not clear and may warrant further investigation into CF screening attitudes in different ethnic groups, particularly because a cost-benefit review of prenatal CF screening in Hispanic patients anticipated a low screen uptake rate. Preliminary counseling placed the choice for testing with the patient. Given the increasing difficulty in the determination of ethnic background in some populations, allowing patients autonomy to determine whether they feel that screening has applicability for them eliminates the unintended exclusion of potential high-risk patients on the basis of the presumption of their ethnicity. Clearly, however, it is incumbent on counseling to advise patients on the limitations in risk reductions on the basis of their designation of ethnicity.

We encountered substantial difficulty in obtaining paternal blood specimens after diagnosis of a maternal mutation. Although this is not unexpected, given the mobile military population, it is likely to be a concern in nonmilitary clinical settings as well and may be compounded by the difficulty of obtaining medical insurance coverage for the cost of paternal testing. For this reason, the timing of formal genetic counseling was changed to offering this at the point of the identification of maternal
carrier status alone. The counseling focused on the nature of CF, population-based risk for affected infants, the need for partner testing, and the option of amniocentesis for fetal evaluation if paternal testing were not feasible or if such testing showed a paternal mutation. For a mutation-positive mother, if the ethnicity of the unavailable partner were white (carrier risk, 1:29, with 92% likelihood for identification of carrier status), the fetal risk to be affected would be 7.9 of 1000 live births, which would justify the 5 in 1000 live birth risk of amniocentesis without even testing the father. Couple screening, rather than sequential screening, could preclude the difficulty of obtaining paternal testing subsequent to maternal testing but may be cost prohibitive as a general screening policy or may be perceived as a limitation to patients whose partners are unavailable. If the partner were able to anticipate that he might not be available for future testing, couple screening rather than sequential screening might be valuable on a case-by-case basis. Obtaining paternal specimens continues to be preferable at our institution instead of moving a priori to prenatal diagnosis on the basis of maternal carrier status information alone. However, the option of amniocentesis for maternal carriers with unobtainable paternal testing may be an unexpected, but necessary, additional factor in the overall screening process.

Use of trained genetics professionals (such as genetic counselors, geneticists, maternal fetal medicine specialists, or genetic nurse specialists) is felt to be optimal for genetic counseling, but such providers are rare and may be unavailable at many facilities. A 2000 to 2001 survey of 190 family physicians from around the United States indicated that 23% of the physicians found it “very difficult or impossible” for their patients to get a consultation with a genetics professional (personal communication, Dr Louise Acheson, Case Western Reserve University, September 1, 2003). This difficulty, in association with an expanding potential for future genetic testing, will require innovative education strategies beyond increasing the numbers of formal genetic counselors and clinical geneticists who are trained annually.

Our study, which was based on pre- and posttest studies, found that patients were counseled effectively about the risks and benefits of CF screening in a group setting that used either a dedicated genetic counselor or an audio-visual presentation. The difference in pre- and posttest scores of those patients who accepted testing compared by type of counseling did show a slightly higher pretest score in those who received audio-visual counseling, with a slightly lower posttest score; this difference was not seen when the group who declined testing was compared. The explanation for this finding is unclear, and its clinical relevance is uncertain, given that both counseled groups showed a significant increase in knowledge by scores. We did observe that, for those patients who received counseling from a genetics professional, sufficient time was allotted before the slide presentation for patients to complete their pretest. This time was not always included when the audiovisual counseling was given, and patients were observed to be completing their pretest while they were watching the slide show, thus potentially increasing their pretest scores. After this observation, more time was allowed for the completion of the pretest before the audiovisual presentation was started.

The choice to have a genetic test does not always reflect an understanding of the significance of the test, and each counseling method may have benefits to different patients, on the basis of learning style. It is possible that an audiovisual presentation may provide greater clarity to some patients and enable them to understand better the implications of testing, particularly if decisions made by face-to-face counseling could be influenced positively or negatively by the personality of the counselor. Use of audio-visual programs could reduce the cost of counseling as part of CF screening programs as well. The process of panethnic education by audio-visual counseling would be amenable to web-based patient education methods, either in a group or an individual computer or kiosk educational process. Computerized educational programs have the advantage of self-pacing but do require language and computer literacy. The continued use of the CF screening audio-visual materials in a variety of settings will allow the further assessment of the value of this counseling approach.

References

Fetal transabdominal anatomy scanning using standard views at 11 to 14 weeks’ gestation

Constantin S. von Kaisenberg, MD, PhD,a Heidi Kuhling-von Kaisenberg, MD,a Elfriede Fritzer,b Sandra Schemm, MD,a Ivo Meinhold-Heerlein, MD,a Walter Jonat, MD, PhDa

Department of Obstetrics and Gynecology, University Hospital, Kiel, Germany,a MedStatistik, Gettorf, Germanyb

Received for publication May 5, 2004; revised August 24, 2004; accepted August 24, 2004

KEY WORDS
Nuchal translucency
Anatomy
Standard views
Scoring system
Visualization

Objectives: This study was undertaken to investigate fetal anatomy with the use of standard views and a scoring system, to investigate interobserver variability, and to compare ultrasound modes simultaneously with the measurement of nuchal translucency (11-14 weeks’ gestation).

Study design: Twelve fetal anatomic regions were defined as standard views (n = 60) and assessed with the use of a scoring system (1 = not seen, 2 = seen uncertainly, 3 = seen acceptably, 4 = well seen, and 5 = very well seen). The variation of scores and interobserver variability were analyzed (n = 40), the B-mode was compared with tissue harmonic and compound imaging (n = 60).

Results: The overall average score (11 + 0 to 13 + 6 weeks) with tissue harmonic and compound imaging was 3.56 (well seen) and increased with gestation. The highest score was for the neck and the lowest for the cerebellum. The proportion of identical scores for each given region showed a range of 58% to 83%. Tissue harmonic and compound imaging was significantly better than the plain B-mode, P < .001 (sign test).

Conclusion: Transabdominal fetal anatomy scanning with standard fetal anatomy views at 11 to 14 weeks of gestation is possible with good reproducibility and demonstrability when harmonic and compound imaging are used.

© 2005 Elsevier Inc. All rights reserved.

A study of 97 pregnancies that used transvaginal ultrasound examined the fetuses from 9 to 14 weeks and was aimed at body contours, long bones, fingers, face, palate, feet, toes, and the heart 4-chamber view, showing the detection of an increasing number of structures with increasing gestation.1 A study of 120 fetuses that compared transvaginal and transabdominal ultrasound in visualizing the first trimester conceptus found that vaginal sonography less than 10 weeks was superior to abdominal sonography and was a valuable tool in complementing abdominal sonography.2,3 A study investigating fetal anatomy at 11 to 14 weeks’ gestation that used both transabdominal and vaginal ultrasound found improved visualization of fetal anatomy with increasing gestation, which was about 98% at 14 weeks.4 In this study, criteria for adequate visualization are defined and the results are given as a percentage of anatomic regions, for which these criteria were met.4 Several studies used scoring systems to analyze individual aspects of first-trimester ultrasound, in particular, nuchal translucency (NT).5,6 This proved...
to be helpful in assessing measurements as part of an ongoing audit and for training purposes. No studies were found that evaluated and quantified the accuracy of an early transabdominal first-trimester fetal anatomy survey at 11 to 14 weeks.

The objective of our study was to investigate visualization of fetal anatomy simultaneous to the measurement of NT (11-14 weeks’ gestation), which, in the majority of cases, is measured transabdominally. We used very clearly defined standard views for this study and a well-defined scoring system was applied to quantify visualization of the various regions. The interobserver variability for the scores was assessed and the various ultrasound modes regarding the best resolution were compared.

Methods

Only viable pregnancies were included in the study. Gestation was 11 + 0 to 13 + 6 weeks corresponding to a crown-rump-length (CRL) of 45 to 84 mm. In all cases, a CRL was taken and NT was 0.7 mm or greater and 3 mm or less and there were no obvious structural fetal anomalies. Fetal loss with unknown karyotype and chromosomal abnormalities were excluded from the study. One case with trisomy 18 showed increased NT, low first-trimester biochemistry, and omphalocele. Another case with trisomy 13 showed alobar holoprosencephaly, proboscis, and symmetric hexadactyly of hands and feet. All ultrasound findings were confirmed by a postmortem examination, and there were no additional anomalies. One fetus had intrauterine fetal death at 34 weeks. Postmortem examination showed a knot in the umbilical cord and histologic signs of placental insufficiency, but no anomalies. All other fetuses were normal at birth. The body mass index (BMI) was calculated in all cases to investigate a potential correlation of the maternal weight to impaired visualization.

Standard fetal measurements (Figure 1) were taken by using 4 planes: (1) biparietal diameter (BPD), fronto-occipital diameter (FOD), head circumference (HC), anterior lateral ventricle (Va), posterior lateral ventricle (Vp), hemisphere (HEM); (2) transcerebellar diameter (TCD), cisterna magna (CM); (3) abdomen anterior posterior (AAP), abdomen transverse diameter (ATD), abdominal circumference (AC); and (3) femur length (FL), as previously described. We used additional views to systematically assess the visualization of fetal anatomy from 11 to 14 weeks in 60 singleton pregnancies using transabdominal ultrasound (Philips ATL HDI 5000 ultrasound machine, software version HDI 5000 4252-0901-05 185.13, Philips-ATL, Solingen, Germany). We used a transabdominal broadband curved array transducer (C5-2) of 2 to 5 MHz and a maximum display depth of 24.6 cm. All possible ultrasound modes were examined before selecting the 2 used for the study. The simplest option was the plain 2-dimensional (2D) B-mode with general resolution and depth. For fundamental Sono CT (Philips-ATL), as well as Harmonic Sono CT (Philips-ATL), overview, and target were available. For the study, we used the 2D B-mode general, subsequently referred to as ATL HDI 5000/B-mode, and the Harmonic Sono CT target, subsequently referred to as ATL HDI 5000/HDI 5000/ tissue harmonic imaging and compound imaging mode, representing the simplest option and the one with the highest resolution. Each fetal anatomic topographic region, including the skull, brain, cerebellum, face, spine, neck, thorax, heart (4-chamber view), abdominal wall, gastrointestinal tract, kidneys, and extremities was systematically assessed by using a scoring system (score 1 = not seen, 2 = seen uncertainly, 3 = seen acceptably, 4 = well seen, and 5 = very well seen). No pregnancy was omitted from the study because of overall poor visualization. All views were obtained in all fetuses. The average time used for 1 investigation of all the standard views described in this article was 14 minutes (range 10-21).

Figure 1 First-trimester anomaly scanning: standard views for fetal biometry (I-IV). Axial standard view of the fetal skull and brain showing intactness of cranium, falx cerebri, lateral ventricles with the plexus chorioideus, at 13 + 3 weeks (left), and the respective measurements BPD, FOD, Va, Vp, HEM (I), posteriorly slightly lower axial view of the head showing a normal shaped cerebellum and cisterna magna (right) for measurement of TCD and CM (II), axial view of the fetal abdomen, showing the spine, ribs, stomach and bifurcation of the portal vein for measurements of AAP, ATD, and AC (III), longitudinal view of the fetal femur for FL measurement (IV), 13 + 3 weeks. All photographs were taken in tissue harmonic and compound imaging mode (reprinted with permission from: von Kaisenberg et al. Fetal transabdominal biometry at 11-14 weeks of gestation. Ultrasound Obstet Gynecol 2002;20:564-74).
For each individual standard view in a subgroup of 40 fetuses, scores were applied during the scan by both the scanning operator as well as by an additional operator to assess the interobserver variability. The results remained undisclosed between the operators. This is a subjective comparison of how satisfied each of these sonographers was with the same view or picture. It is not a comparison of 2 examiners rescanning the same patient. Reproducibility of these findings is therefore not the main topic of this study, but rather individual operator satisfaction with the same examination. The interobserver variability was shown by the proportion of same scores for each given region. In addition, a coefficient for the strength of agreement was calculated (Kappa after Cohen: $<0.1 = \text{no}; \ 0.1-0.4 = \text{weak}; \ 0.41-0.6 = \text{acceptable}; \ 0.61-0.8 = \text{strong}; \ \text{and } 0.81-1 = \text{almost perfect agreement}$). Scores were recorded initially by using the regular B-mode and then repeated again by using the tissue harmonic imaging and compound imaging mode to compare the 2 different modes. The sequence of investigation was, however, randomly changed to reduce bias. Both operators were holders of the certificate of competence of the 11 to 14 weeks scan by the Fetal Medicine Foundation (FMF) and had significant experience in ultrasound.

**Statistical methods**

For all 12 fetal anatomic topographic regions, a sign test was used to compare the scores between the conventional ATL HDI 5000/B-mode and the newer ATL HDI 5000/tissue harmonic imaging and compound imaging mode ($n = 60$), with $P < .05$ considered to be statistically significant. The interobserver variability was reported by the proportion of same scores for each given region. In addition, the Kappa coefficient after Cohen was calculated to describe the strength of agreement as previously explained.

The BMI was derived from the maternal weight and height. A potential correlation of the BMI and the scores was examined by using Spearman rank correlation. The correlation coefficient Spearman rank investigates a potential correlation of the BMI and the scores reflecting the visualization. The value of the correlation coefficient can vary from minus to plus 1. A $-1$ indicates a perfect negative correlation, whereas a $+1$ indicates a perfect positive correlation. A correlation coefficient of zero means there is no relationship between the 2 variables. A negative correlation between the 2 variables indicates that the visibility decreases as the BMI increases. The significance of the correlation coefficient is investigated and $P < .05$ is considered to be significant.

**Definition of scores**

The following is a definition of scores:

1: Not seen; not even suggestive of a structure, no details.
2: Seen uncertainly; suggestive of a structure, but structure cannot be clearly seen, no details.
3: Seen acceptably; structure can be clearly seen, but no details.
4: Well seen; structure and details can be clearly seen.
5: Very well seen; structure and details can be very clearly seen, no better visualization possible.

**Definition of standard views**

We used 4 standard views (I-IV) (Figure 1) to measure first-trimester biometry, as previously described. Each fetal topographic region was then further investigated by using several standard planes described later in the article. One score was given to each 1 of the 12 fetal topographic regions, although in some regions more than 1 plane was examined.

**Skull:** Axial view of fetal skull to observe intactness of cranium (I), (Figure 1).

**Brain:** Axial view of fetal brain showing falx cerebri, anterior and posterior horns of the lateral ventricles with the plexus chorioideus, and for measurement of BPD, FOD, and HC, Va, Vp, and HEM (I) (Figure 1).

**Cerebellum:** Posteriorly slightly lower axial view of the head showing a normal-shaped cerebellum and cisterna magna for measurement of TCD and CM (II) (Figure 1).

**Face:** Orbits with presence of lenses and eyeballs, maxillae and mandibles using parallel axial planes, profile for frontal bone, skin at tip and root of nose, nasal bone, maxillary and mandible and frontal view for nostrils, and upper and lower lips (Figure 2).

**Spine:** Complete vertebral column seen in sagittal, frontal, and axial planes with normal overlying skin (Figure 3).

**Neck:** NT measured according to FMF guidelines.

**Thorax:** Normal shape, echogenicity and hypoechoic interface between abdomen and thoracic cavities (Figures 4 and 5).

**Heart:** 4-chamber view, symmetric ventricles and atria, intact interventricular septum, (Figure 4).

**Abdominal wall:** Axial view of the fetal abdomen, showing the spine, ribs, stomach, and bifurcation of the portal vein for measurements of AAP, ATD, and AC (III), and normal cord insertion (Figures 1 and 5).

**Gastrointestinal tract:** Normal echogenicity of bowel (Figure 5).

**Kidneys:** Cortex and pelvis of both kidneys, bladder (hypoechoic structure in midline of pelvis) (Figure 3).

**Extremities:** Longitudinal view of the fetal femur for FL measurement (IV), tibiae and fibulae, normal posture and shape of feet (toes), humeri, ulnae and radii, and normal posture and shape of hands (fingers) (Figure 5).

**Results**

The mean gestational age was 12 + 3 weeks, the median gestational age was 12 + 4 weeks. The overall average
score (11 + 0 to 13 + 6 weeks) with the use of tissue harmonic and compound imaging was 3.56 (seen acceptably) with a range of 2.67 for the cerebellum and a score of 4 for the neck (Table I). The overall average score (11 + 0 to 13 + 6 weeks) with the use of the plain B-mode was 2.83 (seen uncertainly) with a range of 1.9 for the cerebellum and a score of 3.75 for the neck (Table I).

With tissue harmonic imaging and compound imaging, the anatomy was on average more clearly visible in 66% of the cases, the same as in the conventional B-mode in 33%, and worse in only 1% (Table I). This was statistically significant for all 12 fetal anatomic topographic regions, \( P < .001 \) (sign test; \( n = 60; \) Table I).

The interobserver variability was shown by the proportion of identical scores for each given region and ranged between 58% and 83% (Table II). Often, 2 observers showed the same parallel interpretation for the scores, however, with 1 score difference. In addition, a coefficient for the strength of agreement was calculated (Kappa after Cohen) as previously described. Kappa ranged from 0.16 to 0.61 (abdomen and extremities vs spine; \( n = 40; \) Table II).

Increasing visibility with increasing gestation was investigated by analyzing the data by week for both the plain B-mode and for tissue harmonic and compound imaging. The overall score with the use of tissue harmonic and compound imaging at 11 + 0 to 11 + 6 weeks was 3.17 (seen acceptably) with a range of 2.08 for the cerebellum and a score of 3.92 for the neck (Table III). The overall score with the use of tissue harmonic and compound imaging at 12 + 0 to 12 + 6 weeks was 3.67 (seen acceptably) with a range of 2.73 for the cerebellum and a score of 4.32 for the spine (Table III). The overall score with the use of tissue harmonic and compound imaging at 13 + 0 to 13 + 6 weeks was 3.65 (seen acceptably) with a range of 3.0 for the heart and a score of 4 for the skull, brain and neck (Table III). There was an increase in the scores from 11 to 14 weeks (mean overall average score at 11 weeks: 3.17; at 12 weeks: 3.67; and almost identical at 13 weeks: 3.65 [seen acceptably]). In all cases, tissue harmonic and compound imaging was better when compared with the plain B-mode (Table III).

The BMI was calculated for all patients (\( n = 60 \)), the mean was 24.44 (range 17.30-36.84). The results investigating a potential correlation of the BMI to the

<table>
<thead>
<tr>
<th>Table I</th>
<th>Overall mean scores for fetal anatomy examined in a standardized way from 11 to 14 weeks</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>ATL HDI 5000 Mean score</strong></td>
<td><strong>ATL HDI 5000 Tissue harmonic and compound vs B-mode</strong></td>
</tr>
<tr>
<td></td>
<td>B-mode</td>
</tr>
<tr>
<td>Skull</td>
<td>3.40</td>
</tr>
<tr>
<td>Brain</td>
<td>2.63</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>1.90</td>
</tr>
<tr>
<td>Face</td>
<td>2.57</td>
</tr>
<tr>
<td>Spine</td>
<td>2.82</td>
</tr>
<tr>
<td>Neck</td>
<td>3.75</td>
</tr>
<tr>
<td>Thorax</td>
<td>2.97</td>
</tr>
<tr>
<td>Heart</td>
<td>2.63</td>
</tr>
<tr>
<td>Abdomen</td>
<td>2.83</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>2.85</td>
</tr>
<tr>
<td>Kidneys</td>
<td>2.50</td>
</tr>
<tr>
<td>Extremities</td>
<td>3.12</td>
</tr>
<tr>
<td><strong>Average mean score</strong></td>
<td>2.83</td>
</tr>
</tbody>
</table>

Score 1: not seen, 2: seen uncertainly, 3: seen acceptably, 4: well seen; and 5: very well seen. Tissue harmonic and compound mode is better, the same or worse compared with the B-mode (\( n = 60 \)).

<table>
<thead>
<tr>
<th>Table II</th>
<th>Results from the tissue harmonic and compound imaging mode</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Percentage agreement</strong></td>
<td><strong>Kappa (Cohen)</strong></td>
</tr>
<tr>
<td>Skull</td>
<td>83% (68%-93%)</td>
</tr>
<tr>
<td>Brain</td>
<td>59% (42%-74%)</td>
</tr>
<tr>
<td>Cerebellum</td>
<td>71% (54%-84%)</td>
</tr>
<tr>
<td>Face</td>
<td>66% (49%-80%)</td>
</tr>
<tr>
<td>Spine</td>
<td>78% (62%-89%)</td>
</tr>
<tr>
<td>Neck</td>
<td>80% (65%-91%)</td>
</tr>
<tr>
<td>Thorax</td>
<td>68% (52%-82%)</td>
</tr>
<tr>
<td>Heart</td>
<td>76% (60%-88%)</td>
</tr>
<tr>
<td>Abdomen</td>
<td>63% (47%-78%)</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>73% (57%-86%)</td>
</tr>
<tr>
<td>Kidneys</td>
<td>68% (52%-82%)</td>
</tr>
<tr>
<td>Extremities</td>
<td>58% (42%-74%)</td>
</tr>
</tbody>
</table>

The proportion of 2 operators giving the identical score for each region is reported and the interobserver variability as a coefficient for the strength of agreement was calculated (Kappa after Cohen: <0.1: no; 0.1-0.4: weak; 0.41-0.6: acceptable; 0.61-0.8: strong; and 0.81-1: almost perfect agreement) (\( n = 40 \)).

* Numbers in parentheses are 95% CIs.
scores showed that there was a significant correlation of the B-mode with the BMI \(^{(4.0)}\) and the lowest for the cerebellum (2.67) (Figure 1), whereas most of the other anatomic regions had interobserver variability for the scores (Table II), the variation of the scores with increasing gestation (Table III, Figure 6), and the ultrasound plain B-mode versus tissue harmonic and compound imaging demonstrating the best resolution (Tables I and III, Figure 7). Also, factors known to influence visibility, such as the BMI, were correlated with the results (Table IV). The main findings show an average score of 3.67 (seen acceptably) at 12 weeks by using tissue harmonic and compound imaging (Table III, Figures 2 to 7) and an overall average score (11-14 weeks) of 3.56 (seen acceptably) (Table I, Figures 2 to 7). This indicates good visibility throughout the period of 11 to 14 weeks (Figure 6).

The overall mean scores (tissue harmonic and compound imaging) for fetal anatomy for the entire period of 11 to 14 weeks showed the highest score for the neck (4.0) and the lowest for the cerebellum (2.67) (Figure 1), whereas most of the other anatomic regions had intermediate scores (3-4) (Table I, Figures 2 to 5). A similar distribution was found with the B-mode. This seems logical because at 11 weeks the score for the cerebellum is only 2.08, increasing to 3.2 at 13 weeks (Table III), resulting in a low overall mean score (Table I). The cerebellum shows the largest increase in visibility for the observed period. To investigate the best resolution,
The interobserver variability showed identical results in 58% to 83% (Table II). The highest proportion of correspondence was for the skull (83%) and the lowest for the extremities (58%). Often, 2 observers showed the same parallel interpretation for the scores, however, with 1 score difference, leading to nonindetical results (Table II). A similar assessment was therefore not necessarily reflected in the proportion of giving the identical score for each region. With the Kappa after Cohen coefficient for the strength of agreement, the results were even worse, with Kappa after Cohen values ranging from 0.16 to 0.61 (Table II). The highest Kappa after Cohen value was for the spine (0.61) and the lowest for the extremities and abdomen (0.16). Interestingly, the mean visibility score for tissue harmonic and compound imaging decreased slightly for some anatomic structures from 12 to 13 weeks, in particular for the spine, neck, heart, kidneys, and extremities (Table III). No apparent reason for this could be identified by simply looking at the images and raw data. However, it became obvious that “dense” tissues, such as the spine, skull, and heart, showed a low variation during the period of 11 to 14 weeks, whereas “soft” tissues, such as the cerebellum and the brain, showed the highest increase in the scores (Table III, Figure 6). In all cases, tissue harmonic and compound imaging was better when compared with the plain B-mode (Table III, Figure 7). We also investigated whether the BMI affected fetal visibility. There was, however, no obvious correlation between the BMI and fetal visualization.

It seems that a systematic approach to measure fetal biometry at 11 to 14 weeks (Figure 1), as well as to investigate fetal anatomy using well-defined standard planes (Figures 2 to 7), may yield important information regarding fetal anatomy and may be the basis for the further management of a pregnancy.

In conclusion, this study shows that transabdominal fetal anatomy scanning with standard fetal anatomy views at 11 to 14 weeks of gestation is possible with good reproducibility and demonstrability when harmonic and compound imaging are used.

**Acknowledgments**

We thank MedStatistik for study planning, statistical assessment, advice, and critical comments in preparing the manuscript.

**References**

Fetal and neonatal alloimmune thrombocytopenia in pregnancies involving in vitro fertilization: A report of four cases

Brian R. Curtis, MS,a,* James B. Bussel, MD,b Marilyn J. Manco-Johnson, MD,c Richard H. Aster, MD,a Janice G. McFarland, MDa

The Blood Center of Southeastern Wisconsin, Milwaukee, Wis,a New York Presbyterian Hospital, New York, NY,b Department of Pediatrics, University of Colorado Health Sciences Center, Aurora, Colo,c

Received for publication March 15, 2004; revised August 11, 2004; accepted September 2, 2004

KEY WORDS
Fetal and neonatal alloimmune thrombocytopenia
Assisted reproductive technology
In vitro fertilization
Human platelet antigen-1a

Objective: We report four cases of neonatal alloimmune thrombocytopenia (NATP) in pregnancies achieved with in vitro fertilization.

Study design: Three cases used surrogate carriers, and the fourth a donor egg. Sera from gestational carriers were tested for platelet antibodies by flow cytometry and enzyme-linked immunosorbent assay. Platelet antigen genotyping of biologic mothers, fathers, and surrogates was performed by amplification of DNA by using polymerase chain reaction with sequence-specific primers.

Results: In all 4 cases, NATP resulted from an incompatibility between the fetus and gestational carrier for the platelet-specific alloantigen HPA-1a. Four infants were born severely thrombocytopenic (platelets <50,000/μL), 2 had antenatal intracranial hemorrhage, and 1 fetus expired in utero at 29 weeks.

Conclusion: NATP can occur in the setting of assisted reproductive technology. Because of the great costs, both financial and emotional, associated with these pregnancies, we strongly recommend that all women be typed for HPA-1a before serving as a surrogate mother.

© 2005 Elsevier Inc. All rights reserved.

Fetal and/or neonatal alloimmune thrombocytopenia (NATP) occurs when a pregnant woman produces an alloantibody that reacts with a paternal platelet-specific antigen carried on fetal platelets. The frequency of NATP is approximately 1:1000 live births1,2 and accounts for 3% of all neonatal thrombocytopenias and 27% of severe cases (platelets <50,000/μL).3 Twenty-three different platelet antigens have been implicated as targets for antibodies in NATP with the platelet antigen PlA1 (HPA-1a) being the causative antigen in 85% of cases.1,5,6 Once maternal immunoglobulin G (IgG) antibodies cross the placenta and react with the corresponding antigen(s) on fetal platelets, the resulting fetal and/or neonatal thrombocytopenia leads to intracranial hemorrhage (ICH) in 10% to 20% of cases.7,8

Supported in part by grant HL-13629 from the National Heart Lung and Blood Institute.

* Reprint request: Brian R. Curtis, MS, MT(ASCP)SBB, The Platelet and Neutrophil Immunology Laboratory, The Blood Center of Southeastern Wisconsin, PO Box 2178 Milwaukee, WI 53201-2178.
E-mail: brcurtis@bcsew.edu

0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.
and death in 1% to 3%. Because 60% of NATP cases occur in first pregnancies and prenatal typing of women for platelet antigens is not performed, the diagnosis is usually not made until after birth of an affected infant.

The use of in vitro fertilization (IVF) and related procedures (assisted reproductive technology [ART]) has increased steadily since 1981 when the first infant was conceived from IVF in the United States. In 1998, 0.7% of 3.9 million births resulted from the use of ART, and 35,025 infants were born after ART procedures performed in 2000. As in naturally conceived pregnancies, in those resulting from ART, screening for parental platelet antigen incompatibility is not performed nor is it performed on potential surrogates. Thus, a small but significant number of ART births are also at risk of being affected by NATP. In addition, unlike in a naturally conceived pregnancy, it is possible NATP could develop in an ART pregnancy, in which the fetus is homozygous for the platelet antigen to which maternal antibodies have been produced (Figure). This scenario creates the possibility of an even more severely affected infant.

We describe the first reported cases of NATP in the setting of ART. All 4 cases involved IVF procedures, and 3 were surrogate pregnancies. In each case, there was incompatibility between the gestational carrier and the fetus for the platelet-specific antigen HPA-1a and 3 were homozygous HPA-1a/1a. Three infants were born with severe thrombocytopenia, 2 had ICH develop, and 1 had fetal demise at 29 weeks’ gestation.

Case reports

Case 1

A 30-year-old white woman was implanted with a fertilized embryo from 2 other individuals. It was her first surrogate pregnancy, and it resulted in the birth of term twins. Both had petechiae and bruising and were found to be thrombocytopenic (platelet counts of 27,000/μL and 23,000/μL) without ICH. They were treated with platelet transfusions and intravenous immunoglobulin (IVIG) and both recovered uneventfully. Three years later she served as a surrogate for another couple. After successful implantation with the embryo, the previous history of the thrombocytopenic twins was discovered. A serologic evaluation at 11 weeks’ gestation revealed the egg and sperm donors were both incompatible with the surrogate for the platelet-specific antigen HPA-1a. Because of the high risk to the fetus of NATP, the surrogate was treated with IVIG at 1 g/kg per week beginning at week 16. Periumbilical vein blood sampling (PUBS) was performed at 22, 30, and 38 weeks’ gestation revealing fetal platelet counts of 159,000/μL, 129,000/μL, and 176,000/μL. At 38 weeks, a normal, healthy infant was delivered with a cord blood platelet count of 171,000/μL.

Case 2

A young woman with no previous history of affected neonates was implanted with an embryo resulting from an IVF procedure that used her sister’s egg and sperm from the sister’s husband. At 37 weeks’ gestation, the surrogate delivered twins spontaneously, both of whom were severely thrombocytopenic (platelets <5,000/μL), and covered with petechiae over the face and trunk. Cranial ultrasounds and computed tomographic scans revealed ICH in both twins that were determined to have occurred antenatally. The twins were treated with a single random donor platelet transfusion that resulted in a modest rise in platelet levels to 80,000/μL, and 1 twin also received a single dose of 1 g/kg of IVIG. Both recovered uneventfully thereafter.

Case 3

A 28-year-old woman was implanted with an embryo produced by IVF that used sperm and egg from an unrelated couple. This was her first surrogate pregnancy. She presented at 11 weeks and revealed to the physician during examination that she had delivered 3 previous infants of her own, all born with significant thrombocytopenia. A NATP workup revealed that the surrogate was incompatible with the fetus for HPA-1a with strong HPA-1a-specific antibodies in her serum, and from these results a decision was made to check the fetal platelet count. A PUBS performed at 26 weeks showed the fetus had a platelet count of 40,000/μL. The mother consented to be enrolled in an antenatal treatment study and was randomly assigned to steroids (prednisone, 0.5 g/kg per week). The protocol called for a repeat PUBS at 30 weeks, and a failure to respond would result in IVIG being added to her therapy. Unfortunately, at 28 weeks’ gestation the surrogate reported lack of fetal movement, and an ultrasound at 29 weeks revealed the fetus had expired, but there was no evidence of ICH. However, no information could be obtained as to the cause of death because an autopsy of the fetus was not performed.

Case 4

A 49-year-old woman, gravid 1 para 1, was implanted with an embryo produced by IVF that used her husband’s sperm and an egg from an unrelated donor. After an uncomplicated term pregnancy, she gave birth to an apparently normal, healthy infant. Bruising on the limbs and scattered petechiae were noted on day 1 of life, and a complete blood cell count revealed a platelet count of 19,000/μL. The infant was admitted to the neonatal intensive care unit and ampicillin, cefotaxime, and a single dose of IVIG (1 g/kg) were administered. A repeat platelet count 1 hour after IVIG infusion was...
41,000/µL. A head ultrasound showed no ICH. The platelet count rose steadily over the next 3 days to 128,000/µL at discharge, eventually reaching normal levels.

Material and methods

Patients studied

Blood samples from patients in all 4 cases had been referred to the Platelet and Neutrophil Immunology Reference Laboratory of The Blood Center of Southeastern Wisconsin for routine serologic evaluation to confirm a diagnosis of NATP. Institutional Review Board approval was obtained for retrospective analysis of test data.

Laboratory evaluation for NATP

Sera from the gestational carriers were screened for platelet antibodies by flow cytometry, and antigen-capture enzyme-linked immunosorbent assay (ELISA [ACE]), as described previously. HPA-1a antibody specificity was determined by modified ACE (MACE), in which target platelets of known HPA alloantigen phenotype are sensitized by test serum, washed, and lysed with the nonionic detergent Triton X-100. The released membrane glycoproteins (GPs) are then captured in the wells of microtiter plates containing immobilized monoclonal antibodies specific for individual membrane GPs, i.e., GPIIb/IIIa. IgG antibodies captured with the GPs are then detected by ELISA with the use of alkaline phosphatase-labeled goat antihuman IgG. Details of the assay have been described previously.

Genotyping of patient DNA for HPA polymorphisms was performed by polymerase chain reaction (PCR) amplification with sequence-specific primers, followed by electrophoresis of PCR products on ethidium bromide–stained agarose gels, and inspection for specific allelic bands, as reported previously.

Results

HPA-1a incompatibility caused NATP in all 4 cases

In 3 of the cases, IVF was performed by using eggs from the biologic mothers and sperm from the biologic fathers, and the resulting embryos were implanted in and carried by surrogates (cases 1, 2, and 3). In case 4,
IVF was performed by using an egg from an anonymous donor and sperm from the husband of the woman whom carried the implanted embryo. Each father typed homozygous (HPA-1a/1a) for HPA-1a obligating the fetus in each case to be HPA-1a positive, and creating an incompatibility for this antigen between the fetus and gestational carrier (Table). The gestational carrier in each of the 4 cases typed negative (HPA-1b/1b) for the PlA1 platelet alloantigen putting them at risk to produce antibodies when exposed to HPA-1a positive fetal platelets. In cases 1, 3, and 4, the biologic mothers, like the fathers, typed homozygous for HPA-1a obligating the fetus conceived through IVF to be homozygous for HPA-1a (Table).

PlA1 antibodies were detected in sera from 3 of 4 gestational carriers

Strong IgG antibodies specific for the HPA-1a platelet alloantigen were detected by MACE in sera from the gestational carriers in cases 2, 3, and 4 (Figure). HPA-1a-specific IgG platelet antibodies were detected in serum from the surrogate in case 1 after her first surrogate pregnancy (data not shown), but no platelet-specific antibodies could be detected in her serum throughout gestation of her second surrogate pregnancy during which she received IVIG (Figure).

Comment

American couples have increasingly used ART since the advent of these procedures over 20 years ago. Although ART has enabled many couples to have a child who otherwise could not, the use of this technology is not without its risks. Studies have shown that infants conceived through ART procedures are more likely to have low birth weights and increased rates of major birth defects. In this report, we present 4 pregnancies conceived by using ART that resulted in 5 infants affected with NATP, a disorder not previously described in the setting of ART. In all 4 cases, NATP resulted from an incompatibility between the fetus and gestational carrier for HPA-1a, the platelet alloantigen implicated in 85% of NATP cases. Interestingly, in 3 of the 4 cases, the fetuses were homozygous for the HPA-1a antigen, which could not occur in a naturally conceived pregnancy in which there is an incompatibility between the parents for HPA-1a (mother HPA-1b/1b, father HPA-1a/1a or -1a/1b). Because platelets from a homozygous HPA-1a/1a fetus would express twice as much incompatible antigen, it is conceivable they could be more highly sensitized by maternal antibody, resulting in a more severely thrombocytopenic fetus or infant with increased risk of hemorrhage. Whether this actually occurred in the current cases is not known. However, in 2 cases all 3 infants were born with platelet counts below 28,000/μL, and in the third case, the fetus had a platelet count of only 40,000/μL at 26 weeks’ gestation and expired 3 weeks later (Table).

Cases 1 and 3 confirm the importance of taking a careful history from a surrogate mother before implantation of a fertilized ovum. Taking a simple history for prior episodes of NATP from the surrogates in these cases could have prevented this serious complication. Fortunately, in case 1, the history of NATP was discovered in time to begin prenatal treatment, which was effective. All 4 cases support the recommendation by some that all pregnant women be screened for HPA-1a.

The financial cost of a typical pregnancy achieved through IVF is significant ($12,000-$15,000) and even

<table>
<thead>
<tr>
<th>Case</th>
<th>Gestational Carrier</th>
<th>ART Procedure</th>
<th>Platelet antigen type</th>
<th>Antenatal Therapy</th>
<th>Birth Platelet ct.</th>
<th>ICH (Yes/No)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (first pregnancy—twins)</td>
<td>Surrogate</td>
<td>IVF with maternal egg</td>
<td>NT NT NT NT None 27,000/μL 23,000/μL No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2 (twins)</td>
<td>Surrogate</td>
<td>IVF with maternal egg</td>
<td>HPA-1a/1a HPA-1a/1a HPA-1b/1b HPA-1a/1a IVIG 1 gm/kg/wk 171,000/μL No</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>Surrogate</td>
<td>IVF with maternal egg</td>
<td>HPA-1a/1a HPA-1a/1a HPA-1b/1b HPA-1a/1b None 5,000/μL Both twins Yes Both twins</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>Biologic mother</td>
<td>IVF with donor egg</td>
<td>HPA-1b/1b HPA-1a/1a NA HPA-1a/1a prednisone 0.5 gm/kg/wk Fetal demise - 29 weeks Unknown</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

NA. Not applicable; NT, not tested.
greater when using a surrogate carrier, and the emotional costs suffered by all parties involved when a fetus or infant is affected with NATP are immeasurable. For a relatively small additional cost ($100-$150), the 3 surrogate mothers described in this report could have been typed for HPA-1a/1b, and NATP could have been prevented. For these reasons, it should probably be mandatory that all women be typed for HPA-1a/1b before serving as a surrogate mother. We strongly recommend that physicians who evaluate prospective surrogate mothers: (1) obtain thorough prepregnancy histories, especially regarding any previous children born with bleeding and/or thrombocytopenia; (2) consider routine prepregnancy typing of gestational carriers (especially surrogates) for HPA-1a; and (3) be mindful that a fetus of homozygous HPA-1a/1a platelet type is possible and could be more severely affected with NATP than would be possible in a natural pregnancy.

References

8. Radder CM, Brand A, Kanhai ETTHHH. Will it ever be possible to balance the risk of intracranial hemorrhage in fetal or neonatal alloimmune thrombocytopenia against the risk of treatment strategies to prevent it? Vox Sang 2003;84:318-25.
Role of *Helicobacter pylori* infection in iron deficiency during pregnancy

Maria Weyermann, PhD,a,* Dietrich Rothenbacher, MD,a Lydia Gayer,a Günter Bode, PhD,a Guido Adler, MD,b Dieter Grab, MD,c Felix Flock, MD,c Hermann Brenner, MDa

Department of Epidemiology, The German Centre for Research on Ageing, Heidelberg, Germany, a Departments of Internal Medicine I, b and Obstetrics and Gynecology, c University of Ulm, Ulm, Germany

Received for publication March 1, 2003; revised August 7, 2004; accepted August 23, 2004

**Objective:** We investigated the possible role of *Helicobacter pylori* infection in iron deficiency during pregnancy in a large group of mothers in Germany after the birth of their baby under special consideration of iron supplementation.

**Study design:** All women who were delivered of their baby between November 2000 and November 2001 at the Department of Gynecology and Obstetrics at the University of Ulm, Germany, were recruited for the study. Hemoglobin levels at various points of time during pregnancy were obtained from the mothers’ health charts. Current *H pylori* infection was determined by 13C-urea breath test. We used multiple linear regression analyses to assess the impact of infection status on hemoglobin level at the beginning of pregnancy and on hemoglobin change during pregnancy.

**Results:** Twenty-three percent of the 898 mothers had a *H pylori* infection, and 20% of the mothers had a hemoglobin level below 12 g/dL at the beginning of pregnancy. Compared with uninfected mothers, mothers with *H pylori* infection had a lower mean hemoglobin level at the beginning of pregnancy (-0.25 g/dL; 95% CI, -0.49, -0.003) and a more unfavorable change in hemoglobin level during pregnancy (-0.14 g/dL; 95% CI, -0.38, 0.10).

**Conclusion:** This study supports a possible moderate, but still relevant, independent role of *H pylori* infection in iron deficiency during pregnancy.© 2005 Elsevier Inc. All rights reserved.

Despite the apparent availability of a high-quality diet, in Europe and in the United States, iron deficiency is considered one of the main nutritional deficiency disorders.1,2 Among fertile women, 80% have iron reserves that are below the required minimum during pregnancy, and one half of them have virtually no iron stores. During pregnancy, iron absorption increases, but not enough to compensate for the strongly increasing demand for absorbed iron.3 Placebo-controlled studies on iron treatment in pregnancy have shown that iron-treated pregnant women have greater iron reserves, higher hemoglobin levels, and a lower prevalence of iron-deficiency anemia and that an iron supplement of 65 mg/d during 20 weeks of gestation is adequate to prevent iron-deficiency anemia.
Physiologic and epidemiologic evidence suggest that *Helicobacter pylori* may interfere with iron metabolism. Barabino suggested that the *H pylori*-infected antrum could act as a sequestering focus for serum iron by means of outer membrane receptors of the bacterium. The latter are able to capture and use iron for growth from human lactoferrin in vitro. However, there is a lack of large-scale epidemiologic studies regarding a possible role of *H pylori* infection in anemia during pregnancy, when iron demand is particularly high.

The aim of this study was to determine a possible role of an *H pylori* infection in anemia at the beginning and during pregnancy in a large group of mothers in Germany and a possible interaction between *H pylori* infection and iron supplementation during pregnancy.

**Methods**

**Study design and population**

All women who came to the Department of Gynecology and Obstetrics at the University of Ulm between November 2000 and November 2001 for the delivery of their baby were recruited for the study. In Germany, women on average stay in the hospital for approximately 5 days after delivery, and recruitment was done during this time window. The Department of Gynecology and Obstetrics is the only major department of obstetrics in the study area and serves most of the childbearing women in the city of Ulm and the nearby communities.

To obtain a birth cohort of healthy babies, we excluded 544 women who were at <32 gestational weeks, with a baby of <2500 g birth weight or who had transfer of their infants to inpatient pediatric care immediately after delivery. Furthermore, we excluded women with no understanding of German, Turkish, or Russian language and all women who left the hospital immediately after birth. Overall, 1066 of the 1593 eligible mothers (67%) participated in the study.

Participation was voluntary, and informed consent was obtained in each case. The study was approved by the Ethics Board of the University of Ulm and the Physicians’ Boards of the states of Baden-Wuerttemberg and Bavaria.

**Data collection**

All mothers underwent standardized interviews that were conducted by trained interviewers during hospitalization after delivery. Interviews contained detailed questions about housing and living conditions, lifestyle factors, medical history, and health status during pregnancy. In particular, we asked participants about history and duration of iron supplementation during pregnancy. The interview was also offered in Turkish and Russian, because this was the mother’s tongue of a large proportion of mothers. A standardized form was used to collect laboratory and anthropometric data during pregnancy from the mother’s pregnancy health chart.

**Laboratory analyses**

After the interviews were conducted, active infection with *H pylori* among the mothers was determined with the 13C-urea breath test (UBT). First, an initial breath sample was collected in a plastic bag. The women then received 200 mL of apple juice that contained 75 mg of non-radioactive labeled 13C-urea (Mass Trace, Woburn, Mass). After 30 minutes, a second breath sample was collected. The breath samples were analyzed with an isotope selective nondispersive infrared spectrometer (NDIRS: Wagner Analytical Systems, Bremen, Germany). A change of the 13CO2/12CO2 ratio over baseline of > 4 permille was considered positive. Sensitivities and specificities of the UBT close to 100% have been reported consistently, which suggests the test to be the gold standard in subjects in whom endoscopy is not indicated.

**Statistical analyses**

We first carried out descriptive analyses concerning sociodemographic characteristics of mothers. We then assessed the relation between *H pylori* infection status of mothers and hemoglobin level at the beginning of pregnancy. Hemoglobin level at the beginning of pregnancy was defined as the first measurement within the series of routine prenatal visits during pregnancy. Adjustment was made by multiple linear regression with hemoglobin level as the dependent variable and the following potential confounders as covariates: age (in years), school education (≤9 years; ≥10 years), nationality (German, Turkish, other), body mass index (<20 kg/m2; ≥20 to < 25 kg/m2; ≥25 to < 30 kg/m2; ≥30 kg/m2), alcohol consumption during pregnancy (no alcohol consumption, ≤1 drink per week, >1 drink per week), parity (1, 2, ≥3), and gestational age (weeks) at the time of hemoglobin measurement.

Furthermore, we used multiple linear regression analyses to assess the independent contribution of *H pylori* infection status to the degree of hemoglobin change during pregnancy (dependent variable) under consideration of iron therapy during pregnancy. Hemoglobin change during pregnancy was defined as the difference between first and last hemoglobin measurement within the series of routine prenatal visits during pregnancy. This analysis was restricted to mothers with a first hemoglobin measurement within the first 12 weeks and a last hemoglobin measurement after the 34th week of pregnancy. An interaction term between *H pylori* infection status and iron therapy during pregnancy was fitted into the multiple linear regression
analyses to examine whether the influence of iron therapy on hemoglobin change depends on the *H pylori* infection status of mothers. Adjustment in the multiple linear regression model was made by consideration of hemoglobin level at the beginning of pregnancy and of the same potential confounders as listed earlier.

All analyses were carried out with the SAS statistical software package (version 8; SAS Institute Inc, Cary, NC).

### Table I  Basic characteristics of mothers in the study sample (N = 898)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Measure</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, n (%)</td>
<td>Mean ± SD (range)</td>
</tr>
<tr>
<td>16-25 y</td>
<td>164 (18.2)</td>
</tr>
<tr>
<td>26-35 y</td>
<td>569 (63.4)</td>
</tr>
<tr>
<td>36-45 y</td>
<td>165 (18.4)</td>
</tr>
<tr>
<td>School education, n (%)</td>
<td>≤ 9 y</td>
</tr>
<tr>
<td>164 (24.5)</td>
<td></td>
</tr>
<tr>
<td>10-11 y</td>
<td>339 (37.8)</td>
</tr>
<tr>
<td>≥ 12 y</td>
<td>313 (34.9)</td>
</tr>
<tr>
<td>Unknown</td>
<td>26 (2.9)</td>
</tr>
<tr>
<td>Nationality, n (%)</td>
<td>German</td>
</tr>
<tr>
<td>755 (84.1)</td>
<td></td>
</tr>
<tr>
<td>Turkish</td>
<td>51 (5.7)</td>
</tr>
<tr>
<td>Other</td>
<td>92 (10.2)</td>
</tr>
<tr>
<td>Alcohol consumption during pregnancy, n (%)</td>
<td>None</td>
</tr>
<tr>
<td>742 (82.8)</td>
<td></td>
</tr>
<tr>
<td>1 drink/wk</td>
<td>135 (15.1)</td>
</tr>
<tr>
<td>&gt; 1 drink/wk</td>
<td>19 (2.1)</td>
</tr>
<tr>
<td>Iron supplementation during pregnancy, n (%)</td>
<td>No</td>
</tr>
<tr>
<td>365 (40.6)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>533 (59.4)</td>
</tr>
<tr>
<td>Duration of therapy, n (%)</td>
<td>1-13 wk</td>
</tr>
<tr>
<td>232 (25.8)</td>
<td></td>
</tr>
<tr>
<td>14-26 wk</td>
<td>151 (16.8)</td>
</tr>
<tr>
<td>&gt; 26 wk</td>
<td>138 (15.4)</td>
</tr>
<tr>
<td>Mean body mass index at the beginning of pregnancy (± SD)</td>
<td>&lt; 20 kg/m²</td>
</tr>
<tr>
<td>112 (12.5)</td>
<td></td>
</tr>
<tr>
<td>20-25 kg/m²</td>
<td>528 (59.0)</td>
</tr>
<tr>
<td>25-30 kg/m²</td>
<td>183 (20.5)</td>
</tr>
<tr>
<td>≥ 30 kg/m²</td>
<td>72 (8.0)</td>
</tr>
<tr>
<td>Parity, n (%)</td>
<td>1</td>
</tr>
<tr>
<td>420 (47.1)</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>333 (37.3)</td>
</tr>
<tr>
<td>≥ 3</td>
<td>139 (15.6)</td>
</tr>
<tr>
<td>Mean hemoglobin level (g/dL)</td>
<td>At the beginning of pregnancy (range)</td>
</tr>
<tr>
<td>Anemia (&lt; 12 g/dL)</td>
<td>176 (19.7)</td>
</tr>
<tr>
<td>At the end of pregnancy (range)</td>
<td>12.1 ± 1.2 (7.7-17.3)</td>
</tr>
<tr>
<td>Anemia (&lt; 12 g/dL)</td>
<td>410 (46.3)</td>
</tr>
</tbody>
</table>

### Table II  *H pylori* infection (HP+) according to UBT by sociodemographic characteristics and potential confounders

<table>
<thead>
<tr>
<th>Variable</th>
<th>N</th>
<th>N %</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total</td>
<td>898</td>
<td>206</td>
<td>22.9</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>16-25 y</td>
<td>164</td>
<td>62</td>
<td>37.8</td>
</tr>
<tr>
<td>26-35 y</td>
<td>569</td>
<td>120</td>
<td>21.1</td>
</tr>
<tr>
<td>36-45 y</td>
<td>165</td>
<td>24</td>
<td>14.6</td>
</tr>
<tr>
<td>School education</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤ 9 y</td>
<td>220</td>
<td>89</td>
<td>40.4</td>
</tr>
<tr>
<td>10-11 y</td>
<td>339</td>
<td>65</td>
<td>19.1</td>
</tr>
<tr>
<td>≥ 12 y</td>
<td>313</td>
<td>42</td>
<td>13.4</td>
</tr>
<tr>
<td>Nationality</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>German</td>
<td>755</td>
<td>115</td>
<td>15.2</td>
</tr>
<tr>
<td>Turkish</td>
<td>51</td>
<td>38</td>
<td>74.5</td>
</tr>
<tr>
<td>Other</td>
<td>92</td>
<td>53</td>
<td>57.6</td>
</tr>
<tr>
<td>Alcohol consumption during pregnancy</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>742</td>
<td>183</td>
<td>24.7</td>
</tr>
<tr>
<td>≥ 1 drink/wk</td>
<td>135</td>
<td>20</td>
<td>14.8</td>
</tr>
<tr>
<td>&gt; 1 drink/wk</td>
<td>19</td>
<td>2</td>
<td>10.5</td>
</tr>
<tr>
<td>Intake of iron supplement</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>No</td>
<td>365</td>
<td>77</td>
<td>21.1</td>
</tr>
<tr>
<td>Yes</td>
<td>533</td>
<td>129</td>
<td>24.2</td>
</tr>
<tr>
<td>Body mass index at the beginning of pregnancy (kg/m²)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; 20</td>
<td>112</td>
<td>23</td>
<td>20.5</td>
</tr>
<tr>
<td>≥ 20-&lt;25</td>
<td>528</td>
<td>118</td>
<td>22.4</td>
</tr>
<tr>
<td>≥ 25-&lt;30</td>
<td>183</td>
<td>43</td>
<td>23.5</td>
</tr>
<tr>
<td>≥ 30</td>
<td>72</td>
<td>20</td>
<td>27.8</td>
</tr>
</tbody>
</table>

* Chi-squared test for difference between groups.

### Results

Overall, 1066 among the 1593 eligible mothers (67%) participated in the study. For 1 mother, no UBT result was available; 167 mothers who had antibiotic treatment during the past 4 weeks before the conduct of the UBT were excluded from this analysis because of the possibility of false-negative breath test results, which led to a final sample size of 898 mothers.

Table I shows basic characteristics of the mothers in the study sample. The mean age was 30.7 ± 5.2 years, and approximately 25% of the mothers had <10 years of school education. Most women (84.1%) were of German nationality; 5.7% and 10.2% were of Turkish or other nationality, respectively. Most of the mothers (59.4%) had received iron supplementation during pregnancy. The mean body mass index at the beginning of pregnancy was 23.8 kg/m². Distributions of hemoglobin levels at the beginning and at the end of pregnancy were unimodal and symmetric (hemoglobin level at the beginning of pregnancy: mode, 12.8 g/dL; median, 12.9 g/dL; interquartile range, 1.6 g/dL; hemoglobin...
level at the end of pregnancy: mode, 11.9 g/dL; median, 12.0 g/dL; interquartile range, 1.6 g/dL). The mean hemoglobin level at the beginning of pregnancy was 12.9 g/dL, and 19.7% of mothers had hemoglobin levels \( \leq 12 \) g/dL. At the end of pregnancy, the mean hemoglobin level was 12.1 g/dL, and 46.3% of mothers had hemoglobin levels \( \leq 12 \) g/dL.

Of the 898 mothers, 206 mothers (22.9%) had a positive UBT (Table II). Prevalence of infection was higher in younger mothers and in mothers with a low level of school education. Of the 755 German mothers, only 115 mothers (15.2%) were infected with \( H \) \textit{pylori}, whereas 74.5% and 57.6% of the mothers with Turkish or other nationality were infected with \( H \) \textit{pylori}, respectively. The prevalence of \( H \) \textit{pylori} infection decreased with increasing alcohol consumption during pregnancy. The prevalence of \( H \) \textit{pylori} infection was slightly higher in mothers with iron supplementation (24.2%) compared with mothers without iron supplementation during pregnancy (21.1%), but this difference was not statistically significant.

The relation between \( H \) \textit{pylori} infection status and hemoglobin level at the beginning of pregnancy is shown in Table III. The mean hemoglobin level at the beginning of pregnancy was lower among infected women (12.59 g/dL) than among uninfected women (13.05 g/dL). After adjustment for age, this difference was 0.45 g/dL (95% CI, 0.003 to 0.25).

### Table III
Relation between \( H \) \textit{pylori} infection according to UBT and hemoglobin level at the beginning of pregnancy: Regression coefficients (\( \beta \)) and their 95% CIs in multiple linear regression (n = 888)

<table>
<thead>
<tr>
<th>( H ) \textit{pylori} infection</th>
<th>Uninfected women</th>
<th>Infected women</th>
<th>Adjusted for age</th>
<th>Adjusted for multiple covariates*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Anemia (%)</td>
<td>Mean hemoglobin level (g/dL)</td>
<td>N</td>
</tr>
<tr>
<td>All</td>
<td>685</td>
<td>17.5</td>
<td>13.05</td>
<td>203</td>
</tr>
<tr>
<td>German</td>
<td>633</td>
<td>17.1</td>
<td>13.06</td>
<td>115</td>
</tr>
<tr>
<td>Other</td>
<td>52</td>
<td>23.1</td>
<td>12.91</td>
<td>88</td>
</tr>
</tbody>
</table>

* Age, nationality, school education, body mass index, alcohol consumption, number of pregnancies, and gestational age at time of hemoglobin measurement.

† Reference group.

‡ Prevalence of anemia at the beginning of pregnancy.

### Table IV
Relation between \( H \) \textit{pylori} infection status and hemoglobin change during pregnancy (Hb change [g/dL]) according to iron therapy during pregnancy: Regression coefficients (\( \beta \)) and their 95% CIs in multiple linear regression (n = 633)

<table>
<thead>
<tr>
<th>( H ) \textit{pylori} infection</th>
<th>Uninfected women</th>
<th>Infected women</th>
<th>Crude</th>
<th>Adjusted for age</th>
<th>Adjusted for age and hemoglobin level at the beginning of pregnancy*</th>
<th>Adjusted for multiple covariates†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Anemia change (g/dL)</td>
<td>N</td>
<td>Hemoglobin change (g/dL)</td>
<td>N</td>
<td>Anemia change (g/dL)</td>
<td>( \beta )</td>
</tr>
<tr>
<td>All</td>
<td>-0.95</td>
<td>501</td>
<td>-0.87</td>
<td>132</td>
<td>54.6</td>
<td>0.09</td>
</tr>
<tr>
<td>No iron therapy</td>
<td>-1.04</td>
<td>215</td>
<td>-0.93</td>
<td>47</td>
<td>38.3</td>
<td>0.12</td>
</tr>
<tr>
<td>Iron therapy</td>
<td>-0.88</td>
<td>286</td>
<td>-0.83</td>
<td>85</td>
<td>63.5</td>
<td>0.05</td>
</tr>
</tbody>
</table>

* Hemoglobin level at the beginning of pregnancy, gestational age at time of hemoglobin measurement.

† Hemoglobin level at the beginning of pregnancy, age, nationality, school education, alcohol consumption, body mass index, number of pregnancies, and gestational age at time of hemoglobin measurement.

‡ Reference group.

§ Prevalence of anemia at the end of pregnancy.
was reduced to 0.25 g/dL (95% CI, −0.49 to −0.003) after adjustment for multiple covariates but was still statistically significant. An inverse association between *H pylori* infection and hemoglobin level at the beginning of the pregnancy was observed both among German women and among women with other nationality. The hemoglobin change during pregnancy could be derived from pregnancy health charts of 633 women (70.5%). The relations between *H pylori* infection status and hemoglobin change during pregnancy are shown in Table IV. The mean hemoglobin change during pregnancy was −0.95 g/dL among uninfected women and −0.87 g/dL among infected women (ie, the mean hemoglobin level decreased by 0.95 g/dL and 0.87 g/dL, respectively, during pregnancy). The crude regression coefficient for this relation was 0.09 g/dL (95% CI, −0.19 to 0.37), which indicates that hemoglobin loss during pregnancy was slightly lower among infected women compared with uninfected women. However, after adjustment for age and hemoglobin level at the beginning of pregnancy, the hemoglobin loss during pregnancy was 0.22 g/dL (95% CI, −0.44 to 0.006) higher among women with *H pylori* infection compared with uninfected women. After further adjustment for multiple covariates, this difference was reduced to 0.14 g/dL (95% CI, −0.38 to 0.10) and no longer statistically significant. The relation of *H pylori* infection to increased hemoglobin loss during pregnancy was stronger among women with iron therapy during pregnancy (−0.18 g/dL; 95% CI, −0.48 to 0.11) than among women who reported no iron therapy during pregnancy (−0.04 g/dL; 95% CI, −0.41 to 0.33), but like all other associations that are shown in Table IV, the difference between both groups was not statistically significant.

Comment

In this study, we investigated the possible role of *H pylori* infection in iron deficiency during pregnancy under special consideration of iron therapy in a large group of mothers after the birth of their baby. In the presence of *H pylori* infection, we found a significantly lower mean hemoglobin level at the beginning of pregnancy and a decrease in hemoglobin level during pregnancy. The latter association was not statistically significant, however.

To our knowledge, no previous study specifically has addressed a potential role of *H pylori* infection on anemia or iron deficiency during pregnancy, but a few studies have addressed these issues in population samples. In 1996, Plojoto et al found no association between *H pylori* infection and blood levels of hemoglobin, iron, ferritin or transferrin among 102 asymptomatic subjects >65 years of age. Yip et al first reported a positive role of *H pylori* gastritis in iron deficiency in an Alaskan native population. This association was confirmed among samples of adolescent Alaskan natives and of women from Victoria, Australia, and in Korean adolescent girls. Berg et al analyzed the association of *H pylori* infection and serum ferritin in a population-based sample of 1806 adults in Germany. Infection with *H pylori* that was measured serologically by enzyme-linked immunosorbent assay was associated with a 17% decrease of the serum ferritin concentration in a multivariate linear regression model. In a seroepidemiologic survey comprising 2794 Danish adults, Milman et al found that the seroprevalence of *H pylori* infection was not related to hemoglobin, but serum ferritin levels were significantly lower in men and in postmenopausal women who were immunoglobulin G positive than in seronegative individuals. Among 1060 subjects who were selected randomly from the Christchurch population (New Zealand), Collett et al found lower serum iron levels in the presence of *H pylori* infection only in male subjects, whereas no significant differences in serum ferritin between seropositive and seronegative subjects was seen in male or in female subjects.

Taken together, these previous studies suggest a role of *H pylori* infection in iron deficiency, which appears not to be strong enough to lead to manifest anemia in the general population. Our study suggests that a possible impact on hemoglobin levels may become clinically manifest, albeit still to a moderate degree, in pregnancy, when iron demand is particularly high.

In our study population, the reduction of hemoglobin levels during pregnancy in the presence of *H pylori* infection seemed to be slightly higher among women with iron therapy during pregnancy compared with women without. Even though the difference between both groups was not statistically significant, this pattern would be consistent with the hypothesis of a possible increase in bacterial density by iron therapy, which might in turn reduce the benefit from iron therapy, because microbiologic and ferrokinetic studies suggested that outer membrane receptors of *H pylori* in vitro are able to capture iron from human lactoferrin and use it for growth. However, this hypothesis must be confirmed in even larger studies.

On the basis of UBT results, 22.9% of the mothers included in our study had a current *H pylori* infection. In agreement with findings from previous studies that were conducted in the same study area, the prevalence of infection was much higher in Turkish mothers (74.5%) and in mothers with other than German or Turkish nationality (57.6%) than in mothers of German nationality, whose prevalence of infection was only 15.2%. Our study is also consistent with previous findings that *H pylori* infection in adults is strongly associated with certain sociodemographic factors like school education. The inverse association between...
H pylori infection and age in our bivariate analysis appears surprising on first view but can be explained by confounding by nationality and socioeconomic factors (data not shown).

The following limitations of our study have to be kept in mind. Whereas multiple measurements of hemoglobin levels were recorded in health charts of most mothers, serum levels of ferritin, which reflects the magnitude of the mobilizable iron stores in the body, were not available. Furthermore, measurement of H pylori infection was done after delivery only. However, given the very low rate of acquisition and loss of infection during adulthood, infection status at the beginning and at the end of pregnancy is likely to have been identical in most of the women. Furthermore, infection was measured by UBT, which is a more reliable measure of current infection status than serologic tests that were used in most previous epidemiologic studies. Information on iron therapy during pregnancy was collected retrospectively. However, the women were unaware of the value of the UBT at the time of the interview, and therefore selective recall is very unlikely.

In summary, our large-scale epidemiologic study suggests a moderate independent role of H pylori infection in iron deficiency at the beginning of pregnancy. A possible impact of H pylori infection on the aggravation of anemia during pregnancy, in particular with respect to iron therapy, must be confirmed in even larger studies.

References

Chronic maternal and fetal *Porphyromonas gingivalis* exposure during pregnancy in rabbits

Kim A. Boggess, MD,a,b Phoebus N. Madianos, DDS, PhD,b John S. Preisser,c Kenneth J. Moise Jr, MD,a Steven Offenbacher, DDS, PhDb

Department of Obstetrics and Gynecology, University of North Carolina School of Medicine,a Center for Oral and Systemic Inflammatory Diseases, University of North Carolina School of Dentistry,b and Department of Biostatistics, University of North Carolina School of Public Health,c Chapel Hill, NC

Received for publication June 14, 2004; revised August 12, 2004; accepted September 2, 2004

**Objective:** This study was undertaken to develop a rabbit model of maternal exposure to *Porphyromonas gingivalis* and determine whether fetal or placental exposure occurs.

**Study design:** Subcutaneous steel chambers were implanted in 8 New Zealand White female rabbits. On day 7 of pregnancy, 4 rabbits were inoculated through the chamber with 5 × 10⁸ CFU/mL live *P gingivalis*, and 4 rabbits with broth (controls) and sacrificed at term. Polymerase chain reaction was used to detect *P gingivalis* in maternal and fetal liver and placenta. Fisher exact test was used to compare *P gingivalis* detection between groups.

**Results:** Among exposed does, *P gingivalis* was detected in 33% of the maternal livers, 49% of placentas, and 34% fetal livers compared with none from controls (*P* < .001).

**Conclusion:** Chronic maternal exposure to *P gingivalis* results in systemic dissemination, transplacental passage, and fetal exposure. This model may be useful to study placental and fetal effects of this oral pathogen and to study microbial dissemination across the placenta.

© 2005 Elsevier Inc. All rights reserved.

Periodontal disease is an infectious disease manifested by the progressive change of healthy resident commensal oral microbes to pathogenic ones within the biofilm of the tooth and gingiva. Maternal periodontal disease has recently been linked to several adverse pregnancy outcomes. In an early case-control study we found that maternal periodontal disease was associated with delivery of a preterm low-birth weight infant, a finding confirmed by 2 large prospective studies. We have also demonstrated that maternal periodontal disease is associated with delivery of significantly smaller infants, and with the development of preeclampsia. Systemic dissemination of oral microbes with subsequent maternal, fetal, and/or placental inflammatory responses has been suggested as a possible causal mechanism. Data from a pregnant mouse model of infection demonstrate that oral organisms translocate to the placenta and adversely affect fetal growth. Furthermore, in a large cohort study, we found that 317 (58%) of 546 umbilical cord samples collected were positive for immunoglobulin M (IgM) to 1 or more...
specific oral pathogens,9 suggesting systemic dissemination and fetal exposure.

The purpose of this investigation was to create a rabbit model of chronic maternal exposure to Porphyromonas gingivalis, an oral pathogen associated with periodontal disease, to determine an appropriate inoculum for maternal exposure without systemic illness, and to determine whether systemic dissemination occurs and results in placental or fetal exposure.

Materials and methods

The Institution Animal Care and Use Committee of the University of North Carolina approved this study. Reproductive-aged female, Pasteurella-free, New Zealand White rabbits (O cuniculus) were purchased from a local breeder (Robinson Services, Greensboro, NC). Rabbits were maintained in an animal housing facility in stainless steel cages and fed antibiotic-free Purina Laboratory Chow (Purina Mills, Inc, St Louis, Mo). For breeding, a rabbit buck was placed into the cage with the female for 5 minutes on 2 consecutive days.

After acclimatization, animals were transported to the animal surgical facility, anesthetized with intramuscular ketacet (25 mg/kg) and domitor (0.5 mg/kg). Cylindrical surgical grade steel chambers, each 2 cm in length and 0.75 cm in diameter (Figure 1, A), were then surgically implanted subcutaneously on the dorsal side, between the scapulae (Figure 1, B). The skin was then closed in 1 layer with poliglecaprone 25 suture (Ethicon Inc, Somerville, NJ). Anesthesia was reversed with subcutaneous antisedan (0.5 mg/kg). Animals were observed for 4 hours and then daily.

Porphyromonas gingivalis strain A7436 was grown in anaerobic blood agar (Remel, Lenexa, Kan) in a Coy anaerobic chamber for 10 days. The bacterial cells were subcultured and grown until pure, and then colonies were inoculated into Wilkins-Chalgren anaerobic broth (Remel) and grown for approximately 18 hours.

Ten to 14 days after chamber placement, rabbits were sedated with 0.9 mL (10 mg/mL) acepromazine maleate, then inoculated with P gingivalis through the chamber. Dosing experiments were performed to determine the concentration of P gingivalis that would result in chronic exposure without systemic maternal illness. Microbial exposure consisted of 2 inoculums introduced into the implanted chamber: the first, heat-killed P gingivalis, which was followed 14 to 21 days later by inoculation with live P gingivalis. For heat-killed inoculums, live organisms were boiled for 10 minutes then cooled to room temperature.10 Central ear artery blood sampling was performed on the day of inoculation with heat-killed P gingivalis, then on days 1, 5, 10, 15, 20, and 25 after exposure. The blood was allowed to clot, centrifuged at 2000g, with the resultant serum stored in 1-mL aliquots at −80°C. To confirm sensitization, P gingivalis-specific IgG was assayed by using a checkboard immunoblot, as previously described.11

After testing doses of P gingivalis ranging from 10⁹ CFU/mL to 10⁷ CFU/mL, we determined that using 10⁹ CFU/mL heat-killed, followed by 5 × 10⁸ CFU/mL live P gingivalis, allowed for adequate maternal exposure (as demonstrated by doe serum P gingivalis-specific IgG) without inducing systemic illness or death.

Five female rabbits were inoculated through the chamber with 10⁹ CFU/mL heat-killed P gingivalis and 5 were inoculated with an equal volume of sterile media (controls); all were observed daily for systemic signs of infection (temperature, feeding, behavior) for 3 to 5 days after inoculation and then mated 14 days later. On day 7 of gestation, corresponding with day of implantation, does were sedated, and those previously inoculated with heat-killed P gingivalis were inoculated through the chamber with 5 × 10⁸ CFU live P gingivalis; controls were inoculated with sterile media. Animals were observed daily for systemic signs of infection (rectal temperature, feeding, behavior) for 5

Figure 1 A, Surgical grade steel chambers used for P gingivalis inoculation. B, Placement of chamber into rabbit.
days. Fever was defined as 104°F or greater, which is 2 SDs above the mean for normal rabbit temperature.12

Central ear artery blood sampling to obtain serum was performed on the day of the first inoculation (preimmune), the day of live challenge (day 7 of gestation), and at days 8, 13, 18, and 23. Does were killed at term, on day 29 to 30 of gestation, by intravenous pentobarbital (100 mg/kg). Fetuses were killed at term, and immediately weighed on a Sartorius (model 1002 MP9) 500.0 g scale. All newborn kits were then exsanguinated with an intracardiac blood sample. After death, the kit’s abdomen was opened sharply with sterile scissors, the liver identified and biopsied. After delivery of all kits, the maternal liver was identified and biopsied. Placentas were trimmed of membranes with sterile scissors, weighed, and biopsied. A portion of maternal liver, placenta, and fetal liver biopsies were placed in RNase-free solution and portions frozen for polymerase chain reaction (PCR). Briefly, samples about 20 to 30 mg were cut by using sterile scissors, treated with Dneasy Tissue Kit (Qiagen, Valencia, Calif) for DNA extraction, transferred into sterile 1.5-mL tubes with 180 µL lysis buffer and 20 µL protease K, and incubated at 55°C. Digested samples were mixed with DNA binding buffer, then subjected to a spin column provided by the manufacturer to filter all non-DNA particles, leaving DNA on the column. The quality and quantity of extracted DNA was determined spectrophotometrically. Two primers for P gingivalis 16S rRNA gene were synthesized at the University of North Carolina Pathology Oligonucleotide Synthesis Facility: 5’AGGCCAGTTGGCCATCTGCAG3’ and 5’ACTGTAGCAACTACGGATGT3’ and 2 nested primers developed from the first amplicon with Cprimer (IU Bio-Archive at http://www.iubio.bio.indiana.edu): 5’T- GCGACTGACACTGAAGCAC3’ and 5’TACATAAGCCCGAAGGAG3’.13 An Entrez nucleotide database was used to search for any conflicting homology of the nested primer sequences.

Nested PCR was performed in a 0.2-mL microcentrifuge tube by using Taq DNA Polymerase recombinant (Invitrogen, Carlsbad, Calif). For the first set of primers, the PCR conditions used were 10 µL (0.25-3.5 µg) of DNA solution as the template, 5 µL of 10× PCR buffer solution, 1.5 µL of 50 mmol/L MgCl2, 1 µL of 10 mmol/L dNTPs, 0.625 µL of 20 µmol/L each primer, 0.5 U Taq polymerase in a final volume of 50 µL. Eppendorf Mastercycler system was used for amplification (a 15-minute 95°C denature cycle, followed by 36 cycles at 95°C for 30 seconds, 1 cycle at 57.9°C for 1 minute, and 1 cycle at 72°C for 11 minutes). For the second PCR reaction using nested primers, 2 µL of the first reaction mixture as the template, 5 µL 10× PCR buffer solution, 1.5 µL of 50 mmol/L MgCl2, 1 µL of 10 mmol/L dNTPs, 1.25 µL of 20 µmol/L each primer, 0.5 U Taq polymerase in a final volume of 50 µL were cycled as previously described. A negative control using distilled water as template and a positive control using P gingivalis, strain A7436, was run in all batches. The 15 µL of amplified material was subjected to electrophoresis on 2% TAE agarose gel, stained with SYBR gold, and photographed under ultraviolet light. The presence of P gingivalis was determined by the existence of a 404-base pairs (bp) band.

Fisher exact test was used to compare proportion of P gingivalis positive maternal liver, placental, or fetal liver specimens between exposed and control rabbits (SigmaStat, SPSS Inc, San Rafael, Calif). A P value of less than .05 was considered significant.

### Results

Pregnancy was achieved in 8 of 10 rabbits (4 in each group). The 4 does exposed to P gingivalis resulted in 35 kits and 1 resorption; 33 placentas were available for analysis as 2 were inadvertently discarded. Three maternal liver specimens were available for analysis as 1 biopsy sample yielded insufficient DNA. The 4 controls resulted in 31 kits and no resorptions; 31 placentas were available for analysis and all 4 maternal liver specimens were adequate. There were no signs of systemic illness or elevated temperature above baseline among infected or control does (data not shown).

All preimmune serum specimens were negative for P gingivalis–specific IgG. After inoculation with heat-killed P gingivalis, IgG was detected in all 4 does on day 1, with an increasing response 5 and 10 days after exposure. There was not a substantial increase in antibody response beyond 10 days (data not shown). As expected, none of the controls demonstrated P gingivalis–specific IgG.

P gingivalis PCR results are shown in the Table. In the 1 doe with a positive liver, 9 (90%) of 10 placentas were also positive, compared with only 2 (14%) of 14 placentas between the 2 does whose livers were negative for P gingivalis (P < .001). Among the exposed does, 8 (50%) of 16 positive placental specimens were associated with a positive fetal liver. P gingivalis was not detected.

<table>
<thead>
<tr>
<th>Table</th>
<th>Proportion of specimens with P gingivalis detected by PCR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Control</td>
</tr>
<tr>
<td>Maternal liver</td>
<td>0/4</td>
</tr>
<tr>
<td>Placenta</td>
<td>0/31</td>
</tr>
<tr>
<td>Fetal liver</td>
<td>0/31</td>
</tr>
<tr>
<td>Data presented as number (percent).</td>
<td></td>
</tr>
</tbody>
</table>
among any of the specimens collected from control rabbits.

**Comment**

Periodontal disease is a chronic, exacerbating, and remitting microbial oral infection that has received recent attention as a possible risk factor for adverse pregnancy outcome. Several studies have shown an association between maternal periodontal disease and preterm birth, fetal growth restriction, and preeclampsia, but the underlying mechanism is unknown.

To begin to understand and study possible mechanisms behind our findings in humans, we created a rabbit model of chronic exposure to *P. gingivalis*. We chose *P. gingivalis* because it is one of the strongest bacterial markers for periodontal disease. We used rabbits because, like humans, rabbits have a hemochorial placentation, and there is evidence that maternal to fetal immunoglobulin transport in rabbits is similar to humans. This is in distinction to rodents such as mice and rats, where there is limited immunoglobulin transport across the placenta. In addition, in utero blood sampling of the rabbit fetus has been reported, enabling future study to examine fetal responses before delivery. Sensitization of the animals with heat-killed organisms before exposure to live organisms establishes a chronic rather than acute exposure, resulting in a circulating antibody response to the heat-killed organism, protecting against secondary ulcerations at the chamber site, rejection or infection of the chamber site, and animal death, but allowing colonization of the live inoculum introduced later.

In this study, we demonstrate exposure of the fetal and placental compartments to *P. gingivalis* after maternal exposure at a site distant from the reproductive tract in the rabbit. Microbial DNA was recovered from one third of maternal livers, one third of placentas, and almost half of fetuses, suggesting maternal systemic dissemination and transplacental passage to the fetal compartment. All fetuses demonstrating exposure by PCR also had evidence of placental exposure. The dissemination and exposure rate may have been more frequent than we detected, as organism recovery is a function of sampling and only a portion of maternal liver, placenta, or fetal liver was used for PCR analysis.

We have demonstrated that after chronic exposure to *P. gingivalis*, some pregnant rabbits experience systemic dissemination and translocation of the organism. This model will allow further investigation of the factors regulating systemic exposure, as well as placental and fetal effects of this common human oral pathogen, and exploration of the mechanisms of microbial dissemination across the placenta.

**References**

Reduced flow-mediated vasodilation is not due to a decrease in production of nitric oxide in preeclampsia

Tamao Yamamoto, MD, Yoshikatsu Suzuki, MD, PhD,* Kazuhisa Kojima, MD, PhD, Kaoru Suzumori, MD, PhD

Department of Obstetrics and Gynecology, Graduate School of Medical Sciences, Nagoya City University, Nagoya, Japan

Received for publication April 8, 2004; revised August 1, 2004; accepted August 23, 2004

KEY WORDS
Preeclampsia
Flow-mediated vasodilation
Hyperemia
Cyclic guanosine monophosphate

Objective: Our aim was to determine the reduced function of endothelial nitric oxide in preeclampsia by use of noninvasive techniques in vivo.

Study design: With the use of a high-resolution ultrasound transducer, diameters of brachial artery were measured after reactive hyperemia in 20 nonpregnant women, 20 normotensive pregnant women, and 15 women with preeclampsia. The concentrations of cyclic guanosine monophosphate were measured in samples of platelets from all groups.

Results: Flow-mediated vasodilation at 1 minute after deflation was higher in the normotensive pregnant women (115.1% ± 6.5%) than in the nonpregnant women (108.7% ± 3.9%); flow-mediated vasodilation was lower in women with preeclampsia (106.8% ± 2.7%) than in the normotensive pregnant women. The concentration of platelet cyclic guanosine monophosphate was higher in the normotensive pregnant women than in the nonpregnant women (2.21 ± 1.10 pmol/mL/10^8 cells vs 0.746 ± 0.381 pmol/mL/10^8 cells). There was no difference between the normotensive pregnant and the preeclamptic group (2.81 ± 1.82 pmol/mL/10^8 cells). Furthermore, the increase in cyclic guanosine monophosphate by sodium nitroprusside in platelet samples that were obtained from the normotensive pregnant women was larger than the samples from the nonpregnant women (6.20 ± 4.2 pmol/mL/10^8 cells vs 1.62 ± 0.81 pmol/mL/10^8 cells). The increase in cyclic guanosine monophosphate from the women with preeclampsia did not differ from that in the normotensive pregnant women (5.84 ± 3.73 pmol/mL/10^8 cells).

Conclusion: These results indicate that reduced endothelial nitric oxide activity might be due to a reduction of nitric oxide–cyclic guanosine monophosphate activity rather than its production in preeclampsia.

© 2005 Elsevier Inc. All rights reserved.

Vascular endothelial cells release endothelium-derived relaxing factors (EDRFs; such as nitric oxide [NO], prostacyclin and endothelium-derived hyperpolarizing factor [EDHF]) and play an important role in the regulation of vascular tone, vascular permeability, and blood coagulation, thus helping to maintain circulatory homeostasis. Vascular endothelial cells release endothelium-derived relaxing factors (EDRFs; such as nitric oxide [NO], prostacyclin and endothelium-derived hyperpolarizing factor [EDHF]) and play an important role in the regulation of vascular tone, vascular permeability, and blood coagulation, thus helping to maintain circulatory homeostasis.
not simple. In women with preclinical and early disease, there is high cardiac output but normal-to-low vascular resistance; with worsening disease, preeclampsia is established and is characterized by a marked increase in vascular resistance.4-6

Among the EDRFs, an abnormality in the role that is played by endothelium-derived NO has been suggested in resistance arteries that were obtained from women with preeclampsia.7-9 Furthermore, on the basis of experiments with human omental resistance arteries, the reduced responsiveness to NO itself was seen in women with preeclampsia.10 In human omental resistance arteries, the synthesis of prostacyclin in endothelial cells is also reduced; therefore, the relaxation that is mediated by endothelium-derived prostacyclin in the presence of bradykinin is down-regulated in preeclampsia.10 Conversely, the function of EDHF is suggested to be preserved in the same human resistance arteries in preeclampsia.10-11 Taken together, it is suggested that, in preeclampsia, the function not only of NO but also of prostaglandins on endothelium-dependent relaxation are down-regulated, although the function of EDHF is to operate normally in human omental resistance arteries.

In in vivo assessment of endothelial function, with vasoactive substances (such as acetylcholine and nitroglycerin), vascular responses have been studied extensively in the coronary and in the forearm circulation.12,13 Because of the invasive nature of these tests, they are not suitable for the study of pregnant women. A noninvasive technique has evoked the evaluation of flow-mediated vasodilation (FMD), an endothelium-dependent function, in the brachial artery.14 This stimulus provokes the endothelium to release EDRFs with subsequent vasodilation that can be imaged and quantitated as an index of vasomotor function. Increased production of NO in endothelium by shear stress has been proposed as the main mechanism underlying the increase in FMD that is induced by reactive hyperemia.15-17

Endothelial NO activates soluble guanylate cyclase and subsequently transfers guanosine triphosphate to cyclic guanosine monophosphate (cGMP) in the smooth muscle cells, thus working in NO-mediated vasodilation as a second messenger.3 NO from both endothelium and NO donors activates soluble guanylate cyclase and increases the concentration of cGMP in platelets,18,19 which are known to contain predominantly the soluble form of guanylate cyclase.20 Therefore, platelets offer a convenient material for the clinical measurement of intracellular cGMP instead of biopsied vascular smooth muscle cells. In a previous study of nitroglycerin tolerance in ischemic heart disease, the intracellular production of cGMP could be evaluated by the measurement in platelets.21

In this study, we investigated whether the activity of endothelial NO is reduced in women with preeclampsia by the use of a noninvasive approach in comparison with normotensive pregnant and nonpregnant women.

We also investigated the concentrations of cGMP in platelets that were obtained from each of the groups to evaluate the production of NO and cGMP in possible resistance arteries in women with preeclampsia.

Material and methods

Subjects

Twenty healthy nonpregnant women, 20 normotensive pregnant women, and 15 women with preeclampsia participated. Preeclampsia was diagnosed according to the criteria laid down by the National High Blood Pressure Education Program.4 Subject details are summarized in Table I. Because both severe hypertension (162 ± 9 mm Hg in systolic and 106 ± 12 mm Hg in diastolic) and proteinuria (4.1 ± 2.1 g/L) were prevalent in the preeclamptic group, most women with preeclampsia who participated in this experiment were diagnosed as having severe disease. The platelet count was lower in the preeclamptic group than in the normotensive pregnant women (15.9 ± 4.9 × 10^4/μL vs 23.0 ± 4.8 × 10^4/μL); liver enzymes did not differ between the 2 groups (Table I). Women with a history of smoking or a history that was complicated by certain diseases (diabetes mellitus, renal disease, collagen disease, anti-phospholipid syndrome, or disinfections) were excluded because the function of endothelium is known to be changed by smoking or these diseases. Furthermore, none of the women were taking any regular medication, which included antihypertensive drugs or MgSO4 before and during the experiment, and none of the control subjects included antihypertensive drugs or MgSO4 before and during the experiment. The study was approved by the ethics committee of the Nagoya City University Hospital, and all subjects gave informed consent.

FMD

FMD, a noninvasive technique to assess endothelium-dependent and -independent vasorelaxation in a medium-sized artery, was assessed in accordance with established protocols.22 A high-resolution ultrasound transducer was placed over the brachial artery to measure its diameter before, during, and after reactive hyperemia. Each subject rested at least 10 minutes before a baseline scan was acquired. The right brachial artery was scanned with a 7- to 13-Hz linear array transducer (Logic 500; GE Yokokawa, Tokyo, Japan) over a longitudinal section 5 to 7 cm above the right elbow. A cuff with 140-mm width was placed on the upper arm and inflated to 50 mm Hg above the systolic blood pressure for 5 minutes. The radial artery diameter was measured before inflation (baseline) and after deflation of the cuff. Inflation of the cuff was started within 1 minute after the measurement of baseline radial...
were kept frozen at −70°C until analysis.

The aqueous phase was then assayed for cGMP with a commercially available radioimmunoassay kit (Yamasa Shoyu, Tokyo, Japan) as in a previous study, and the results were expressed as picomoles per 10⁸ platelets. The coefficients of variation averaged 3.4% for intraassay error and 11.9% for interassay error.

**Statistics**

All data that were used are expressed as means ± SD. Two-way repeated analysis of variance and the Scheffe’s post hoc F test were used for the statistical analysis, or the Student unpaired t test with the F test were used. Probabilities <5% (P < .05) were considered significant.

**Results**

**FMD**

The brachial artery diameter before inflation was larger in the pregnant women (3.3 ± 0.4 mm) and the women with preeclampsia (3.6 ± 0.2 mm) than in the nonpregnant women (3.0 ± 0.4 mm; P = .04 and P < .0001, respectively; Figure 1, A). The diameter in radial artery after deflation was increased markedly and reached maximum dilation at 1 minute after deflation in all 3 groups. The increase in radial artery diameter was significantly larger in normotensive pregnant women than that in the women with preeclampsia and the nonpregnant women (P = .02 and P = .01, respectively) by 2-way analysis of variance (Figure 1, A). FMD at 1 minute after deflation was significantly higher in the normotensive pregnant women (115.1% ± 6.5%) than that in the nonpregnant women (108.7% ± 3.9%; P = .02), although FMD was not different in the women with preeclampsia (106.8% ± 2.7%) from in the nonpregnant women (P = .746; Figure 1, B).

### Table I  Patient details

<table>
<thead>
<tr>
<th>Variable</th>
<th>Nonpregnant women</th>
<th>Normotensive pregnant women</th>
<th>Women with preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>20</td>
<td>20</td>
<td>15</td>
</tr>
<tr>
<td>Age (y)*</td>
<td>30 ± 4</td>
<td>30 ± 1</td>
<td>30 ± 2</td>
</tr>
<tr>
<td>Parity: primiparous/multiparous (N/n)</td>
<td>15/5</td>
<td>12/3</td>
<td></td>
</tr>
<tr>
<td>Gestational age (wk)*</td>
<td>33 ± 3</td>
<td>34 ± 3</td>
<td></td>
</tr>
<tr>
<td>Blood pressure (mm Hg)*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Systolic</td>
<td>109 ± 5</td>
<td>110 ± 3</td>
<td>162 ± 9†</td>
</tr>
<tr>
<td>Diastolic</td>
<td>63 ± 4</td>
<td>72 ± 9</td>
<td>106 ± 12†</td>
</tr>
<tr>
<td>Proteinuria (g/L)*</td>
<td>—</td>
<td>4.1 ± 2.1</td>
<td></td>
</tr>
<tr>
<td>Platelet (×10⁴/μL)*</td>
<td>23.0 ± 4.8</td>
<td>15.9 ± 4.9†</td>
<td></td>
</tr>
<tr>
<td>Aspartate aminotransferase (U/L)*</td>
<td>19.1 ± 10.2</td>
<td>27.6 ± 18.2</td>
<td></td>
</tr>
<tr>
<td>Alanine aminotransferase (U/L)*</td>
<td>14.9 ± 12.0</td>
<td>25.1 ± 28.5</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL)*</td>
<td>0.46 ± 0.07</td>
<td>0.83 ± 0.35†</td>
<td></td>
</tr>
<tr>
<td>Uric acid (mg/dL)*</td>
<td>4.1 ± 0.9</td>
<td>8.5 ± 1.8†</td>
<td></td>
</tr>
<tr>
<td>Urine calcium/creatinine ratio*</td>
<td>0.199 ± 0.128</td>
<td>0.013 ± 0.004†</td>
<td></td>
</tr>
</tbody>
</table>

* Data are expressed as means ± SD.
† Significance was assumed if the probability value was <.05 by Student t test.
Furthermore, FMD was significantly lower in the women with preeclampsia than that in the normotensive pregnant women ($P = 0.049$; Figure 1, B).

**Platelet cGMP level**

The platelet cGMP level was significantly higher in the normotensive pregnant women than in the non-pregnant women ($2.21 \pm 1.10$ pmol/mL/10$^8$ cells vs $0.746 \pm 0.381$ pmol/mL/10$^8$cells; $P < 0.05$). However, there was no difference between the normotensive pregnant group and the preeclamptic group ($2.81 \pm 1.82$ pmol/mL/10$^8$cells; $P < 0.05$; Figure 2). Sodium nitroprusside had increased the concentration of cGMP in all platelet samples. The increase in cGMP by sodium nitroprusside in platelet samples that were obtained from the normotensive pregnant women was significantly larger than those in the nonpregnant women ($6.20 \pm 4.20$ pmol/mL/10$^8$ cells for the normotensive pregnant women vs $1.62 \pm 0.81$ pmol/mL/10$^8$cells for the nonpregnant women; Figure 2). Furthermore, the increase in the concentration of cGMP in platelet samples that were obtained from women with preeclampsia did not differ from that in the normotensive pregnant women ($5.84 \pm 3.73$ pmol/mL/10$^8$cells; Figure 2).

### Comments

**Enhancement of endothelial NO during pregnancy**

The EDRFs, which include NO, may work progressively during pregnancy. Systemic vascular resistance remains lower throughout pregnancy, whereas cardiac output and heart rate gradually increase, to plateau by the end of the second trimester. Although pregnancy is associated with profound hemodynamic changes, the arterial blood pressure shows a progressive fall in the first and middle trimesters.$^6$

In this study, the FMD in the normotensive pregnant women was larger than that in the nonpregnant women. These results are accordant with a previous study.$^{24}$ There are at least 3 possible explanations for the increase in the action of NO by shear stress during pregnancy: (1) an increase in the production of NO in endothelium, (2) an increase in the production of cGMP in smooth muscle cells, and (3) an enhancement of the action of cGMP in smooth muscle cells.

In this study, the levels of cGMP were higher in platelet samples that were obtained from the normotensive pregnant women than those that were obtained from the nonpregnant women. Furthermore, the increase in cGMP levels of platelet samples that were...
obtained from the pregnant women by sodium nitroprusside (NO donor) was larger than the samples for the nonpregnant women. These results suggested that the production of cGMP might be enhanced, at least, in vascular smooth muscle cells during pregnancy. The estrogens might involve it because supplementation with estrogens or estriol might enhance the action on FMD by reactive hyperemia in postmenopausal women. Recently, 17 β-estradiol enhanced vasorelaxation because of an increase in the production of NO through the Akt/PKB pathway. This area must be further clarified in the future.

Reduction of endothelial NO activity in preeclampsia

The hemodynamics of preeclampsia are complex and controversial. Although in women with preclinical and early preeclampsia, the hemodynamics are characterized by high cardiac output and normal-to-low vascular resistance, some women show abrupt vasoconstriction in resistance arteries with worsening preeclampsia. Preeclampsia that is established is characterized by a marked increase in vascular resistance and vascular permeability together with a disturbance of blood coagulation. Indeed women who show a reduction of FMD with elevated serum concentrations of asymmetric dimethylarginine (because of the inhibition of NO synthase in the endothelium) at mid trimester subsequently have preeclampsia in late pregnancy. In the present study, the reduction of FMD in brachial arteries by hyperemia was seen in women with established preeclampsia. This is in agreement with a previous study and suggests that the reduced action of endothelial NO might be evident in women with preeclampsia, because FMD depends mainly on NO release from endothelium.

The mechanisms underlying the reduced action of endothelial NO in preeclampsia are not fully understood. There are at least 3 possible explanations: (1) a decrease in the amount of NO by reduced production or acceleration of break-down in endothelium, (2) a reduction in the synthesis of cGMP, and (3) the reduced action of cGMP in smooth muscle cells. In the present study, the production of cGMP in platelet samples did not differ in the women with preeclampsia from the normotensive pregnant women, which suggests that the concentration of cGMP might not be decreased in smooth muscle cells. On the basis of experiments that use human omental resistance arteries, reduced responsiveness to cGMP itself was seen in the women with preeclampsia. This is in agreement with the present results, which suggests that the reduced action of endothelial NO is not mainly due to the reduced production of NO (possibly down-regulated action of cGMP). Endothelium-independent vasodilation after sublingual nitroglycerin is often used as a control. In in vivo experiments, the dilation of the forearm artery by the NO donor was not different in the women with preeclampsia by plethysmography. In this study, the production of cGMP by NO donors was not altered in platelet samples that were obtained from the women with preeclampsia in comparison with normotensive pregnant women. Interestingly, the concentration of cGMP was more increased by NO donors in platelet samples that were obtained from the women with preeclampsia because of the compensation for the decrease in endothelial NO. It must be clarified in the future.

These results suggested that, in the women with preeclampsia, the relaxation of endothelial NO might be attenuated because of the reduced action of endothelial NO or cGMP rather than the decrease in the production of NO, which is in agreement with previous in vitro experiments by the use of omental resistance arteries.

References


Risk factors for *Toxoplasma gondii* infection in mothers of infants with congenital toxoplasmosis: Implications for prenatal management and screening

Kenneth M. Boyer, MD,*a Ellen Holfels, BS,b Nancy Roizen, MD,c Charles Swisher, MD,e Douglas Mack, PhD,b Jack Remington, MD,g Shawn Withers, RN,h Paul Meier, PhD,i Rima McLeod, MD,b,d,* the Toxoplasmosis Study Groupa-i

Rush University Medical Center, Chicago, Ill,a University of Chicago, Chicago, Ill,b SUNY Upstate Medical University, Syracuse, NY,c Michael Reese Hospital, Chicago, Ill,d Children’s Memorial Hospital and Northwestern University, Chicago, Ill,e Illinois Institute of Technology, Chicago, Ill,f Stanford University, Stanford, Calif,g Cermak Health Services of Cook County, Chicago, Ill,h and Columbia University, New York, NYi

Received for publication March 25, 2004; revised June 23, 2004; accepted July 19, 2004

**KEY WORDS**
Congenital toxoplasmosis
*Toxoplasma gondii*
Maternal screening
Risk factor

**Objective:** The purpose of this study was to determine whether demographic characteristics, history of exposure to recognized transmission vehicles, or illness that was compatible with acute toxoplasmosis during gestation identified most mothers of infants with congenital toxoplasmosis.

**Study design:** Mothers of 131 infants and children who were referred to a national study of treatment for congenital toxoplasmosis were characterized demographically and questioned concerning exposure to recognized risk factors or illness.

**Results:** No broad demographic features identified populations that were at risk. Only 48% of mothers recognized epidemiologic risk factors (direct or indirect exposure to raw/undercooked meat or to cat excrement) or gestational illnesses that were compatible with acute acquired toxoplasmosis during pregnancy.

**Conclusion:** Maternal risk factors or compatible illnesses were recognized in retrospect by fewer than one half of North American mothers of infants with toxoplasmosis. Educational programs might have prevented acquisition of *Toxoplasma gondii* by those mothers who had clear exposure risks. However, only systematic serologic screening of all pregnant women at prenatal visits or of all newborn infants at birth would prevent or detect a higher proportion of these congenital infections.

© 2005 Elsevier Inc. All rights reserved.

Congenital toxoplasmosis is a disease that affects an estimated 500 to 5000 newborn infants in the United States each year. Most infected infants have no apparent physical abnormalities at birth, but, without treatment, most of the infected infants will have significant morbidity that is related to chorioretinitis, hydrocephalus, or neurologic damage by the end of
adolescence.2-6 Treatment of infected infants in the first year of life substantially improves outcomes,9-14 and treatment of a mother with acute Toxoplasma gondii infection during pregnancy can prevent vertical transmission or initiate treatment of the congenitally infected fetus.9,15-21 Therefore, strategies for early recognition of maternal or infant infection and the institution of effective treatment could have a substantial impact on the incidence and morbidity that are associated with this congenital infection.9,23

In the Chicago Collaborative Treatment Trial, we have followed up a cohort of infants who were referred to us for congenital toxoplasmosis over the past 20 years.9,14 In the present study, we have analyzed the mother’s history of exposure to potential vehicles of transmission of T gondii and the history of illness that is compatible with acquired toxoplasmosis during gestation. This analysis defines the potential for prevention of congenital toxoplasmosis through educational efforts, obstetric management of illness during pregnancy, selective maternal screening, or universal maternal or neonatal screening.

Material and methods

Between 1983 and 1998, a total of 131 infants and children were referred to the Chicago Collaborative Treatment Trial with laboratory-confirmed congenital toxoplasmosis.9-23 Of the 131 patients, 122 patients were referred as infants within the first months of life. All studies that involve the Collaborative Treatment Trial participants are approved by the University of Chicago’s Institutional Review Board in accordance with National Institutes of Health guidelines.

In the course of multidisciplinary evaluations of these infants, demographic data were acquired, and mothers were questioned regarding their possible exposure to recognized vehicles of transmission of T gondii and their history of compatible illness during pregnancy. Specifically, demographic data were acquired with regard to maternal residence, maternal age, maternal race/ethnicity, family’s Hollingshead index (a measure of socioeconomic status),24 and method of payment for care. Age and race/ethnicity distributions were compared with those of the general US population using χ² tests. Mothers were questioned regarding their exposure to cats. Specifically, they were asked whether they owned a cat, had emptied a litter pan, had gardened, had exposure to sand boxes, or had any combination of the above. Mothers were also questioned regarding their possibility of exposure to raw meat, specifically whether they had a history of preparation of foods with raw meat, had eaten any dishes that contained raw or undercooked meat, or had consumed any other raw foods (such as unpasteurized milk or raw eggs). The nature of exposure during pregnancy and the trimester in which it may have occurred were questioned specifically. Mothers were also asked about the occurrence of any illness during pregnancy that was compatible with infection and were queried specifically regarding fever or night sweats, flu-like illness or myalgia, headache, or lymphadenopathy.

For referred children, congenital infection was confirmed with standard laboratory tests in a reference laboratory (J. Remington, Toxoplasma Serology Laboratory, Palo Alto)11 that included the Sabin-Feldman dye test, immunoglobulin M, immunoglobulin A, and immunoglobulin E enzyme-linked immunosorbent assays or immunosorbent agglutination assays (ISAGA); subinoculation or polymerase chain reaction (PCR) of blood, amniotic fluid, or cerebrospinal fluid, or compatible findings in infants who were born of acutely infected mothers when other diagnoses were excluded.11 Maternal infection with T gondii was documented with serologic tests that included the Sabin-Feldman dye test, immunoglobulin M, immunoglobulin A, or immunoglobulin E enzyme-linked immunosorbent assay or ISAGA, and the differential agglutination test.25,26

Results

Demographic parameters

Hollingshead indices

Congenital toxoplasmosis was found to affect infants who were born to families of all socioeconomic classes (Table I), although our population had a higher than predicted proportion of Hollingshead indices 1 and 2.

Payment for care

The method of payment for medical care and type of health insurance in referred families was comparable to that of the US population as a whole (Table I).

Hometown size

One half the families were from urban settings, approximately one quarter each from suburban or rural hometowns (Table I).

Maternal age and ethnicity

The median maternal age was 25 to 29 years (Table II). Ethnicities are shown in Table III, with an over-representation of Asian/Pacific Islander subjects and an underrepresentation of African American subjects. Age differences were not statistically significant compared to the general US population, but racial differences were.

Risk factors

Although 75% of women who were delivered of an infant with congenital toxoplasmosis could recall a conceivable exposure, only 39% of the women specifically recalled
exposure to cat litter or raw meat dishes (Table IV). Surprisingly, 25% of the women could not identify any possible exposure to cats or any ingestion of even undercooked meat.

### Compatible clinical illness

Although 48% of the mothers noted an illness that might have included toxoplasmosis as a cause, only 27% of the women recalled fever or night sweats, and only 23% of the women recalled lymphadenopathy (Table V). Fifty-two percent of the mothers could not recall an infectious illness of any kind during pregnancy.

Overall, 60 mothers (48%) recognized signs and symptoms that were considered typical of toxoplasmosis or had known, specific risk factors (Table VI).

### Prenatal serologic testing

Only 10 women (8%) had serologic tests for toxoplasmosis before delivery of their infant. Of these 10 women, 3 women were American expatriates living in France who were tested during their pregnancies as a component of routine obstetric care. Interestingly, all 3 of these women also recalled compatible symptoms (eg, lymphadenopathy, malaise, myalgia, fever, chills, or “flu-like” illness), but screening occurred as part of the standard practice of care in France and not because of their symptoms. Of the remaining 7 women, all had compatible illness and/or identified risk factors; however, 1 woman was tested because of ascites that was noted on prenatal ultrasound scans in both her twins; 3 women were tested only because their physicians were looking for the cause of compatible illness and/or identified risk factors, and 3 women were tested because their physicians used routine screening practices.

### Comment

This study addresses whether a report of exposure to epidemiologic risk factors or etiologic investigation of a compatible illness during pregnancy would lead to the identification of most or all women who are at risk for transmitting *T gondii* to their unborn babies. If the identification of most such women could identify most infected infants, then serologic screening would not be

---

**Table I** Hollingshead indices,* methods of payment for care, and hometown size for 131 families with infants with congenital toxoplasmosis in the National Collaborative Treatment Trial (1981-1998)

<table>
<thead>
<tr>
<th>Socioeconomic status score</th>
<th>N (%)</th>
<th>Type of insurance N (%)</th>
<th>Hometown size N (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>32 (26)</td>
<td>Insured/health maintenance organization</td>
<td>77 (61)*</td>
</tr>
<tr>
<td>2</td>
<td>32 (26)</td>
<td>Public aid</td>
<td>41 (33)</td>
</tr>
<tr>
<td>3</td>
<td>21 (17)</td>
<td>Self-pay</td>
<td>8 (6)*</td>
</tr>
<tr>
<td>4</td>
<td>21 (17)</td>
<td>Unknown</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>17 (15)</td>
<td>Unknown</td>
<td>Rural 35 (27)</td>
</tr>
<tr>
<td>Unknown</td>
<td>8</td>
<td>Unknown</td>
<td>Unknown 1</td>
</tr>
</tbody>
</table>

* The Hollingshead index includes factors, such as education and income, that are used to calculate a socioeconomic status score; a socioeconomic status score of 1 is the highest.  
† 70% in the United States.  
‡ 16% in the United States.  
§ Unknown for reasons such as the child was adopted, and this information about birth parents was not available to us.

**Table II** Maternal age for 131 women who were delivered of infants with congenital toxoplasmosis

<table>
<thead>
<tr>
<th>Age (y)</th>
<th>N (%)</th>
<th>Percentage in the United States*</th>
</tr>
</thead>
<tbody>
<tr>
<td>15-19</td>
<td>16 (13)</td>
<td>12.3</td>
</tr>
<tr>
<td>20-24</td>
<td>23 (18)</td>
<td>24.5</td>
</tr>
<tr>
<td>25-29</td>
<td>38 (30)</td>
<td>27.5</td>
</tr>
<tr>
<td>30-34</td>
<td>29 (23)</td>
<td>22.6</td>
</tr>
<tr>
<td>35-39</td>
<td>15 (12)</td>
<td>10.8</td>
</tr>
<tr>
<td>40-44</td>
<td>4 (3)</td>
<td>2.1</td>
</tr>
<tr>
<td>Unknown</td>
<td>6</td>
<td>—</td>
</tr>
</tbody>
</table>

All comparisons were \( P > .05 \).


**Table III** Maternal race/ethnicity for 131 women with infants with congenital toxoplasmosis

<table>
<thead>
<tr>
<th>Race/ethnicity</th>
<th>N (%)</th>
<th>Percentage in United States*</th>
<th>( P ) value</th>
</tr>
</thead>
<tbody>
<tr>
<td>White</td>
<td>96 (73)</td>
<td>72.2</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Hispanic†</td>
<td>20 (15)</td>
<td>10.9</td>
<td>&gt; .05</td>
</tr>
<tr>
<td>Asian/Pacific Islander</td>
<td>11 (8)</td>
<td>3.7</td>
<td>.003</td>
</tr>
<tr>
<td>African American</td>
<td>3 (2)</td>
<td>12.5</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Native American</td>
<td>1 (1)</td>
<td>0.7</td>
<td>&gt; .05</td>
</tr>
</tbody>
</table>

† Hispanic ethnicity includes women of any race.
needed. Simply obtaining a careful history would lead to appropriate testing and management. Our data demonstrate that a careful history would identify, at most, 48% of mothers who have acquired toxoplasmosis during pregnancy. Thus, only serologic screening would have identified the rest.

Implications of our findings for prevention with education are clear. Even if education about the risks of toxoplasmosis became a component of standard obstetric practice, only approximately one half of the women had risk factors that might have been recognized and thus could have been eliminated by education. Our observation that only 8% of women in our study were screened for toxoplasmosis during pregnancy is consistent with the relatively infrequent screening of pregnant women in the United States for this disease. In France and Austria, where educational measures have been incorporated into routine obstetric care, reductions in rates of infection by 50% have been reported. Thus, other measures appear to be necessary to prevent or identify a higher proportion of cases of this congenital disease.

Our study is retrospective, because we elicited histories only from women whose infants already had been diagnosed with congenital toxoplasmosis. In many instances, the babies had substantial handicaps, and their mothers were knowledgeable about toxoplasmosis and understandably actively seeking explanations for their infection. Thus, the bias in ascertainment of risk behaviors and compatible illnesses in our experience is in favor of the identification of a higher proportion of mothers with risk factors. It is likely that the proportion of these women who reported risk exposures or compatible illnesses, if queried prospectively, would have been considerably lower. The degree to which US populations of pregnant women are aware of the risks of exposure to cats and cat excrement and of consuming raw or undercooked meat was not addressed specifically in our study and deserves further investigation. There are no means for determining percentages of women with similar symptoms and risk factors in a demographically comparable, concomitant control group during the past 20 years. This type of control group, established prospectively, might have helped identify whether any factor was seen more commonly in the mothers in our studies or whether nothing in the history was distinctive. However, this information was not available and does not effect the conclusion of this work.

In the course of the study, several additional observations and demographic factors were noted. First, the proportion of African American women with infants who were infected with toxoplasmosis in the study was quite low, representing only 2% of the total, whereas the proportion of African American women in the US population is 12% (\( P < .001 \)). Whether this low prevalence is due to different exposure rates, to poorer
quality of primary health care for African American women and infants, or possibly to a genetically based, increased resistance to the transmission of the disease remains to be determined. If the lower than expected proportion of African American infants is due to poorer quality of health care, systematic screening would help to remedy the problem of differential access to proper diagnosis and health care. Second, despite reporting nonspecific symptoms of infection to their physicians (such as prolonged fever or lymphadenopathy), many of the women indicated that toxoplasmosis was seldom considered as a possible explanation of the symptoms. This observation points out the importance of greater recognition by obstetricians of the pediatric implications of maternal infection and infectious symptoms during pregnancy.27

Other new epidemiologic observations have been made recently and suggest the possibility of additional modes of transmission of *T gondii* in North America. A high number of sea otters have died off the coast of California since 1995, and investigators have found that *T gondii* infection is 1 of the causes.28 The suggested epidemiologic factor is that the otters ingest *Toxoplasma* oocysts in sea water, where oocysts can persist up to 6 months, concentrated in mussels or other shellfish. Researchers hypothesize that oocysts infect shellfish through cat excrement in litter that people discard into toilets or watershed areas, which then arrive in coastal waters where otters live. Similarly, a large community epidemic of toxoplasmosis took place in Victoria, British Columbia, in 1995.29 No conventional transmission vehicle was identified, but case-control studies showed significant associations between acute infection and residence in the distribution system of one reservoir that supplied unfiltered water to greater Victoria. A recent study from Brazil, where toxoplasmosis is hyper-endemic, also supports the hypothesis of transmission by unfiltered drinking water.30 In our study, we did not ask about the consumption of shellfish35 or sources of water consumption in our inquiries about possible risk factors.

There have been economic analyses, Cochran Database reviews, and metareviews concerning screening programs for toxoplasmosis and their outcomes.1,9,32-40 Some of these have assigned equal value to well-performed studies and to dissimilar cohorts in studies that sometimes are designed, controlled, performed, or interpreted inadequately. Some of these analyses have noted the absence of perfectly designed and performed prospective, placebo-controlled, randomized studies with long follow-up, which included economic analyses, that clearly document savings in costs and efficacy of newborn infant or maternal screening.33-36 Some authors who reviewed these available data have concluded that, in the absence of better prospective studies, it may be too costly or unwarranted to perform universal screening or even testing and treatment at all, even to prevent suffering, health care–related costs, loss of productivity, and limitation in quality of life that are associated with untreated congenital toxoplasmosis.36 Some authors have commented that such screening could cause anxiety because of false-positive test results or unnecessary pregnancy terminations caused by serologic testing that was not confirmed in a high-quality reference laboratory or to counseling that was sub-optimal.38 In contrast, other analyses have concluded that screening, in conjunction with careful confirmation in a high-quality reference laboratory and knowledgeable and caring counseling is important to facilitate identification and treatment.19,20,32,37-43 We critically reviewed these analyses and the concerns they raise. We conclude that there is rigorous and careful work that indicates that systematic detection of this infection in pregnant women and the treatment of the infected fetus, as described9,37-40 results in improved outcomes for affected children. Careful confirmation of serologic testing in a high-quality, reliable reference laboratory and knowledgeable and empathetic medical care and counseling are essential parts of this process.

From our analysis of the risk factors and illnesses in mothers of congenitally infected children, we conclude that the most effective way to prevent or detect a higher proportion of infants with this congenital infection is by systematic serologic screening. It is difficult to imagine that any informed mother or father would choose not to include this screening in their prenatal care, considering that almost all untreated infants who are infected with *T gondii* in utero experience ophthalmologic and/or neurologic disease4-6 and that treatment of the fetus and infant clearly reduces these risks.11-21

An implication of our data is that education of pregnant women concerning risk factors for acquiring *Toxoplasma* during gestation would be useful in the prevention of congenital toxoplasmosis. Also, although uncommon,9 the recognition of signs and symptoms of this infection by obstetricians is important for prevention. Another implication of our findings is that only systematic serologic screening would detect a substantial proportion of mothers who are infected during gestation and those fetuses and infants with congenital toxoplasmosis. For many of these mothers, risk factors and signs or symptoms were not identified. Thus, our approach to prevention also is to include serologic screening to prevent congenital toxoplasmosis. Delays in treatment have been shown to result in more severe clinical manifestations. Therefore, our approach to how frequent serologic screening is performed, without limitation of resources, includes preconception testing of women and the identification of women who are infected acutely during pregnancy with an initial test for *Toxoplasma* infection at the first prenatal visit in the first trimester. Thereafter, monthly testing of seronegative
women to identify seroconversion and testing of newborn infants to identify congenital infection should be performed. We recognize that this optimal approach may not be economically feasible at this time in the United States, where seroprevalence is relatively low, resources are limited, and automated testing procedures are not widely available. In this country, testing seronegative pregnant women once each trimester (eg, at 8-10, 18-20, and 28-30 weeks’ gestation) and all newborn infants might be considered. However, it should be recalled that the best outcomes derive from monthly screening approaches.

10. T gondii—specific immunoglobulin G and M assays are used for screening pregnant women and newborn infants. Acute infection in the mother should be confirmed by a Toxoplasma serologic reference laboratory (eg, in the United States: www.pamf.org/serology). Toxoplasma-specific immunoglobulin A, differential agglutination test, and avidity assays are used to document the timing of the acquisition of an infection during gestation more precisely. The avidity assay is an important, recently developed test that can be used in the first 12 to 16 weeks of gestation (based on the test kit used) to accurately date the acquisition of infection before conception. High avidity of Toxoplasma-specific antibody in a single serum that has Toxoplasma-specific immunoglobulin G and M antibodies and that has been obtained in the first 12 or 16 weeks of gestation indicates that infection has occurred before conception and therefore is not likely to threaten the fetus.

This approach to the diagnosis and treatment of women who are suspected or proved to have acquired the infection during gestation also includes fetal ultrasound scans and amniocentesis with PCR on the amniotic fluid at 18 weeks of gestation or thereafter to determine whether the fetus is infected. Whereas the specificity of PCR testing on amniotic fluid approaches 100%, the sensitivity is significantly less. Thus, a negative PCR does not rule out infection in the fetus definitively. If the PCR is negative, spiramycin is used to attempt to reduce the transplacental transmission of T gondii to the fetus. When infection is acquired by the pregnant woman before mid gestation and there is no fetal infection that is documented by amniocentesis or suggested by fetal ultrasound scans, spiramycin is continued until term. If the fetus is determined to be infected, the administration of pyrimethamine and sulfadiazine to the mother provides treatment for the fetus as well. Dosages of these medicines for the pregnant woman are pyrimethamine (50 mg per day) and sulfadiazine (2 g, twice daily) with leucovorin (10 mg per day) for its marrow-protective effects. Pyrimethamine is not administered before 12 weeks of gestation.

Management of the pregnant woman who becomes infected later in gestation is controversial. Some physicians use the same approach as described earlier to differentiate the exposed and infected fetus. However, because the likelihood of transplacental transmission and an infected fetus is very high late in gestation, other physicians treat all such mothers and their infants with pyrimethamine, sulfadiazine, and leucovorin.

Our approach to newborn infants who are born to mothers who are suspected or proved to have acquired the infection during gestation is to evaluate the infant clinically and serologically, with an attempt to isolate the parasite from placental tissue. The results of that testing are used to determine whether the treatment of the infant should be initiated or continued. Testing for immunoglobulin M antibodies by a sensitive method like the immunoglobulin M ISAGA test and immunoglobulin A antibodies by enzyme-linked immunosorbent assay must be performed. Serologic testing and subcultivation of the placenta for the infant are performed by a Toxoplasma serologic reference laboratory. Treatment in utero reduces clinical manifestations, the ability to isolate the parasite from the placenta, and the serologic markers of infection in the newborn infant. The infected infant is treated throughout the first year of life.

Analyses of cost and efficacy of screening programs are important in public health policy decision making. Rigorous analyses clearly are needed. However, congenital toxoplasmosis eventually has devastating clinical consequences for nearly all infected infants. Early and aggressive antimicrobial therapy has a clear benefit. The disease occurs with an incidence that is comparable to or higher than a number of genetic and metabolic diseases (eg, phenylketonuria, congenital hypothyroidism, and congenital adrenal hyperplasia) for which neonatal screening is mandated by law in most states. The data herein support the conclusion that “the time has come” to screen for acute acquired Toxoplasma infection in pregnant women and congenital toxoplasmosis in infants to prevent the potentially devastating outcomes from untreated disease.

Acknowledgments

We thank the patients with congenital toxoplasmosis and their families for their generous cooperation; the physicians and other participants in the Chicago Collaborative Toxoplasmosis Treatment Study Group (Ilona Buscher, Audrey Cameron, Esther Castro, Diana Chamot, Barbara Danis, Peter Heydemann, MD, Joyce Hopkins, PhD, Lara Kallal, Kristin Kasza, MS, Michael Kipp, MD, Michael Kirisits, PhD, James McAuley, MD, Marilyn Mets, MD, Sanford Meyers, MD, Ernest Mui, Gwen Noble, MD, Dushyant Patel, MD, Jeanne Perkins, PhD, Linda Pfiffer, MD, Peter Rabiah, MD, Laszlo Stein, MD (deceased), Mark Stein, PhD, Andrew Suth, PhD, Marie Weissbord, PhD, HuiYuan Zhang, MD); the airlines and hotels that provided complimentary transportation to and accommodations

Boyer et al 569
in Chicago; the pharmaceutical companies that provided medications without charge to medically indigent patients; Pam Stipeck-Biek, Dorothy Gibbons, Marie Paul, Nannette Cannon, and Meg Davis for their technical assistance in Dr Jack Remington’s laboratory; Dr Theodore Karrison for his assistance in initial study design, helpful discussions, and assistance with statistical analyses; Drs Jacques Couvreur, George Desmonts, and Philippe Thulliez for their insight, helpful suggestions, and advice; and Judy Darbro for her assistance in preparation of the manuscript.

References


Remodeling of myometrial radial arteries in preeclampsia

Stephen S. Ong, MRCOG, Philip N. Baker, DM, Terry M. Mayhew, PhD, William R. Dunn, PhD

School of Human Development, University of Nottingham, City Hospital, Nottingham, United Kingdom, Maternal and Fetal Health Research Centre, St. Mary’s Hospital, University of Manchester, United Kingdom, and Centre for Integrated Systems Biology and Medicine, School of Biomedical Sciences, University of Nottingham, Queen’s Medical Centre, Nottingham, United Kingdom

Received March 16, 2004; revised July 29, 2004; accepted August 17, 2004

Objective: This study was undertaken to test for structural differences between myometrial radial arteries isolated from women having normal pregnancies and pregnancies complicated by preeclampsia and intrauterine growth restriction.

Study design: Pressure myography was used to study myometrial radial arteries obtained at cesarean section. With the use of a transilluminating system, lumen diameter, wall thickness, wall/lumen ratio, distensibility and stress-strain relationship were studied through a range of pressures. Arteries were then fixed in glutaraldehyde, embedded in resin, cross-sectioned, and studied in greater detail by light and electron microscopy.

Results: Pressure myography showed that arteries from women with preeclampsia had a reduced lumen diameter, thicker wall, and greater wall/lumen ratio compared with vessels isolated from women with normal pregnancy. Light microscopy indicated an identical media content remodeled around a smaller lumen. Electron microscopy indicated enlarged extracellular spaces in the media but no change in myocyte profile size or number. There was no clear evidence of structural changes in myometrial radial arteries isolated from women with intrauterine growth restriction compared with normal pregnancy. No differences in vessel distensibility or stress-strain relationships were detected in complicated pregnancies.

Conclusion: The changes observed in myometrial radial arteries isolated from women with preeclampsia are due to inward eutrophic remodeling. Alterations in these vessels may contribute to increased uterine vascular resistance in preeclampsia.

Preeclampsia and intrauterine growth restriction (IUGR) are conditions in which the placenta is believed to be poorly perfused. In support of this assertion, measurements reflecting uterine blood flow, using a variety of techniques, including radioisotope studies, Doppler ultrasound, and magnetic resonance imaging, have shown that flow is impaired in these conditions. The mechanisms that contribute to reduced uterine perfusion are not wholly understood. However, they are likely to reflect an alteration in either the structural or functional properties of the uteroplacental arterial vasculature.

KEY WORDS
Preeclampsia
Intrauterine growth restriction
Resistance arteries
Myography

$0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.

doi:10.1016/j.ajog.2004.08.015$
It has been proposed that placental blood flow is compromised in preeclampsia as a consequence of impaired endothelium-dependent vasorelaxatory responses of myometrial small arteries. Whether this change reflects the primary reduction in placental blood flow, however, is a matter of some debate, because it has been proposed that the impairment of endothelial function is brought about by the release of a placenta-derived circulating factor in response to placental underperfusion. In this model, the reduced placental perfusion occurs as a consequence of inadequate structural changes that occur within the spiral arteries. In normal pregnancy, the processes of trophoblast invasion and placentation convert spiral arteries into low-pressure high-flow vessels and, in consequence, the major site of uterine vascular resistance is shifted upstream as well as to nonplacental bed sites. Thus, an important determinant of uterine vascular resistance in normal pregnancy is the diameter of maternal uterine radial arteries. To date, no studies have examined whether the structure of radial arteries differs between normal and compromised pregnancies.

In this study we have used physiologic and morphometric methodology to determine whether there are structural differences in myometrial radial arteries isolated from women with normal pregnancy compared with pregnancies complicated by preeclampsia and IUGR.

**Material and methods**

The Ethics Committee at Nottingham City Hospital gave approval for this work and all women who participated gave written informed consent. Women with essential hypertension and medical complications such as diabetes and renal disease were excluded from the study.

The definition of preeclampsia in this study conforms with that used by the International Society for the Study of Hypertension in Pregnancy. Preeclampsia was defined as the clinical syndrome in which a previously normotensive woman, has hypertension and proteinuria develop after the 20th week of pregnancy. Hypertension was defined as a blood pressure of at least 140/90 mm Hg on 2 or more occasions, separated by more than 4 hours. Proteinuria was defined as 2+ on urinalysis, or at least 300 mg/L or 500 mg per 24 hours in a 24-hour collection of urine in the absence of a urinary tract infection. Blood pressure and proteinuria needed to have returned to normal levels by the sixth week postpartum.

Women with IUGR were selected on the basis of clinical or ultrasound suspicion of growth restriction. The individualized birth weight ratio was used as the final arbiter in defining growth restriction. The individualized birth weight ratio is the birth weight adjusted for maternal age, parity, ethnicity, gestation, and fetal sex. The individualized birth weight ratio provides a better correlation with perinatal outcomes than birth weight for gestational age alone. An infant with an individualized birth weight ratio of less than 10th percentile was considered growth restricted. For the purposes of this study, women recruited to the IUGR study group did not have preeclampsia, but 1 woman had pregnancy-induced hypertension.

Myometrial biopsy samples were obtained after delivery of the infant and placenta at cesarean section. A full thickness biopsy was obtained from the upper margin of the uterine incision within the lower uterine segment. These samples were placed in a container of calcium-free physiologic salt solution (Ca2+-free PSS) and transported to the laboratory on ice.

The decidual and serosal portions of the myometrial sample were initially identified. The sample was pinned out in a dissecting dish such that the decidual portion was on the right and the serosal portion was on the left hand side. The sample was viewed under a light microscope and an artery was dissected free of surrounding connective tissue. Care was taken to ensure that the artery chosen was distant (at least 1 cm) from the decidua.

Myometrial arteries were mounted on a Halpern pressure-perfusion myograph; segments of the vessels were secured between 2 cannulae and tied with a double strand (1 cm long) of surgical braided nylon suture. One cannula was closed and the other connected to a system containing Ca2+-free PSS, which in turn was linked to a pressure-servo unit. This allowed the intraluminal pressure to be changed. The arteriograph was a 10-mL vessel chamber with an input and output channel to allow superfusion of Ca2+-free PSS. The blood vessels were imaged with a video camera. The arteriograph in which the vessel was secured was connected to a 200-mL reservoir of Ca2+-free PSS, which was bubbled with a 5% CO2/95% O2 gas mixture and circulated with a Masterflex pump (Cole-Parmer, London, UK) at a rate of 10 mL/min. This ensured that the arteriograph volume was exchanged once per minute. Temperature was maintained at 37°C.

Internal diameter and wall thickness measurements were made possible by transilluminating the vessel. These measurements were made through the pressure range of 5 to 100 mm Hg. At each pressure, 6 measurements of arterial wall thickness and 3 measurements of internal diameter were made along the length of the artery. When pressurized, arterial segments underwent some elongation. To compensate for any elongation, vessels were retracted with a micrometer to a length at which buckling was no longer apparent for each pressure. In general, very little adjustment was required. Once these measurements had been obtained, intraluminal pressure was set to the mean arterial pressure.
(MAP) of the patient, and the vessel was subsequently exposed to Ca\(^{2+}\)-free PSS containing 1.5% glutaraldehyde solution at 37°C for 30 minutes, which was then allowed to cool to room temperature over an additional 60 minutes to fix the vessels. This procedure minimizes shrinkage or contraction artifacts. The cannulae to which the arterial segments were attached were broken and used to transport the vessel to a Petri dish, in which the segment was washed 3 times in Ca\(^{2+}\)-free PSS, 3 times in distilled water, and stored overnight at 4°C.

After investigation on the pressure myograph system, arteries that were fixed in glutaraldehyde were subsequently stained with 1% osmium tetroxide and dehydrated with acetone before being embedded with Araldite CY212 epoxy resin (TAAB Laboratories, Berkshire, UK). Semithin (1 µm thick) transverse sections were cut on a Reichert Jung OMU3 ultramicrotome (Reichert Jung, Wien, Austria) by using glass knives, placed on glass microscope slides, and stained with 1% toluidine blue. With light microscopy, structural analysis of these sections was carried out with a randomly superimposed morphometric grid, and luminal and medial cross-sectional areas were estimated. Provided test points are randomly superimposed on sections, and the area equivalent of each test point is known (taking into account magnification), the number of points provides an unbiased estimator of component sectional areas. Because of the approximately circular nature of the cross section, values for lumen internal diameter and average media thickness could subsequently be calculated.

Myometrial arteries isolated from women with preeclampsia and normal pregnancy were further examined with electron microscopy. Ultrathin sections (80 nm thick) were cut, contrasted with lead citrate/uranyl acetate and mounted on copper support grids. These were examined with a Philips EM410 electron microscope (Philips, Eindhoven, The Netherlands), photographed, and printed to a final linear magnification of ×6800 with the aid of external calibration standards. Smooth muscle nuclei, smooth muscle cytoplasm, endothelium, fibroblasts, collagen, and extravascular space were identified and their respective quantities (total and mean profile areas) were also estimated with stereologic test point counting. The number of nuclear profiles of smooth muscle cells within the vessel wall section and the profile sizes of the smooth muscle cells in each vessel were also calculated.

The following chemicals were used: Osmium tetroxide (Johnson Matthey, London, UK), Araldite CY212 epoxy resin (TAAB Laboratories), and glutaraldehyde (TAAB Laboratories). The composition of Ca\(^{2+}\)-free PSS was as follows (in mmol/L): NaCl 119, NaHCO\(_3\) 24, KCl 4.7, KH\(_2\)PO\(_4\) 1.17, MgSO\(_4\) • 7H\(_2\)O 1.17, glucose 5.5, and EGTA 0.5.

For a detailed assessment of the mechanical properties of vessels, the following parameters were determined according to the method outlined by Baumbach and Heistad,\(^{20}\) and incremental distensibility, circumferential stress, and circumferential strain.\(^{11}\)

Nonparametric analysis of apparent differences between the study groups was performed with the Kruskal-Wallis or Mann-Whitney U test as appropriate. For the investigation of vessel wall and lumen parameters over a range of pressures, we used 2-way analysis of variance (ANOVA) to test for significant differences caused by pressure, patient group and interaction (pressure × group) effects. Because this test is not ideal when used on related samples, we compared pressure differences within patient groups using repeated measures ANOVA. Therefore, P values for pressure differences refer to the repeated measures ANOVA results, whereas P values for group and interaction effects refer to results obtained with 2-way ANOVA.

Results

Details of patient characteristics are outlined in Table I. Women with preeclampsia were more often nulliparous, were delivered at an earlier gestation, had smaller infants for gestational age, and had a higher mean arterial pressure at delivery compared with normal pregnant women. Women with IUGR were delivered at an earlier gestation and had smaller infants for gestational age compared with normal pregnant women. Of the 6 women with IUGR, 4 had a prior normal pregnancy. Some women with preeclampsia also had IUGR diagnosed (n = 3). Subanalysis of this group showed that the myometrial arteries were remodelled similarly to women with preeclampsia alone (n = 7), and so these groups were combined.

In total, myometrial small arteries were studied on the pressure myograph system from 15 women with normal pregnancy, 11 women with preeclampsia, and 6 women with IUGR. Subsequent examination with light microscopy was successfully completed on arteries from 11 women with normal pregnancy, 10 women with preeclampsia, and 6 women with IUGR. Further examination with electron microscopy was conducted on arteries from 7 women with normal pregnancy and 8 women with preeclampsia.

The pressure myograph system

Regardless of patient group, arteries showed increases in lumen diameter with increasing intraluminal pressure (repeated measures ANOVA, P < .05). Arteries isolated from women with preeclampsia had a smaller lumen diameter compared with vessels isolated from normal pregnancy at all pressures (2-way ANOVA: P < .01). The lumen diameters of arteries isolated from women...
with IUGR were similar to those isolated from normal pregnancy (Figure 1, A). No significant interaction (pressure × group) effects were detected.

Wall thickness tended to decrease with increasing intraluminal pressure in all groups (repeated measures ANOVA, \( P < .05 \)). Arteries from women with preeclampsia had thicker walls compared with those from normal pregnancy (2-way ANOVA: \( P < .001 \)). Arteries from women with IUGR also appeared to have thicker walls compared with normal pregnancy, but this difference was not statistically significant (Figure 1, B). Again, no significant interaction effects were demonstrable.

Wall/lumen ratios also declined with increases in pressure (repeated measures ANOVA, \( P < .05 \)). In arteries isolated from women with preeclampsia, the wall/lumen ratio was greater than that seen in normal pregnancy throughout the pressure range examined (2-way ANOVA: \( P < .001 \)). In contrast, arteries from women with IUGR were not different from vessels isolated from normal pregnancy (Figure 1, C). No significant interaction effects were detected.

There were no differences in the distensibility of vessels isolated from women with normal or complicated pregnancies (Figure 2, A). Nor were there differences in vessel wall stress or strain, or the stress-strain relationship between vessels isolated from different study groups (Figures 2, B). This was shown by the slope of the tangential elastic modulus versus stress, which was not different between vessels isolated from each of the patient groups. Group means (SEM) for the different pregnancies were 12.5 (1.8) for normal, 10.3 (1.8) for preeclampsia, and 11.1 (2.6) for IUGR (\( P = .70 \), Kruskal-Wallis test).

**Light microscopy**

Myometrial arteries fixed in glutaraldehyde were subjected to more detailed histologic examination with the use of stereologic methods. This allowed greater discrimination of media content and composition from the wall content as a whole (Figure 3).

In histologically examined vessels, arteries isolated from women with preeclampsia appeared to have a smaller lumen diameter compared with normal pregnancy, although this difference was not statistically significant (Table II). Vessels isolated from women with preeclampsia had a significantly greater media thickness and a significantly greater media/lumen ratio. In vessels from women with preeclampsia, apparent reductions in both the internal and external circumference were not statistically significant. The total media content was not significantly different from that found in vessels isolated from women with normal pregnancy (mean [SEM]: normal 21,353 (4,197) \( \mu \text{m}^2 \), preeclampsia 26,038 (3,397) \( \mu \text{m}^2 \)).

The lumen diameter of arteries from women with IUGR was similar to that from normal pregnancy (Table II). The increased media thickness in IUGR was not statistically significant. There was no difference in media/lumen ratio, although there was a tendency for vessels isolated from women with IUGR to be slightly larger and to have a greater media content compared with vessels isolated from women with normal pregnancies.

**Electron microscopy**

Because differences were apparent in the media thickness and media/lumen ratio in vessels isolated from women with preeclampsia, further assessment of media composition was determined with the use of electron microscopy.

Analysis showed that the total area occupied by smooth muscle cytoplasm was not different between vessels isolated from women with preeclampsia and normal pregnancy (Table III). The profile area occupied per smooth muscle cell (used as an indicator of hypertrophy) appeared to be increased in preeclampsia, but this was not statistically significant. The number of profiles of smooth muscle nuclei in the media wall (used as an indicator of hyperplasia) was not significantly different from that in normal pregnancy.

---

**Table I**  Patient demographics

<table>
<thead>
<tr>
<th></th>
<th>Normal n=15</th>
<th>PE n=11</th>
<th>IUGR n=6</th>
<th>Kruskal-Wallis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>30.9 (3.8)</td>
<td>27.3 (6.8)</td>
<td>29.5 (9.0)</td>
<td>NS</td>
</tr>
<tr>
<td>Proportion nullipara</td>
<td>4/15</td>
<td>9/11</td>
<td>2/6</td>
<td></td>
</tr>
<tr>
<td>Gestation at delivery (wk)</td>
<td>38.1 (0.5)</td>
<td>32.5 (4.3)</td>
<td>34.7 (1.6)</td>
<td>( P &lt; .05^* )</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3441 (506)</td>
<td>1883 (956)</td>
<td>1863 (813)</td>
<td>( P &lt; .05^* )</td>
</tr>
<tr>
<td>Individualized birth weight ratio</td>
<td>56.0 (32.5)</td>
<td>22.5 (33.6)</td>
<td>1.5 (2.0)</td>
<td>( P &lt; .05^* )</td>
</tr>
<tr>
<td>MAP at delivery (mm Hg)</td>
<td>89.2 (8.4)</td>
<td>115.7 (4.5)</td>
<td>89.2 (12.6)</td>
<td>( P &lt; .05^* )</td>
</tr>
</tbody>
</table>

Values are quoted as mean (SD). PE, Preeclampsia. Dunn’s post-hoc test was statistically significant for:

* Normal vs PE.
\( ^* \) Normal vs IUGR.
\( ^* \) PE vs IUGR.
The total area occupied by extracellular space (which was not occupied by collagen) was significantly increased in arteries from women with preeclampsia. The areas occupied by collagen, cytoplasm of fibroblasts, and the endothelium were not different between study groups.

Comment

The main finding from this study is that myometrial arteries isolated from women with preeclampsia are altered in structure such that the lumen diameter is smaller and the vessel wall thicker compared with normal pregnancy, resulting in an increased wall/lumen ratio. This structural change is a result of remodeling of similar amounts of media content around a smaller lumen. In myometrial arteries isolated from women with IUGR, no such change in structure was evident compared with vessels from normal pregnancies.

Preeclampsia

The results from the 3 methods (pressure myography, light microscopy, and electron microscopy) used to study the myometrial arteries were in general agreement. Results from the pressure myograph system showed that, throughout a range of pressures, arteries from women with preeclampsia had a smaller lumen diameter, thicker walls, and a larger wall/lumen ratio. More detailed investigations by light microscopy supported these findings and, in addition, showed that vessels isolated from women with preeclampsia had a smaller internal and external circumference, despite similar media content, compared with vessels isolated from normal pregnant women. This could only occur if similar media content is organized around a smaller lumen. Electron microscopy provided confirmation that the total cross-sectional area of cytoplasm was
similar in vessels isolated from women with preeclampsia and normal pregnancy. The latter analysis provided no clear evidence of hypertrophy or hyperplasia. The fact that the overall media area was unaltered with no significant change in cell profile size or number was consistent with inward eutrophic remodeling.²⁰

One of the potential difficulties in assessing vascular structure in vitro is the possibility of systematic selection bias, whereby vessels may be selected from biopsy samples from a single patient group partly on the basis of their size (diameter). Given the fact that group differences resided solely in the preeclampsia group, and that its variables showed similar levels of subject-to-subject variation to those found in normal and IUGR groups, we believe that the observed differences are due to physiologic effects rather than sampling biases. In any event, we placed particular emphasis on deriving wall/lumen ratio measurements and this ratio is known to remain relatively constant in different-sized vessels sampled from within a single vascular bed.¹⁷ The wall/lumen ratio in vessels isolated from women with preeclampsia was almost twice that of normal pregnancy. This indicates that substantial structural change has occurred in preeclampsia.

The reason why there was a greater quantity of extracellular space within the media of vessels from women with preeclampsia is unclear. It is possible that this is associated with an increased permeability of fluid into the extracellular space, a common feature of preeclampsia that manifests itself as edema.

Arterial diameter can be reduced as a consequence of the artery being less distensible.¹⁸ However, no differences in stress-strain relationships or distensibility were apparent in vessels isolated from women with preeclampsia compared with normal pregnancies.

The data presented in this study indicate that, in addition to structural alterations of spiral arteries, an altered structure of myometrial radial arteries could contribute to the increased uterine vascular resistance characteristic of preeclampsia. The question as to whether remodeling of myometrial arteries is a cause or consequence of preeclampsia is difficult to ascertain. Inadequate invasion of spiral arteries by trophoblast relates to placentation and is believed to be the origin of reduced placental perfusion,²¹ whereas the attenuation of endothelium-dependent responses of myometrial arteries is believed to be a consequence of reduced placental perfusion mediated through a circulating factor(s).²²,²³ Our previous data have demonstrated that omental arteries are not remodeled in preeclampsia.¹¹ In other words, systemic hypertension does not lead to remodeling of omental arteries. Therefore, it seems

### Table II
Light microscopy. Dimensions of myometrial radial arteries fixed in glutaraldehyde at the predelivery MAP. Results are expressed as the median (range).

<table>
<thead>
<tr>
<th></th>
<th>Normal n = 11</th>
<th>PE n = 10</th>
<th>IUGR n = 6</th>
<th>Kruskal Wallis test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lumen diameter (µm)</td>
<td>311 (210-561)</td>
<td>256 (165-388)</td>
<td>302 (203-509)</td>
<td>P = .52</td>
</tr>
<tr>
<td>Media thickness (µm)</td>
<td>17 (12-32)</td>
<td>26 (13-42)</td>
<td>32 (11-38)</td>
<td>P = .03*</td>
</tr>
<tr>
<td>Media-to-lumen ratio (×100)</td>
<td>5.3 (3.8-8.6)</td>
<td>9.5 (5.1-25.5)</td>
<td>7.5 (4.4-15.5)</td>
<td>P &lt; .01*</td>
</tr>
<tr>
<td>Media area (µm²)</td>
<td>16,969 (7,992-50,160)</td>
<td>26,292 (11,846-48,593)</td>
<td>34,201 (9,285-58,194)</td>
<td>P = .20</td>
</tr>
<tr>
<td>Internal circumference (µm)</td>
<td>1,009 (649-1,760)</td>
<td>809 (504-1,219)</td>
<td>942 (634-1,612)</td>
<td>P = .48</td>
</tr>
<tr>
<td>External circumference (µm)</td>
<td>1,081 (712-1,957)</td>
<td>965 (793-1,665)</td>
<td>1,156 (860-1,839)</td>
<td>P = .70</td>
</tr>
</tbody>
</table>

* Dunn’s post-hoc test was statistically significant for normal vs PE.

### Table III
Electron microscopy. Media contents of myometrial radial arteries

<table>
<thead>
<tr>
<th></th>
<th>Normal n = 7</th>
<th>PE n = 8</th>
<th>Mann-Whitney U test</th>
</tr>
</thead>
<tbody>
<tr>
<td>Area of media</td>
<td>16,242 (8,779-48,243)</td>
<td>21,586 (11,407-47,193)</td>
<td>P = .40</td>
</tr>
<tr>
<td>Area of endothelium</td>
<td>727 (399-1,917)</td>
<td>585 (122-1,400)</td>
<td>P = .40</td>
</tr>
<tr>
<td>Area per smooth muscle cell</td>
<td>133 (58-184)</td>
<td>173 (76-623)</td>
<td>P = .12</td>
</tr>
<tr>
<td>Number of smooth muscle nuclei within media</td>
<td>77 (51-213)</td>
<td>78 (29-123)</td>
<td>P = .12</td>
</tr>
<tr>
<td>Area of fibroblast cytoplasm</td>
<td>121 (0-1,085)</td>
<td>296 (0-2,000)</td>
<td>P = .42</td>
</tr>
<tr>
<td>Area of collagen</td>
<td>4,612 (1,995-16,294)</td>
<td>6,266 (2,457-10,398)</td>
<td>P = .61</td>
</tr>
<tr>
<td>Area of extracellular space (excluding collagen)</td>
<td>789 (399-3,195)</td>
<td>2,646 (1,580-6,399)</td>
<td>P = .03</td>
</tr>
</tbody>
</table>

Areas are calculated in square microns (µm²). Results are expressed as the median (range).
unlikely that remodeling of myometrial arteries is a consequence of systemic hypertension. It remains a matter of conjecture as to whether a circulating factor such as a growth factor could play a part in vascular remodeling of myometrial arteries and thus exacerbate increased uterine vascular resistance.

We have argued that in preeclampsia, myometrial arteries are remodeled such that a similar amount of tissue is arranged around a smaller lumen. However, it is equally plausible that, in normal pregnancy, myometrial arteries are remodeled such that existing tissue is reorganized around a larger lumen, and that this does not take place in preeclampsia.

The results of this study have to be interpreted with caution. Women with preeclampsia delivered at an earlier gestation and were more likely to be nulliparous. These confounders have not been accounted for in this study. Another limitation of this work is the site from which vessels were obtained. Although samples obtained from the lower uterine segment demonstrate clear structural alterations in arteries obtained from normal pregnancies and preeclamptic women, caution should be applied in extrapolating these findings to the whole uterus.

**IUGR**

Results from the pressure myograph system suggested that arteries isolated from women with IUGR had a similar lumen diameter but a thicker wall compared with vessels from normal pregnant women, although the difference was not statistically significant. As mentioned previously, we place great importance on the wall/lumen ratio to minimize potential sampling bias. The wall/lumen ratio was similar in both the IUGR and normal pregnancy study groups, indicating that no substantial structural changes had occurred. These findings were further confirmed by light microscopy, which showed that lumen diameter and internal circumference of vessels isolated from women with IUGR were not different to normal pregnancy. Although it appeared that the media area and external circumference in vessels isolated from women with IUGR was increased, this difference was not statistically significant. Taken together, the results suggest that overall, there was no alteration in structure in vessels isolated from women with IUGR compared with normal pregnancy.

The lack of difference in lumen diameter of myometrial arteries suggest that, under conditions of maximal vasodilatation, blood delivery to the uterus would not be compromised in women with IUGR at this level of the maternal uterine vasculature. Thus, placental perfusion would only be compromised in this condition if there was a significant reduction in structure and/or function of the spiral arteries. There is evidence to suggest that in IUGR, the inadequate transformation of spiral artery structure is not as defective as in preeclampsia. Brosens et al\(^\text{24}\) showed that in normotensive women with IUGR, the depth of spiral artery transformation was either normal or restricted to the decidua, whereas in hypertensive women with IUGR, the depth of spiral artery transformation was restricted to the decidua in all women. In the current study, 5 of 6 women with IUGR were normotensive. The data presented here are in support of other workers who caution that uteroplacental blood flow is not the only mechanism giving rise to IUGR, and that placental transport and growth mechanisms must be taken into consideration.\(^\text{25}\) We classified IUGR on the basis of individualized birth weight ratios.\(^\text{14}\) It remains possible that the lack of structural difference between arteries isolated from normal and IUGR pregnancies reflects an oversimplification of this classification system and does not allow discrimination between IUGR occurring because of different causes.

In conclusion, myometrial radial arteries from women with preeclampsia, are altered such that existing

![Light microscopy of pressurized myometrial arteries isolated from women with a normal pregnancy (top) and pregnancy complicated by preeclampsia (bottom). The vessel isolated from the women with preeclampsia had a reduced lumen diameter, increased wall thickness, and increased wall/lumen ratio. Original magnification, $\times 300$.](image-url)
media tissue is remodeled around a smaller lumen. This could reduce vascular supply to the placenta in pre-eclampsia. These changes were not seen in vessels isolated from women with IUGR.

References


Prevalence of seat belt use among reproductive-aged women and prenatal counseling to wear seat belts

Laurie F. Beck, MPH, a,* Brenda Colley Gilbert, PhD, b Ruth A. Shults, PhD a

Division of Unintentional Injury Prevention, National Center for Injury Prevention and Control, a and Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, b Centers for Disease Control and Prevention, Atlanta, Ga

Received for publication March 25, 2004; revised June 23, 2004; accepted July 19, 2004

KEY WORDS
Injury
Seat belt
Counseling
Prenatal care

Objective: The purpose of this study was to determine the prevalence of counseling to wear seat belts during pregnancy and seat belt use among women of reproductive age.

Study design: Self-reported data from 2 population-based surveys were used to examine counseling to wear seat belts during pregnancy and seat belt use among reproductive-aged women.

Results: The prevalence of counseling to wear seat belts during pregnancy ranged from 36.7% to 56.5% across 19 states. The prevalence of seat belt use among reproductive-aged women ranged from 69.5% to 91.4% across 19 states. Younger, non-Hispanic black, and less educated pregnant women were more likely to report counseling, but reproductive-aged women with these characteristics were less likely than older, non-Hispanic white, and more educated women to use seat belts.

Conclusion: Most women are not counseled about seat belt use during pregnancy. Providers should ensure that this topic is discussed with each patient.

© 2005 Elsevier Inc. All rights reserved.
the crash) and low birth weight. A recent population-based study by Hyde et al showed that unrestrained pregnant drivers were at an increased risk of experiencing fetal death and excessive maternal bleeding, whereas restrained drivers were not at any higher risk for adverse pregnancy outcomes than pregnant women who were not involved in crashes.

Nearly all pregnant women in the United States receive prenatal care. Consistent with occupant safety recommendations from the US Preventive Services Task Force, the American Academy of Pediatrics (AAP) and the American Academy of Obstetricians and Gynecologists (ACOG) recommend that health care providers counsel all pregnant women on the proper use of seat belts during pregnancy. Prenatal care visits provide a unique opportunity for health care professionals to educate women on the importance of using seat belts during pregnancy and to demonstrate the correct positioning of the seat belt throughout pregnancy.

Clinic-based surveys have found that <50% of pregnant women were counseled about seat belt use during prenatal care. In the late 1990s, the Pregnancy Risk Assessment Monitoring System (PRAMS) found that 53% of pregnant women were counseled on the subject. This report summarizes findings regarding seat belt counseling from the 2000 PRAMS survey and provides population-based estimates of seat belt use among pregnant women and women of reproductive age from the 2002 Behavioral Risk Factor Surveillance System (BRFSS).

Material and methods

PRAMS and BRFSS are ongoing, population-based surveillance systems that are administered by the Centers for Disease Control and Prevention. PRAMS collects self-reported data on maternal behaviors and experiences that occur before, during, and after pregnancy. Women who are delivered of live-born infants are sampled from birth certificates at 2 to 6 months after delivery. PRAMS uses a standardized data collection method, which was approved by the Institutional Review Board of the Centers for Disease Control and Prevention. Data are collected with mailed, self-administered surveys or with telephone interviews. Details of the PRAMS methods have been described elsewhere.

In 2000, information on prenatal care counseling about seat belt use during pregnancy was collected in 19 states (Alabama, Alaska, Arkansas, Colorado, Florida, Hawaii, Illinois, Louisiana, Maine, Nebraska, New Mexico, New York, North Carolina, Ohio, Oklahoma, South Carolina, Utah, Washington, West Virginia). New York data did not include New York City. Response rates, which were weighted to reflect the sampling design, ranged from 72% to 86% across states.

Respondents were asked whether, during any prenatal care visit, a doctor, nurse, or other health care worker talked with them about using a seat belt during pregnancy. We examined seat belt counseling by selected indicators. Maternal sociodemographic variables (age, race/ethnicity, education, parity) were obtained from birth certificates; all other variables were self-reported on the PRAMS questionnaire. Age was categorized into 4 groups: ≤17 years, 18 to 24 years, 25 to 29 years, and ≥30 years. Race/ethnicity was categorized as Hispanic, non-Hispanic white, non-Hispanic black, and non-Hispanic other. Education was defined as less than high school, high school, and more than high school. Parity was dichotomized by whether the mother had any previous live births. Payment source for prenatal care was defined as Medicaid or non-Medicaid. Prenatal care providers were categorized as either public or private. Timing of entry into prenatal care was defined by the trimester in which a woman had her first prenatal care visit (first trimester vs second/third trimester). Women who did not receive any prenatal care were excluded from all analyses; the prevalence of not receiving any prenatal care ranged from 0.3% to 1.5% across states. All missing observations were also excluded (missing data for the variables that were examined ranged from 0.02% to 6.8%).

BRFSS is an ongoing, population-based survey that collects self-reported data on a variety of health-related topics. All 50 states, the District of Columbia, and 3 territories participate. A disproportionate stratified sample of adults (at least age 18 years) is selected for the 50 states and the District of Columbia; a simple random sample design is used for the territories. Data are collected by telephone with an interviewer-administered survey. Institutional review board approval is not needed because BRFSS is a public health surveillance system for which data are collected anonymously with the voluntary consent of participants. Details of the BRFSS methods have been described elsewhere. In 2002, the median response rate, as defined by the Council of American Survey Research Organizations, was 58% (range, 42-83).

In 2002, information on seat belt use was collected on the BRFSS interview. Respondents were asked how often they used seat belts in a car. For this analysis, seat belt use was defined as a dichotomous variable (always vs nearly always, sometimes, rarely, or never). Respondents who reported that they never rode in cars were excluded from the analysis (0.2%). The analysis was limited to women of reproductive age (range, 18-44 years) in the 50 states and the District of Columbia. Pregnancy status was assessed at the time of the interview (currently pregnant or not). We examined seat belt use among women of reproductive age by sociodemographic variables (age, race/ethnicity, education). Age was categorized as 18 to 24 years, 25 to 29 years,
and 30 to 44 years. Race/ethnicity was categorized as Hispanic, non-Hispanic white, non-Hispanic black, and non-Hispanic other. Education was defined as less than high school, high school, and more than high school. We also examined seat belt use by the type of seat belt law that was present in the state during 2002. States were classified as having a primary enforcement law (ie, motor vehicle occupants can be ticketed solely for not wearing a seat belt) or a secondary enforcement law (ie, motor vehicle occupants can be ticketed for not wearing a seat belt only after they have been stopped for another reason). Washington implemented a primary enforcement law during the study period and was excluded from this portion of the analysis. All missing observations were excluded from the analysis (missing data for the variables that were examined ranged from 0.0%-1.3%).

To accommodate the complex survey designs, SUDDAAN software (Research Triangle Institute, NC) was used for analysis. Prevalence estimates and 95% confidence intervals (CIs) for being counseled to use a seat belt during pregnancy were calculated by state. Prevalence estimates and 95% CIs were calculated to provide national estimates of seat belt use among all reproductive-aged women and for the subset of pregnant women. Because of sample size concerns among the pregnant women, the descriptive analysis of the BRFSS data was performed for all women of reproductive age. We performed the BRFSS analysis using aggregated data for all 50 states and the District of Columbia. Because results did not differ from the results that we observed when we limited the analysis to 19 states, we reported only those data from the 19 states for which data on prenatal counseling were also available.

**Results**

PRAMS data indicate that the prevalence of pregnant women who reported prenatal counseling to use a seat belt was 48.2% overall; the prevalence ranged from 36.7% to 56.5% across the 19 states (Table I). The women who were least likely to report having been counseled to wear seat belts were at least 30 years of age, were non-Hispanic white, had more than a high school education, were not receiving Medicaid, or were receiving prenatal care from a private provider (Table II). The prevalence of being counseled decreased as the level of education increased.

In 2002, BRFSS data show that self-reported seat belt use in the United States (50 states and the District of Columbia) was 83.8% (95% CI, 83.2, 84.4) for reproductive-aged women and 84.1% (95% CI, 81.9, 86.3) for pregnant women. Seat belt use among reproductive-aged women ranged from 59.8% to 94.1% across all states; Table III shows seat belt use in the 19 states for which prenatal counseling about seat belt use was assessed. Aggregated data for these 19 states showed that, among women of reproductive age, non-Hispanic black women were slightly less likely to wear seat belts than non-Hispanic white women, that women aged 29 years were slightly less likely to wear seat belts than older women, and that women with a high school or less than high school education were slightly less likely to wear a seat belt only after they have been stopped for another reason). Washington implemented a primary enforcement law during the study period and was excluded from this portion of the analysis. All missing observations were excluded from the analysis (missing data for the variables that were examined ranged from 0.0%-1.3%).

To accommodate the complex survey designs, SUDDAAN software (Research Triangle Institute, NC) was used for analysis. Prevalence estimates and 95% confidence intervals (CIs) for being counseled to use a seat belt during pregnancy were calculated by state. Prevalence estimates and 95% CIs were calculated to provide national estimates of seat belt use among all reproductive-aged women and for the subset of pregnant women. Because of sample size concerns among the pregnant women, the descriptive analysis of the BRFSS data was performed for all women of reproductive age. We performed the BRFSS analysis using aggregated data for all 50 states and the District of Columbia. Because results did not differ from the results that we observed when we limited the analysis to 19 states, we reported only those data from the 19 states for which data on prenatal counseling were also available.

**Comment**

Although the American Academy of Pediatrics and ACOG recommend that prenatal care providers counsel

| Table I Prevalence (%) of prenatal counseling to use seat belts during pregnancy: Pregnancy Risk Assessment Monitoring System, 19 states, 2000 |
|-----------------|-----------------|-----------------|
| State           | Sample (n)      | Prevalence (95% CI) |
| Total           | 32,517          | 48.2 (47.2-49.2)  |
| Alabama         | 1536            | 49.9 (46.8-53.0)  |
| Alaska          | 1430            | 49.9 (47.0-52.8)  |
| Arkansas        | 1604            | 36.7 (33.2-40.2)  |
| Colorado        | 2118            | 48.3 (45.6-51.0)  |
| Florida         | 1957            | 45.9 (42.6-49.2)  |
| Hawaii          | 2443            | 48.8 (46.6-51.0)  |
| Illinois        | 1936            | 50.3 (47.9-52.7)  |
| Louisiana       | 2220            | 52.2 (49.7-54.7)  |
| Maine           | 1123            | 55.4 (52.1-58.7)  |
| Nebraska        | 2066            | 50.8 (48.1-53.5)  |
| New Mexico      | 1571            | 55.7 (53.2-58.2)  |
| New York*       | 1220            | 39.0 (35.5-42.5)  |
| North Carolina  | 1764            | 55.9 (52.8-59.0)  |
| Ohio            | 1611            | 46.7 (43.4-50.0)  |
| Oklahoma        | 1932            | 42.9 (39.4-46.4)  |
| South Carolina  | 1563            | 51.9 (48.0-55.8)  |
| Utah            | 1610            | 42.8 (39.7-45.9)  |
| Washington      | 1540            | 56.5 (53.0-60.0)  |
| West Virginia   | 1273            | 46.9 (43.6-50.2)  |

* Data do not include New York City.
all women about seat belt use during pregnancy, we found that fewer than one half of pregnant women (48%) reported that they were counseled about seat belt use. Petersen et al\textsuperscript{22} reported a 53% prevalence in 14 PRAMS states during 1997 and 1998. Prevalence in 2000 may be attributed to a revision to the survey question in 2000 and may not represent a true decrease in the prevalence of seat belt counseling.\textsuperscript{28} No other population-based estimates of seat belt counseling were identified, but our findings were consistent with clinic-based studies that reported that fewer than 50% of the women recalled prenatal counseling about seat belts.\textsuperscript{19-21,29,30}

In clinic-based surveys that were conducted in the 1990s, 45% to 86% of pregnant women reported always wearing seat belts.\textsuperscript{20-21} Our population-based national estimate of pregnant women who always wore seat belts was 84%. This finding is consistent with a recent national estimate that 14% of pregnant women who are involved in police-reported crashes were not belted.\textsuperscript{31} Sociodemographic differences in seat belt use are well documented, with lower rates consistently reported for males, young adults, blacks, and people with lower levels of education or income.\textsuperscript{32-35} Although our findings about seat belt use among reproductive-aged women support the existing literature, the observed differences among groups were modest. This may be because our analysis, by definition, excluded men, and gender is 1 of the strongest predictors of seat belt use.\textsuperscript{32-35}

Our findings suggest that, rather than counseling all patients as recommended, providers may be targeting groups that are less likely to wear seat belts (eg, younger women, black women, or women with lower levels of education) for counseling. The PRAMS data showed that older mothers (at least 30 years of age), non-Hispanic white mothers, and mothers who had more than a high school education were less likely to report prenatal counseling. In addition, non-Medicaid recipients and women with private health care providers were less likely to report counseling. None of the studies that we reviewed reported on associations between prenatal counseling about seat belt use and sociodemographic factors.\textsuperscript{19-21,29,30}

The PRAMS data are subject to several limitations. First, because PRAMS did not collect information about seat belt use during pregnancy, the effect of prenatal counseling on seat belt use could not be assessed. Other studies, however, have reported a positive association between prenatal counseling and proper seat belt use.\textsuperscript{20,21,29} Second, recall bias may be an issue; some women who were counseled may not recall being

### Table II

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Risk ratio (unadjusted)</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal race/ethnicity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.25</td>
<td>1.18-1.32</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>1.37</td>
<td>1.32-1.43</td>
</tr>
<tr>
<td>Non-Hispanic other</td>
<td>1.23</td>
<td>1.15-1.32</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Maternal age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>≤17</td>
<td>1.19</td>
<td>1.09-1.29</td>
</tr>
<tr>
<td>18-24</td>
<td>1.17</td>
<td>1.12-1.23</td>
</tr>
<tr>
<td>25-29</td>
<td>1.10</td>
<td>1.04-1.16</td>
</tr>
<tr>
<td>≥30</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Maternal education</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt; High school</td>
<td>1.31</td>
<td>1.24-1.37</td>
</tr>
<tr>
<td>High school</td>
<td>1.16</td>
<td>1.11-1.22</td>
</tr>
<tr>
<td>&gt; High school</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.02</td>
<td>0.98-1.06</td>
</tr>
<tr>
<td>≥2</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Prenatal care payer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Medicaid</td>
<td>1.21</td>
<td>1.16-1.25</td>
</tr>
<tr>
<td>Non-Medicaid</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Prenatal care provider</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Public</td>
<td>1.25</td>
<td>1.20-1.30</td>
</tr>
<tr>
<td>Private</td>
<td>Referent</td>
<td></td>
</tr>
<tr>
<td>Entry into prenatal care</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd/3rd trimester</td>
<td>1.01</td>
<td>0.96-1.06</td>
</tr>
<tr>
<td>1st trimester</td>
<td>Referent</td>
<td></td>
</tr>
</tbody>
</table>

### Table III

<table>
<thead>
<tr>
<th>State</th>
<th>Sample (n)</th>
<th>Prevalence (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Alabama*</td>
<td>848</td>
<td>85.6 (82.7-88.5)</td>
</tr>
<tr>
<td>Alaska</td>
<td>800</td>
<td>73.9 (69.6-78.2)</td>
</tr>
<tr>
<td>Arkansas</td>
<td>916</td>
<td>69.5 (66.0-73.0)</td>
</tr>
<tr>
<td>Colorado</td>
<td>1085</td>
<td>81.1 (78.2-84.0)</td>
</tr>
<tr>
<td>Florida</td>
<td>1463</td>
<td>84.6 (82.4-86.8)</td>
</tr>
<tr>
<td>Hawaii*</td>
<td>1398</td>
<td>91.4 (89.2-93.6)</td>
</tr>
<tr>
<td>Illinois</td>
<td>692</td>
<td>75.0 (69.5-80.5)</td>
</tr>
<tr>
<td>Louisiana*</td>
<td>1427</td>
<td>80.9 (78.5-83.3)</td>
</tr>
<tr>
<td>Maine</td>
<td>614</td>
<td>76.6 (72.7-80.5)</td>
</tr>
<tr>
<td>Nebraska</td>
<td>1136</td>
<td>76.3 (73.4-79.2)</td>
</tr>
<tr>
<td>New Mexico*</td>
<td>1086</td>
<td>89.7 (87.5-91.9)</td>
</tr>
<tr>
<td>New York*</td>
<td>1217</td>
<td>83.1 (80.6-85.6)</td>
</tr>
<tr>
<td>North Carolina*</td>
<td>1738</td>
<td>91.3 (89.3-93.3)</td>
</tr>
<tr>
<td>Ohio</td>
<td>1070</td>
<td>78.8 (75.9-81.7)</td>
</tr>
<tr>
<td>Oklahoma*</td>
<td>1601</td>
<td>82.1 (79.7-84.5)</td>
</tr>
<tr>
<td>South Carolina*</td>
<td>1160</td>
<td>76.8 (73.5-80.1)</td>
</tr>
<tr>
<td>Utah</td>
<td>1122</td>
<td>78.7 (75.4-82.0)</td>
</tr>
<tr>
<td>Washington</td>
<td>1294</td>
<td>89.9 (87.9-91.9)</td>
</tr>
<tr>
<td>West Virginia</td>
<td>800</td>
<td>76.1 (72.6-79.6)</td>
</tr>
</tbody>
</table>

* State had primary enforcement belt law in effect January 1, 2002.
counseled. ACOG guidelines call for counseling about seat belt use to occur in the first trimester, whereas most PRAMS respondents complete the survey 2 to 4 months after delivery. Third, to the extent that health care delivery or individual demographic characteristics vary by state, results may not be generalizable to states that are not included in this analysis. Finally, because PRAMS data are self-reported by postpartum women, information on provider characteristics is limited. Thus, we were unable to fully assess differences in counseling by health care system factors (such as provider specialty, years in practice, and time spent with patients). Health care system factors have been shown to influence counseling on other topics (such as physical abuse, human immunodeficiency virus [HIV] testing, and smoking cessation).

Limitations of the BRFSS data included the fact that numbers were not sufficient to examine seat belt use among pregnant women at the state level. In addition, BRFSS does not collect data on seat belt use during pregnancy specifically. Although BRFSS captures pregnancy status at the time of the interview, the measurement of seat belt use is not limited to a specific time period; pregnant women may report seat belt use for a period that includes time before and during the pregnancy. Likewise, women who are not pregnant at the time of the interview may report use for a period that includes time before and during the pregnancy. Because comfort or safety concerns regarding seat belts for pregnant women and their fetuses, including the role of an airbag as a supplement to seat belts; the proper placement of the seat belt and belt adjustment throughout pregnancy; and an opportunity for women to ask questions about wearing seat belts. Because comfort or safety concerns regarding seat belt use may arise as the pregnancy advances, it is important to revisit the topic periodically throughout the pregnancy. Repeated messages throughout the course of the pregnancy may reinforce seat belt use.

Finally, BRFSS data are self-reported, and social desirability may result in the over-reporting of seat belt use. A comparison of BRFSS data and observational reports of belt use suggests that social desirability has a diminishing impact on self-reported data as observed levels of belt use increase, which has been the trend in recent years.

Effective strategies for increasing seat belt use among the general population could also influence pregnant women. These strategies include enacting primary enforcement laws and conducting high-visibility enforcement activities (such as seat belt checkpoints). Our findings showed that seat belt use for women of reproductive age was higher in states with primary enforcement laws than in those states without such laws.

In summary, the consistent, proper use of seat belts during pregnancy can reduce motor vehicle-related morbidity and mortality rates among pregnant women and their unborn children. Prenatal care visits provide an important avenue for conveying this life-saving message.

We thank the PRAMS states, represented by the PRAMS Working Group, and the project coordinators in the BRFSS states and territories for their roles in collecting the data.

### References


Use of the placental perfusion model to evaluate transplacental passage of *Trypanosoma cruzi*

Stuart H. Shippey, III, MD, Christopher M. Zahn, MD, Margaret M. Cisar, BS, T. John Wu, PhD, Andrew J. Satin, MD

Department of Obstetrics and Gynecology, Uniformed Services University of the Health Sciences, Bethesda, Md

Received for publication May 6, 2004; revised July 28, 2004; accepted July 30, 2004

Objective: To determine whether *Trypanosoma cruzi* could be identified in placental cells in vitro and in placental tissue using a human ex vivo perfusion model.

Study design: A placental cell line was incubated with trypomastigotes for progressive time periods. Trypomastigotes were also infused as a bolus into the maternal circulation of a placental perfusion model. Maternal and fetal perfusates, and placental tissue, were analyzed for parasitic DNA using polymerase chain reaction; perfused specimens were also examined histologically.

Results: Intracytoplasmic amastigotes were identified in trophoblast of the incubated cell line by 24–48 hours. Following placental perfusion, *T. cruzi* DNA was identified in all postinoculation maternal perfusate samples and postinoculation placental tissue specimens; preinoculation controls were negative.

Conclusion: This is the first description of the use of the human placental perfusion model to study congenital Chagas’ disease, including the presence and time course of early parasitic invasion of the placenta.

Chagas’ disease, caused by the protozoan parasite *Trypanosoma cruzi*, is a significant health problem in Central and South America, and the prevalence is increasing in the southern and southwestern United States. The seroprevalence in pregnant women may be as high as 81%, and congenital infection ranges from less than 1% to as high as 21%. Conditions associated with congenital infection include prematurity, low birth weight, abortion, and stillbirth; it is estimated that approximately 50% of preterm infants born to mothers with Chagas’ disease will die. Surviving infants may suffer hepatosplenomegaly, meningoencephalitis, myocarditis, or severe gastrointestinal manifestations.

The pathophysiology of congenital infection is not well known. It has been suggested that the parasite reaches the fetus via a hematogenous route and is transmitted across the placenta to result in congenital infection; however, data documenting placental involvement and the actual mode of transmission relative to congenital infection are limited.

The placental perfusion model allows for the study of the physiology of the human maternal-fetal circulation unit and transmission of various agents across the placenta. This model has been used to study blood flow and vasoconstriction in the placenta and the effects of experimentally induced acidosis and hypoxia on
placental blood flow. Additionally, the kinetics of transplacental transmission of medications have also been studied using this model.

The purpose of this study was to first determine whether *T. cruzi* parasites are able to invade trophoblastic cells as a necessary mechanism to gain access to a placental villus. After establishing the ability to invade trophoblast, our purpose was to use the placental perfusion model to: (1) determine the minimum time necessary following perfusion for *T. cruzi* to be identified in placental tissue, (2) examine histologic placental tissue sections for parasitic involvement of the placenta, including spatial orientation of the parasite relative to the normal placental structures, and (3) evaluate for the presence of parasitic DNA in placental maternal and fetal circulation and in tissue sections using polymerase chain reaction (PCR). We speculated that these findings might explain the early events of parasitic transmission in the development of congenital Chagas’ disease.

**Material and methods**

This protocol was approved by the Institutional Review Boards at both the Uniformed Services University of the Health Sciences and the National Naval Medical Center (Bethesda, Md). Trypanosomes, grown in a bovine skeletal muscle cell culture, were obtained from Dr. James A. Dvorak (Laboratory of Parasitic Diseases of the National Institute of Allergy and Infectious Disease). The bovine cell culture contained both motile infective forms (trypomastigotes) and intracellular dividing forms (amastigotes).

**Placental cell culture**

As an initial part of this study, a commercially available mixed placental cell line (CRL7502, American Type Culture Collection, Manassas, VA) was obtained and incubated with parasites in an attempt to demonstrate the ability of the organism to gain access and survive within human placental cells. In this experiment, placental cells were incubated in 4-well chamber slides with fresh Dulbecco’s minimal essential medium containing 200,000 trypanastigotes per milliliter and maintained for progressive time periods ranging from 1 to 48 hours. They were then fixed and examined microscopically for evidence of the parasite, using Giemsa staining. Experiments were carried out in duplicate to allow parallel immunohistochemical staining for human chorionic gonadotropin using commercially available kits. Placental tissue served as the positive control and 2 negative controls were performed by omitting primary and secondary antibody, respectively.

**Placental perfusion model**

For the placental perfusion model, human placentas were obtained from women in whom informed consent was obtained before delivery. The placentas were obtained from women with no antepartum or intrapartum complications; placentas were used only if there was no clinically relevant indication for pathologic examination of the placenta and if they were delivered intact. The placenta was placed in sterile saline immediately after delivery and transported to the placental perfusion lab within 30 minutes after delivery. The placental perfusion was performed according to previously described methods with minor modifications. Briefly, an intact cotyledon was selected, and the placenta clamped into a holder that isolated the selected cotyledon and prevented leakage either into or out of this separate area of the placenta. The maternal surface of the cotyledon was placed directly above a sterile funnel, which allowed collection of the maternal effluent in sterile tubes. Free flow of the fetal effluent was obtained from the fetal vein and collected in sterile tubes.

Preparasite perfusion was performed for 1 hour to flush residual blood; preparasite effluent specimens were also collected to serve as negative controls. A solution containing approximately 10 million trypomastigotes was given as a bolus injection via an injection port in the tubing, downstream from the pump, through which the maternal perfusate had been flowing. The number of organisms perfused was based on an approximation from experiments using BALB/C pregnant mice. The trypomastigote form was used because this form of the organism would most closely mimic true hematogenous transmission from maternal vasculature to the placenta. After the bolus infusion of parasites, the maternal and fetal circulation units were perfused with sterile solution for a total of 2 hours. Maternal and fetal effluents were collected in a sterile fashion at predetermined time intervals of 30, 60, 90, and 120 minutes after perfusion of the parasite and stored in a –20°C freezer for subsequent DNA extraction and PCR analysis.

Immediately after 120 minutes of perfusion, a small (approximately 3 × 3 mm) tissue specimen was obtained by dissecting the fetal membrane from the underlying chorion and cutting the specimen from the placenta; this specimen was stored in a –20°C freezer for subsequent DNA extraction and analysis. An additional tissue specimen (approximately 1 × 1 cm) was taken from this same area and fixed for subsequent histologic evaluation. After these specimens were obtained, the perfusion model was disassembled. The isolated placental cotyledon was then placed in sterile Dulbecco’s minimal essential medium/10% fetal bovine serum solution for a total of 24 hours, after which further tissue specimens (approximately 1 × 1 cm) were taken from the same general area as the specimens taken at 120 minutes and placed in fixative. After fixation, histologic slides were prepared and separately stained with both Giemsa and hematoxylin and eosin stains.
Importantly, all tissue specimens were a minimum of 1-2 cm from the site in which the butterfly catheters simulating the maternal circulation unit were placed to minimize the potential for contamination due to a geographic proximity to the insertion site.

**DNA extraction, PCR, and analysis**

DNA extraction was performed on the effluent and tissue specimens using commercially available DNA extraction kits according to the manufacturer’s instructions (QIAamp DNA minikit, lot 11551347, Qiagen, Valencia, CA). DNA extraction was performed on both control (preparasite perfusion) and postparasite perfusion specimens. DNA extracted from centrifuged specimens of pure *T cruzi* organisms served as positive controls.

PCR was performed on the effluent and tissue DNA extraction specimens following methods described by Kirchhoff et al. 18 PCR was performed using 2 sets of primers specific for *T cruzi*. The TCZ1 and TCZ2 primer pair amplifies a 188 base-pair segment of the 195 base-pair repetitive nuclear sequence. 19 The S35 and S36 primer pair amplifies a 332 base-pair minicircle sequence. 20 Previous reports 18 demonstrated that the TCZ1/2 primer pair is more sensitive than the S35/36 primer pair in detecting *T cruzi* DNA. Electrophoresis was performed following PCR; known bands for *T cruzi*
are: TCZ1/2, 188 kb; S35/36, 332 kb. Analysis of test specimens and controls was performed according to these known bands.

The following controls were also applied to this experiment. To minimize the possibility of contamination, DNA from the samples was extracted in one building, and the PCR reactions were performed in another laboratory. Disposable labware was used whenever possible. Additional samples were also collected, processed, and analyzed in parallel with test samples as controls for possible contamination, including preinoculation samples as well as media from a T cruzi infected culture dish. Additional negative and positive controls were also used to evaluate for contamination during PCR reaction set-up.

Results

For the placental cell culture portion of the study, incubation was maintained for 1, 2, 3, 4, 24, and 48 hours prior to fixation and staining. Because amastigotes are the intracellular form of the organism, only amastigotes within cells were considered definite evidence of cellular invasion. A total of 8 separate replicates of the placental cell line was incubated with parasites, and all demonstrated similar findings. There was suggestive evidence of intracellular parasites as early as 3 and 4 hours after incubation; however, definite evidence of amastigotes was not identified until 24 hours of incubation, with florid intracellular presence manifested at 48 hours (Figure 1). Although the placental cell line contains varied cell types, syncytiotrophoblast involvement was identified, as demonstrated in Figure 1.

Immunostaining with human chorionic gonadotropin revealed strong staining of the cells identified as syncytiotrophoblast, thus confirming parasitic involvement of trophoblastic cells.

For the perfusion experiments, effluent and tissue specimens were available from 3 perfused placentas, all demonstrating the same results. Shown in Figure 2 is a histologic section of a placental villus from the cotyledon that was incubated for 24 hours after the perfusion. The villous surface, composed of trophoblast, had a fragmented, markedly irregular appearance, compared with the typically very smooth villous surface from a normal placenta. The appearance of that which constitutes the irregular villous surface is very similar to the amastigote form of T cruzi, which seem to be “attached” to the trophoblast. Hemolytic debris, in contrast, is usually found in the intervillous space. The placental tissue specimen taken at 120 minutes did not reveal this irregular fragmented villous surface; the villi had a smooth surface similar to normal placenta.

For the molecular analysis portion of the study, T cruzi DNA was identified in the maternal effluent specimens taken at all time periods (30, 60, 90, and 120 minutes) but was not identified in the preinoculation (negative control) specimens (Figure 3). T cruzi DNA was not identified in the fetal effluent specimens at any time point or in the preinoculation samples. In the tissue specimens, T cruzi DNA was present in the 120-minute postinoculation sample and was not present in the preinoculation negative control (Figure 4).

Comment

Congenital Chagas’ disease is a significant health problem in areas in which adult Chagas’ disease is
endemic. Congenital infection may occur during any phase of maternal illness, and treatment is limited. The 2 medications currently in use are nifurtimox and benznidazole; these formulations kill only the circulating form of *T. cruzi* and are used to primarily treat the acute illness, which may often be missed because of its nonspecific presentation. Treatment during the chronic phase is often unsuccessful. Additionally, because of toxic side effects and unknown effects of the fetus, the use of these medications in pregnant women is limited and typically used only for treatment during known acute maternal disease. There are no medications that when administered to seropositive pregnant women have been shown to prevent congenital transmission.

Parasitic infection of trophoblast has been theorized as necessary in the pathophysiology of congenital infection; trophoblast involvement would likely be a requirement because the trophoblastic cells serve as the principal barrier in the maternal-fetal transfer of any organism or agent. However, data regarding placental involvement are limited. The largest series of placentas consists of an analysis of 24 cases. The most consistent finding in these placentas was an inflammatory response in the villi and intervillous spaces, which ranged from extensive to minimal. Parasites were identified in 14 of these cases, which were mainly found in the Hofbauer cells in the chorion. However, amastigotes were identified in the trophoblast in only one case; the authors theorized that this was due to recent infection because parasites had not been identified in the trophoblast in any other placentas.

Placental involvement by *T. cruzi* was studied in one other series involving a total of 8 placentas. In this study, the placentas from the 4 stillborn fetuses demonstrated severe inflammation, and numerous parasites were identified. In contrast, the inflammatory response was only slight to moderate in the 4 liveborn infants, and few parasite nests were present.

In our study, we have demonstrated the capability of parasites to invade and grow in trophoblastic cells, identifying the potential time course as well, in that only 24 hours were required to identify a florid intracellular presence of amastigotes in trophoblastic cells. This was further confirmed by the placental perfusion model, demonstrating the presence of parasites in the placenta by PCR in as little as 2 hours, and the highly suggestive histologic appearance after further incubation for 24 hours. The presence of parasitic DNA in the maternal effluent is not surprising because this fluid is basically the fluid “bathing” the villi in the perfusion model, in which the parasite was given as a bolus. However, the maternal effluent was still positive for *T. cruzi* DNA even 2 hours later, implying that some of the parasites did not immediately pass through the model but were instead taken up in the placenta, as confirmed by the PCR analysis of the tissue at 120 minutes. The lack of *T. cruzi* DNA by PCR analysis of the fetal effluents is also not surprising; phagocytosis of the parasite by Hofbauer cells and subsequent release into the fetal circulation, as well as an inflammatory response, would require an in vivo system. Although the perfusion model can be used to evaluate transplacental transmission of chemicals or pharmaceuticals in the effluent specimens, it is unlikely that an ex vivo system could mimic the necessary in vivo interactions to reproduce true congenital infection. The inability to reproduce a placental inflammatory response, which as described was a common finding in prior studies, is a limitation of the perfusion model in investigating congenital infection.

One concern may be that the presence of *T. cruzi* DNA in the placental tissue specimen might be due to contamination from the perfusion medium in which the parasites were provided. However, as described, the histologic analysis of the placenta after further incubation was very suggestive of organisms on the villous surface. Additionally, perfusion of sterile medium continued for 2 hours after the parasite bolus. Moreover, the tissue specimen was washed multiple times before homogenization during the DNA extraction process. Therefore, potential contamination from the medium should not be a factor.

Our findings are the first to demonstrate plausible early findings in the development of congenital Chagas’ disease and corroborate the previously theorized early mechanisms of congenital infection. These results have potential clinical application, including the study of the molecular mechanisms involved in early trophoblastic invasion and the investigation of agents that could potentially block or reduce transmission. Because current therapeutic options are limited, the ability to study new treatment approaches using this model could potentially decrease the significant impact of congenital infection.

### Acknowledgments

We thank James A. Dvorak, PhD (Head, Biophysical and Biochemical Parasitology, Laboratory of Parasitic Diseases, National Institute of Allergy and Infectious Disease), for graciously supplying *T. cruzi* organisms for the study.

### References


Stephen J. Bacak, MPH,* William M. Callaghan, MD, MPH, Patricia M. Dietz, DrPH, Chadd Crouse, MSc

Division of Reproductive Health, National Center for Chronic Disease Prevention and Health Promotion, Centers for Disease Control and Prevention, Atlanta, Ga

Received for publication July 7, 2004; revised October 4, 2004; accepted October 29, 2004

Objective: The purpose of this study was to examine nondelivery, pregnancy-associated hospitalizations in the United States and the factors associated with them.

Study design: Population-based nondelivery hospitalizations during pregnancy were obtained from the 1999 and 2000 National Hospital Discharge Survey. Ratios of hospitalizations per 100 deliveries were calculated and analyzed by age, race, and payment source.

Results: The pregnancy-associated hospitalization ratio for 1999 through 2000 was 12.8 per 100 deliveries (95% CI, 11.8-13.8). Hospitalizations were highest among young women, African American women, and women without private insurance. Preterm labor, nausea and/or vomiting, and genitourinary complications accounted for one half of antenatal hospitalizations.

Conclusion: Pregnancy-associated hospitalizations declined during the 1990s. This may represent a decline in maternal morbidity or a change in management of pregnancy complications. Future research should be expanded to assess trends in morbidity treated in settings outside of hospitals.

Maternal morbidity is a group of physical or psychologic conditions, resulting from or aggravated by pregnancy that adversely affects a woman’s health. Maternal morbidity includes complications and conditions associated with any pregnancy outcome. Conditions resulting in hospitalization prior to admission for labor and delivery represent 1 indicator of maternal morbidity. According to data from managed care and military populations, between 8% and 27% of women are hospitalized at least once during pregnancy.1,2 The most common reasons for nondelivery hospitalizations include preterm labor, vomiting, genitourinary complications, and hypertensive disorders.3,4

National estimates of pregnancy-associated hospitalizations, defined as all nondelivery hospitalizations that occur during pregnancy, have been calculated by using the National Hospital Discharge Survey (NHDS); the most recent estimate was 18 hospitalizations for every 100 US births during 1991 through 1992.3,4 Considerable change in the management of pregnancy complications over the past decade has led to a shift toward outpatient care for many conditions, including preterm labor, mild preeclampsia, and ectopic pregnancy.5-7 However, hospitalization data remain the primary national source for monitoring pregnancy complications. The purpose of our study was to (1) examine
the change in pregnancy-associated hospitalizations during the 1990s and (2) report the relative difference in hospitalization ratios for 1999 through 2000 by age, race, and payment source.

**Material and methods**

**Data source**

We used an aggregated database from the 1999 and 2000 NHDS. Two years of data were needed to provide a sufficient sample size for stratified analysis. The methods and design of the NHDS are described in detail elsewhere and are discussed only briefly here. The NHDS, which is conducted annually by the National Center for Health Statistics, collects data on inpatient utilization from short-stay, noninstitutional, and nonfederal hospitals in the United States and the District of Columbia. The NHDS uses a 3-stage sampling design, and the data are weighted to represent national inpatient hospitalizations. Approximately 300,000 hospitalizations were sampled from 458 hospitals in 1999 and from 434 hospitals in 2000. The NHDS includes a primary diagnosis field and 6 additional diagnosis fields based on International Classification of Diseases, ninth Revision (ICD-9) codes. The NHDS does not contain any unique patient identifying information such as medical record numbers or social security numbers. Therefore, the unit of analysis is hospitalizations and not individuals. This study was reviewed for human subjects concerns and found to be in compliance.

**Definitions and coding decisions**

We defined pregnancy-associated hospitalizations as all hospitalizations in which the woman was pregnant and did not deliver during the hospitalization. Pregnancy-associated hospitalizations were further divided into early pregnancy loss and antenatal hospitalizations. Early pregnancy loss hospitalizations were defined as nondelivery hospitalizations with selected ICD-9 codes from 630-634 and listed in any of the seven diagnosis fields. These included hospitalizations for molar and ectopic pregnancies, spontaneous abortions, and complications related to these diagnoses. Hospitalizations for elective terminations (ICD-9 codes 635 and 636) were not included in the definition and were excluded from all analyses. Antenatal hospitalizations were defined as nondelivery, nonpregnancy loss hospitalizations with selected ICD-9 codes from 640-676. This code range includes diagnoses for complications of pregnancy, childbirth, and the puerperium, as well as codes for labor and delivery. We required diagnosis codes from 640-676 to have a fifth digit of “3,” which indicates that a delivery did not occur. We also included in the definition of antenatal hospitalizations those hospitalizations with any diagnosis listed in combination with a normal pregnancy (V22) or a high-risk pregnancy (V23) code.

Within the early pregnancy loss and antenatal hospitalization groups, we examined hospitalizations by age, race, and payment source. We grouped women into 4 age groups: less than 20, 20 to 24, 25 to 34, and 35 to 54 years. Race was classified as white, African American, other (American Indian/Alaskan Native, Asian, Native Hawaiian or other Pacific Islander, other, and multiple race), and not stated. Information on ethnicity (eg, Hispanic) is not reported by the NHDS and could not be included in our analysis. Payment source was coded as private insurance (Blue Cross/Blue Shield, HMO/PPO, and other private insurance), Medicaid, self-pay, other (worker’s compensation, Medicare, other government, no charge, and other), and not stated.

For antenatal hospitalizations, we examined the leading diagnoses and the length of stay. The primary diagnosis field was used to classify the leading cause of the hospitalization. For example, if the code 644.03 or 644.13 was listed in the primary diagnosis field, we categorized the hospitalization as preterm labor. However, if the antenatal hospitalization had a 648.93 code (other antepartum complication) listed in the primary diagnosis field, we used the code listed in the second diagnosis field to classify the leading diagnosis. For instance, a hospitalization with a 648.93 code listed in the primary diagnosis field and a 644.03 or 644.13 code listed in the second diagnosis field was also classified as preterm labor. We grouped related diagnosis codes into single categories for analytic purposes. For example, mental disorders and genitourinary complications comprise various individual diagnosis codes. Any diagnosis or group of similar diagnoses that accounted for less than 1% of all antenatal hospitalizations was categorized as “other diagnoses.” Documentation of all antenatal and early pregnancy loss classifications is available from the study authors.

**Analysis**

We defined hospitalization ratios as the number of hospitalizations per 100 deliveries. Delivery hospitalizations were identified with a V27 code listed in the primary diagnosis field. Risk ratios (ratios of hospitalization ratios) and 95% CIs were calculated to examine groups at highest risk of hospitalization by age, race, and payment source. For antenatal hospitalizations, we examined the proportion and mean length of stay for the leading diagnoses. Although hospitalization ratios for 1991 through 1992 have been reported, confidence intervals were not. As such, statistical comparisons were not possible between our analysis and the previously published analysis. Therefore, we applied our programming to the 1991-1992 NHDS data to generate
pregnancy-associated hospitalization ratios and associated 95% CIs. All analyses were performed by using SUDAAN software, version 8.0, (Research Triangle Institute, Research Triangle Park, NC) to adjust for the complex sampling design of the NHDS.

Results

The demographic characteristics of the women with pregnancy-associated and delivery hospitalizations are shown in Table I. Overall, the pregnancy-associated hospitalization ratio declined from 17.6 per 100 deliveries in 1991 through 1992 (95% CI 16.4-18.8) to 12.8 hospitalizations per 100 deliveries (95% CI 11.8-13.8) in 1999 through 2000 (Table II). Antenatal hospitalizations declined from 13.3 (95% CI 12.2-14.4) to 10.5 (95% CI 9.5-11.4) and early pregnancy loss hospitalizations declined from 4.3 (95% CI 3.9-4.6) to 2.3 per 100 deliveries (95% CI 2.1-2.6).

Groups with higher antenatal hospitalization ratios included African American women and women without private health insurance (Table III). Women 25 years and older had significantly lower antenatal hospitalizations than women 20 to 24 years. African American women had more than 1.5 times the hospitalization ratio of white women. Self-paying women were nearly twice as likely to be hospitalized and women with Medicaid and other insurance were 50% more likely to be hospitalized for antenatal complications than women with private insurance.

Hospitalization ratios for early pregnancy loss were higher among older women, African American women, and self-paying women (Table III). Women aged 35 years and older were 1.5 times (95% CI 1.11-2.09) more likely to be hospitalized for early pregnancy loss than women aged 20 to 24 years. African American women were more than 2.5 times as likely to be hospitalized for early pregnancy loss as were white women. Self-paying women were 5 times more likely to be hospitalized for early pregnancy loss than privately insured women.

Preterm labor, nausea and/or vomiting, genitourinary complications, hypertensive disorders, and hemorrhage accounted for more than 60% of the primary diagnoses for antenatal hospitalizations (Table IV). Preterm labor was the most common diagnosis (29%), and nausea and/or vomiting was the second most common (11%). Hospitalizations for “other diagnoses” accounted for more than 25% of all antenatal hospitalizations. Spontaneous abortions and ectopic pregnancy accounted for 58% and 41% of early pregnancy loss hospitalizations, respectively.

The overall mean length of hospital stay for antenatal hospitalizations was 2.7 days (95% CI 2.6-2.8) (Table

<table>
<thead>
<tr>
<th>Maternal characteristic</th>
<th>Pregnancy-associated hospitalizations (n = 8792)</th>
<th>Deliveries (n = 68,514)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No.*</td>
<td>Weighted %</td>
</tr>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;20</td>
<td>1,179</td>
<td>15.3</td>
</tr>
<tr>
<td>20-24</td>
<td>2,309</td>
<td>28.8</td>
</tr>
<tr>
<td>25-34</td>
<td>4,062</td>
<td>43.7</td>
</tr>
<tr>
<td>35-54</td>
<td>1,242</td>
<td>12.2</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>4,021</td>
<td>53.3</td>
</tr>
<tr>
<td>African American</td>
<td>1,871</td>
<td>19.4</td>
</tr>
<tr>
<td>Other</td>
<td>768</td>
<td>6.9</td>
</tr>
<tr>
<td>Not stated</td>
<td>2,132</td>
<td>20.4</td>
</tr>
<tr>
<td>Payment source</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Private</td>
<td>4,577</td>
<td>46.9</td>
</tr>
<tr>
<td>Medicaid</td>
<td>2,885</td>
<td>36.1</td>
</tr>
<tr>
<td>Self-pay</td>
<td>748</td>
<td>10.0</td>
</tr>
<tr>
<td>Other</td>
<td>516</td>
<td>5.9</td>
</tr>
<tr>
<td>Not stated</td>
<td>66</td>
<td>1.1</td>
</tr>
</tbody>
</table>

* Unweighted observations.
† Weighted to represent national inpatient hospitalizations.

Table II

<table>
<thead>
<tr>
<th>Hospitalization type</th>
<th>1991-1992 Ratio* (95% CI)</th>
<th>1999-2000 Ratio* (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pregnancy-associated</td>
<td>17.6 (16.4-18.8)</td>
<td>12.8 (11.8-13.8)</td>
</tr>
<tr>
<td>Antenatal</td>
<td>13.3 (12.2-14.4)</td>
<td>10.5 (9.5-11.4)</td>
</tr>
<tr>
<td>Early pregnancy loss</td>
<td>4.3 (3.9-4.6)</td>
<td>2.3 (2.1-2.6)</td>
</tr>
</tbody>
</table>

* Hospitalizations per 100 deliveries.
IV). Women hospitalized for mental disorders and diabetes had the longest mean hospital stays (4.8 and 3.5 days, respectively), whereas women hospitalized for hypertensive disorders had the shortest (2.2 days on average).

**Comment**

Pregnancy-associated hospitalizations declined during the 1990s from 17.6 per 100 deliveries in 1991 through 1992 to 12.8 per 100 deliveries in 1999 through 2000. Younger women, African American women, and women without private health insurance were more likely to be hospitalized for antenatal complications. Hospitalization ratios for early pregnancy loss were higher among older, African American, and self-paying women.

The reason for higher hospitalization ratios among certain groups of women is unknown, but may reflect a greater prevalence of a condition, a lack of access to quality care, or a more severe presentation of...
a condition. For instance, African American women experience higher rates of preterm delivery and, hence, may be more likely to be hospitalized for preterm labor, whereas older women are more likely to experience spontaneous abortion.\textsuperscript{11,12} Younger women and African American women are less likely to receive prenatal care early during pregnancy.\textsuperscript{13,14} The overall low hospitalization ratios in women with private insurance may reflect their greater access to care. The severity of a condition can be affected by access to timely quality care and individual health status, resulting in an increased risk of hospitalization. Early prenatal care would allow for the detection, management, and successful outpatient treatment of some morbidities, such as mild preeclampsia and ectopic pregnancy. This may explain why women without any health insurance had the highest pregnancy-associated hospitalization ratios.

Consistent with Bennett et al,\textsuperscript{4} antenatal hospitalizations were lower among women older than 35 years than among younger women. To examine this association more closely, we performed stratified analyses by race and payment source separately (data not shown). We found that for white women and for women with private health insurance, older women still had lower antenatal hospitalization ratios than younger women. However, for African American women and for women with Medicaid, hospitalization ratios did not vary by age. This suggests that the association between age and antenatal hospitalizations is complex and varies by race groups and by payment source groups. Parity is another important variable to consider in this association, but we did not have this information. Additional studies are needed to further understand why older women had lower antenatal hospitalization ratios than younger women.

The decline in pregnancy-associated hospitalizations parallels the trends in overall hospitalizations, which are decreasing in the United States.\textsuperscript{5,10} Although women may be healthier, there is no evidence to suggest that the incidence and prevalence of pregnancy complications are declining. Conditions that were formerly managed in inpatient settings, such as threatened preterm labor, hypertensive disorders, and ectopic pregnancy, are now being managed through outpatient treatment.\textsuperscript{5-7,15} Although the impact on improving health outcome is unknown, these changing practice patterns may explain the decline in pregnancy-associated hospitalizations.

Despite overall declines in pregnancy-associated hospitalizations, the leading reasons for hospitalization changed little over the decade. Consistent with several other studies, our data showed that more women are hospitalized for preterm labor than for any other reason.\textsuperscript{1,4,16} Although preterm labor, nausea and/or vomiting, genitourinary complications, hypertensive disorders, and hemorrhage accounted for 60% of the diagnoses for antenatal hospitalizations, more than one quarter of diagnoses consisted of a wide range of morbidities. Eliminating all hospitalizations for pregnancy complications is not feasible. However, if hospitalizations for this wide range of conditions are to be reduced, prevention research that addresses the impact of access, quality of prenatal care, and condition-specific etiologies is needed.

Several limitations of the NHDS should be considered when interpreting our results. First, we cannot calculate pregnancy complication rates because the unit of analysis was hospitalizations and not women. We expect that hospitalization ratios are higher than rates based on women because some women are hospitalized more than once during pregnancy. Prior studies reported that 15% to 22% of hospitalized women are hospitalized more than once during their pregnancy.\textsuperscript{1,2} Another limitation is our understanding of prolonged delivery hospitalizations involving pregnancy complications. Many severe pregnancy complications occur during labor and delivery. However, because we could not identify whether a complication occurred before or after a delivery, we had to exclude delivery hospitalizations from our numerator. This resulted in an underestimation of hospitalizations caused by complications during pregnancy. In a recent national managed care study, approximately 10% of women who were hospitalized during pregnancy had an extended stay (≥4 days) before a live birth or a pregnancy loss.\textsuperscript{1}

The number of hospitalizations for a particular condition may also be underestimated because of our decision to use only the primary diagnosis field to identify the leading cause of hospitalization. We chose this methodology to be consistent with the 2 prior hospitalization studies.\textsuperscript{3,4} Furthermore, making a decision on a hierarchy of the 7 diagnosis codes would be arbitrary given the limited information collected by the NHDS. A final limitation is the increasing amount of missing race information in the NHDS. In our aggregated database, 28% of the observations had no information about race, primarily because of an increase in the number of hospitals not reporting race for any patients.\textsuperscript{8,17} A previous study found that race was more likely to be missing for white women and the associations between white and African American hospitalizations do not change when allocating all hospitalizations with missing race to white race.\textsuperscript{17} This suggests that despite the large amount of missing race information in our database, there are genuine differences in hospitalizations between African American and white women.

Hospitalizations do not include the entire spectrum of antenatal morbidity. To capture the full range of complications, conditions treated in settings outside of hospitals would need to be considered. Despite its limitations, the NHDS remains the primary source of national, population-based estimates of hospitalizations.
for pregnancy complications. Although we do not know the optimal proportion of women who should be hospitalized during pregnancy, ie, the proportion that reflects unpreventable hospitalizations and that maximizes health, the persisting disparities in race and payment source suggest that hospitalizations among these groups could be reduced.

References

Resolution of hypertension during pregnancy in familial hyperkalemia and hypertension with the WNK4 Q565E mutation

Haim Mayan, MD,a Meir Mouallem, MD,a Miriam Shaharabany, PhD,b Rachel Pauzner, MD,a Zvi Farfel, MDa,b,*

Department of Medicine E,a and Laboratory of Biochemical Pharmacology,b Sheba Medical Center, Sackler School of Medicine, Tel Aviv University, Tel Hashomer, Israel

Received for publication April 27, 2004; revised June 2, 2004

Objective: Secondary hypertension during pregnancy usually carries high maternal and fetal morbidity and mortality rates. A rare form of monogenic hypertension is familial hyperkalemia and hypertension, which is caused by mutations in the kinases WNK1 or WNK4 and other unknown molecular defects. The purpose of the study was to examine the course of pregnancy in hypertensive women with familial hyperkalemia and hypertension.

Study design: We prospectively studied 2 pregnancies of a woman with familial hyperkalemia and hypertension and the Q565E WNK4 mutation (pregnancies 1 and 2) and retrospectively studied the course of 2 pregnancies in another woman who was an affected member of this largest family described in the literature.

Results: Both women had hypertension (170-190/105-110 mm Hg), hyperkalemia (5.3-6.0 mmol/L), and hypercalciuria, all of which were well controlled by thiazides. During pregnancies, thiazides were discontinued; throughout the pregnancy, the blood pressure remained normal at 120 to 130/75 to 85 mm Hg; however, hyperkalemia and hypercalciuria, which were documented in pregnancies 1 and 2, persisted. Renin and aldosterone levels (which were measured in pregnancies 1 and 2) rose towards their end. Four normal infants were born. A woman with familial hyperkalemia and hypertension of unknown molecular defect who had 2 pregnancies with hypertension exacerbation and premature deliveries was described previously.

Conclusion: In familial hyperkalemia and hypertension with the WNK4 mutation, pregnancy ameliorates hypertension; however, hyperkalemia and hypercalciuria persist. This dissociation may shed light on the pathogenesis of familial hyperkalemia and hypertension, on pregnancy-related hypertension, and on the mechanism of action of WNK4 kinase, a major regulator of cellular ion transport.

© 2005 Elsevier Inc. All rights reserved.
among pregnant women, which is a major risk factor for the development of preeclampsia,\textsuperscript{2,3} is rising because of the current trend of childbearing at an older age. Most pregnant women with chronic hypertension have essential hypertension; however, approximately 10% of pregnant women with chronic hypertension have secondary hypertension.\textsuperscript{2} Usually, the prognosis in these women appears to be poorer.\textsuperscript{4} A study of secondary hypertension during pregnancy may be important to better understand the pregnancy course in affected women and to gain insight into the mechanisms of pregnancy hypertension in general. One form of secondary hypertension is the monogenic disorder familial hyperkalemia and hypertension (FHH), also known as pseudohypoaldosteronism type II (PHA II), which is a rare autosomal dominant disease that is characterized by hyperkalemia, hypertension, and metabolic acidosis.\textsuperscript{5} These abnormalities are corrected by thiazide diuretics, which are specific inhibitors of the Na–Cl cotransporter in the distal nephron. The molecular defect of FHH is heterogeneous. In some families, mutations were found in the WNK1 or WNK4 kinases;\textsuperscript{6} in other families, the molecular defect awaits identification.\textsuperscript{7} Pregnancy in FHH with or without hypertension was described in 4 cases, with various course and outcome.\textsuperscript{5} Here we describe 4 pregnancies in 2 affected hypertensive women from a family with FHH and the Q565E WNK4 mutation, which has been followed by us for almost 3 decades.\textsuperscript{8–12} The course of the pregnancies was characterized by a dramatic resolution of hypertension, which enabled the discontinuation of thiazide therapy. Of interest is the observation that hyperkalemia and hypercalciuria, which occurs in affected subjects of the family,\textsuperscript{11,12} did not resolve during pregnancy. These findings may shed light on the pathogenetic mechanisms of the clinical manifestations of FHH, on pregnancy-related hypertension, and on the mechanism of action of WNK4 kinase, which is a major regulator of cellular ion transport.

Material and methods

The course of 4 pregnancies of 2 related women with FHH and the Q565E WNK4 mutation was studied. In the first woman, FHH was diagnosed at childhood, and her 2 pregnancies were followed prospectively. In the second woman, FHH was recently diagnosed, and the course of her 2 pregnancies that are described here was recorded retrospectively.

Case 1

This 30-year-old woman was found to have hyperkalemia at the age of 4 years and to have hypertension at the age of 18 years (subject V14; Figure 1, A\textsuperscript{12}). Thiazides were administered with easy control of hypertension, hyperkalemia and hypercalciuria (Table; Figure 1). At the age of 27 years, thiazides were discontinued for 2 weeks, and her blood pressure rose to 190/110 mm Hg. One year later, on finding out that she was pregnant, she discontinued her regular hydrochlorothiazide 12.5 mg/d. The course of the pregnancy is shown in the Table and Figure 2. Blood pressure remained normal at 115/75 to 130/85 mm Hg throughout the pregnancy. Serum K rose and ranged between 5.2 and 6.4 mmol/L. Hyperchloremia (108–111 mmol/L) which reflected metabolic acidosis was recorded. Plasma renin values were 0.7 ng/mL/hr on week 21 of gestation and rose to 1.2 ng/mL/hr on week 39 of gestation, which is much higher than the mean levels of 0.18 ± 0.09 ng/mL/hr measured in 8 affected subjects of the kindred\textsuperscript{12} and somewhat lower than values that were measured during pregnancy in hypertensive or normotensive women.\textsuperscript{13,14} Serum aldosterone levels were 36.2 ng/dL on week 21 of gestation and rose to 105.7 ng/dL on week 39 of pregnancy, which is much higher than the mean levels of 15.2 ± 13.8 ng/dL that were measured in 8 affected family members\textsuperscript{12} and within the range of the levels that were measured during pregnancy in hypertensive or normotensive women.\textsuperscript{14} Hypercalciuria persisted throughout pregnancy. At the end of week 40 of pregnancy, her blood pressure rose to 160/100 mm Hg; hydrochlorothiazide was administered, and labor was induced by oxytocin administration. Her blood pressure rose to 160/100 mm Hg, and a normal female infant who weighed 3000 g was delivered uneventfully. Eighteen months later, at the age of 29 years she became pregnant again. The course of this pregnancy was very similar to that of the first pregnancy (Table; Figure 2). She discontinued thiazide therapy on finding out that she is pregnant. Throughout the pregnancy, her blood pressure was 105/75 to 120/80 mm Hg, and laboratory values that included serum K, serum Cl, plasma renin, serum aldosterone, and urinary calcium were very similar to the values that were measured during the figure 1. Blood pressure, serum K, and urinary calcium in case 1. The closed bars denote control prepregnancy values; the hatched bars denote values that were measured during thiazide therapy; the dotted bars denote values that were measured during pregnancy.
first pregnancy. At week 38 of gestation, at a regular follow-up examination, intrauterine growth retardation with an estimated fetal weight of 2160 to 2400 g was suspected. Labor was induced by oxytocin administration, and a normal female infant who weighed 2720 g was delivered. At 2 months after delivery, a blood pressure of 170/110 mm Hg was measured, and hydrochlorothiazide was resumed.

**Case 2**

In this 52-year-old mother of 10 children (subject IV2; Figure 1, A), hypertension was found at the age of 32 years after 8 deliveries, and antihypertensive therapy that included thiazides was started. During both subsequent pregnancies, 4 and 8 years later, she did not take antihypertensive therapy, and her blood pressure remained normal. In both of these pregnancies, her blood pressure rose at the end of week 40 of gestation, which was followed by normal deliveries of normal male infants. FHH was recently diagnosed, and the following values (all of which were typical findings for FHH) were recorded: blood pressure, 170/110 mm Hg; serum K, 5.3 mmol/L; serum Cl, 108 mmol/L; plasma renin, 0.1 ng/mL/hr; serum aldosterone, 24.9 ng/dL; and urinary calcium, 1.17 mmol/mmol creatinine (Table).

**Comment**

We describe here, apparently for the first time, the course of pregnancies in FHH with a defined molecular defect. Previously 2 pregnancies in a single hypertensive patient with FHH were described. In the mother, who was investigated at the age of 13 years, a low salt diet reversed both hypertension and hyperkalemia. This contrasts the findings of the investigation in our kindred in which a low salt diet corrected hypertension but not hyperkalemia. During both pregnancies, this patient was treated by diuretics; preeclampsia appeared, and premature labor occurred at weeks 32 (cesarean delivery) and 34 of gestation, with delivery of infants weighing 1100 and 1890 g, respectively. Of interest is that, during the first labor, plasma renin and serum aldosterone were recorded.

<table>
<thead>
<tr>
<th>Week of gestation</th>
<th>Hydrochlorothiazide therapy</th>
<th>Blood pressure (mm Hg)</th>
<th>Serum K (mmol/L)*</th>
<th>Serum Cl (mmol/L)</th>
<th>Plasma renin activity (ng/mL/hr)</th>
<th>Serum aldosterone (ng/dL)</th>
<th>Urinary calcium (mmol/mmol creatinine)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Case 1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prepregnancy</td>
<td>-</td>
<td>190/110</td>
<td>5.6</td>
<td>111</td>
<td></td>
<td></td>
<td>1.21</td>
</tr>
<tr>
<td>First pregnancy</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>-</td>
<td>115/75</td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>9</td>
<td>-</td>
<td>115/80</td>
<td>5.2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>-</td>
<td>125/85</td>
<td>6.0</td>
<td>109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>-</td>
<td>120/80</td>
<td>5.4</td>
<td>109</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>21</td>
<td>-</td>
<td>130/85</td>
<td>6.4</td>
<td>110</td>
<td>0.7</td>
<td>36.2</td>
<td></td>
</tr>
<tr>
<td>25</td>
<td>-</td>
<td>115/80</td>
<td>6.2</td>
<td>111</td>
<td></td>
<td>71.5</td>
<td></td>
</tr>
<tr>
<td>37</td>
<td>-</td>
<td>120/75</td>
<td>5.5</td>
<td>108</td>
<td>1.2</td>
<td>105.7</td>
<td></td>
</tr>
<tr>
<td>39</td>
<td>+</td>
<td>135/80</td>
<td>5.5</td>
<td>108</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>+</td>
<td>140/95</td>
<td>4.9</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery</td>
<td>+</td>
<td>160/100</td>
<td>4.6</td>
<td>102</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Case 2</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>12 Y after pregnancy</td>
<td>-</td>
<td>170/110</td>
<td>5.3</td>
<td>0.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FHH affected subjects</td>
<td>-</td>
<td>162 ± 30/99 ± 11</td>
<td>5.6 ± 0.2</td>
<td>109.0 ± 1.5</td>
<td>0.18 ± 0.09</td>
<td>15.2 ± 13.8</td>
<td>0.85 ± 0.27</td>
</tr>
<tr>
<td>(n = 16)</td>
<td></td>
<td>(n = 18)</td>
<td>(n = 18)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 8)</td>
<td>(n = 17)</td>
</tr>
</tbody>
</table>

* Normal range: Serum K, 3.5-5.0 mmol/L; serum Cl, 98-106 mmol/L; renin (supine, normal sodium intake), 0.3-3.0 ng/ml/h; aldosterone (supine, normal sodium intake), <8 ng/dL.


**Table** Course of the 2 pregnancies

---

Mayan et al
suppressed, in contrast to the rise in renin and aldosterone levels that were observed in our case 1. The more severe course of these pregnancies may stem from different molecular defects. We await description of the course of pregnancy in hypertensive women with FHH who harbor the same or different mutations in WNK4 and WNK1.

The most dramatic finding in the course of the pregnancies that we describe is the marked amelioration of hypertension, despite the persistence of hyperkalemia and hypercalciuria (Figure 1). Normal pregnancy is accompanied by increased urinary calcium excretion and maybe by some impairment in renal potassium excretion. Although in the pregnancies that we describe, serum K and urinary calcium did not rise during pregnancy, a specific dramatic effect of blood pressure reduction was observed. In the extended FHH kindred that we have studied recently, that contains 19 subjects with the Q565E WNK4 mutation and their 16 unaffected siblings and offspring, we have found that hyperkalemia and hypercalciuria occur apparently simultaneously and that both may precede the appearance of hypertension by many years (at least 13 years). The mechanism of this delayed appearance of hypertension is unknown. It was found recently that, when expressed in frog oocytes, WNK4 inhibits Na-Cl cotransporter function and surface expression and that the mutation Q565E relieves this inhibition, which results in increased sodium reabsorption. In addition, elegant studies in the laboratory of Kahle et al showed that WNK4 inhibits the renal potassium channel ROMK and that the mutant Q565E WNK4 further augments this inhibition, causing decreased potassium secretion, apparently the molecular pathogenesis of hyperkalemia in FHH. Moreover, very recently, it was shown that WNK4 regulates paracellular chloride permeability and epithelial apical and basolateral chloride flux. Based on the presence of hypercalciuria in FHH with WNK4 mutation and normocalciuria in FHH with the WNK1 mutation, we have proposed that WNK4 may interact with a renal calcium channel. In this respect, the effect of pregnancy on the manifestations of FHH is different than that of thiazides, which correct hypertension, hyperkalemia, and hypercalciuria, and is similar to that of young age, which ameliorates hypertension but not hyperkalemia and hypercalciuria (Figure 1). The mechanism that is responsible for this pregnancy-induced amelioration is not clear. Probably the different hormonal environment during pregnancy (such as the high concentration of progesterone, which is considered an aldosterone antagonist) has a role in this effect. This pregnancy-induced hypotensive effect may be a nonspecific vasodilatory effect; however, in other disorders of secondary hypertension during pregnancy such as Cushing’s syndrome, pheochromocytoma, and glucocorticoid-remediable aldosteronism, hypertension is not ameliorated during pregnancy. Alternatively, because WNK4 has a central role in the regulation of cellular ion transport, it is possible that humoral gestational compounds affect some, but not all, downstream WNK4-regulated transport systems. The interaction between WNK4 and the Na-Cl cotransporter may be such a target. A possible effect of sex hormones on the manifestations of FHH may be suggested also by the observation that, in our FHH kindred, there is a trend for hypertension to occur slightly later in women than in men; however, the small numbers preclude statistical significance (unpublished data).

The course of the described pregnancies may also shed light on the role of the renin-aldosterone system in FHH and pregnancy. In the study of the extended pedigree, markedly suppressed renin levels were observed in affected subjects. As occurs in normal gestation, renin
levels rose during the described pregnancies and this was accompanied by high aldosterone concentrations\textsuperscript{13,29}, however, this was not sufficient to reduce serum potassium concentration (Table; Figure 2). Of note is the observation that the concentrations of both renin and aldosterone continued to rise until the end of pregnancy, which is a similar pattern to that observed in normal pregnancy or in pregnancy of women with chronic hypertension not complicated by preeclampsia.\textsuperscript{13,29} In contrast, in pregnant women with chronic hypertension that is complicated by preeclampsia, a fall in renin and aldosterone concentrations during the last trimester of pregnancy was observed.\textsuperscript{13}

It is interesting that the amelioration of hypertension during pregnancy was described in a few cases of renal artery stenosis\textsuperscript{30} and aldosterone-producing adenoma.\textsuperscript{31-33}

\section*{Acknowledgments}
We thank Elizeer J Holtzman, Nephrology Institute, Sheba Medical Center, for helpful discussions.

\section*{References}

Persistent elevation of cell-free fetal DNA levels in maternal plasma after selective laser coagulation of chorionic plate anastomoses in severe midgestational twin-twin transfusion syndrome

Tuangsit Wataganara, MD,a Eduard Gratacos, MD,b Jacques Jani, MD,c Jorge Becker, MD,b Liesbeth Lewi, MD,c Lisa M. Sullivan, PhD,d Diana W. Bianchi, MD,a,* Jan A. Deprest, MD, PhDc

Division of Genetics, Department of Pediatrics, Obstetrics and Gynecology, Tufts-New England Medical Center, Tufts University School of Medicine, Boston, Mass,a Fetal Medicine Unit, Department of Obstetrics and Gynecology, Hospital Universitari Vall d’Hebron, Barcelona, Spain,b Department of Obstetrics and Gynecology, University Hospital Gasthuisberg, Leuven, Belgium,c and Department of Biostatistics, Boston University, Boston, Massd

Received for publication March 14, 2004; revised June 11, 2004

KEY WORDS
Cell-free fetal DNA
Twin-twin transfusion syndrome
Fetoscopic laser ablation
Placental pathology
In utero fetal death

Objective: This study was undertaken to determine whether laser thermocoagulation for twin-twin transfusion syndrome (TTTS) causes increased cell-free fetal DNA levels in maternal plasma, potentially as a result of placental injury.

Study design: We enrolled 34 patients with twin pregnancies complicated by severe TTTS who underwent fetoscopic selective laser ablation of placental vascular anastomoses. Blood samples were drawn before and sequentially after the procedure. Fetal DNA in maternal plasma was quantified by polymerase chain reaction amplification of a Y-chromosome sequence.

Results: Compared with baseline, median elevations of fetal DNA levels were 0.8% at 30 minutes ($P = .32$), 15.8% at 60 minutes ($P = .1$), 179.5% at 24 hours ($P = .003$), and 172.9% at 48 hours ($P = .003$). Factors associated with increased fetal DNA levels at 24 hours after procedure included longer operation time, higher number of vessels ablated, and subsequent in utero fetal death ($P = .01, .04,$ and .04, respectively).

Conclusions: Persistent elevation of fetal DNA levels in maternal plasma after laser ablation suggests that circulating fetal DNA could derive from placental injury. Plasma fetal DNA analysis may be an additional prognostic marker for fetal outcome after laser therapy.

© 2005 Elsevier Inc. All rights reserved.
Twin-twin transfusion syndrome (TTTS) is a serious complication of monochorionic twin gestations. It is believed to be attributable to an imbalance in feto-fetal transfusion over the intertwin vascular anastomoses within the shared placenta. When this occurs in the previable period, the prognosis is inevitably poor and therapy seems mandatory. Serial amniodrainage is one option, but fetoscopic laser coagulation of chorionic plate vascular anastomoses has been proposed as a treatment to interrupt the common vasculature. Although different techniques exist, the majority of surgeons strive to selectively coagulate all connecting vessels, and in so doing create a functional dichorionic placenta. The survival rate of at least 1 fetus ranges from 81% to 86% in uncontrolled case series. The only randomized controlled trial comparing the perinatal outcome between fetoscopic laser surgery versus serial amniocentesis in severe midgestational TTTS showed a survival rate of at least 1 twin after laser photocoagulation of 76.4%. During the fetoscopic laser procedure, neodymium: yttrium-aluminium-garnet (Nd:YAG) or Diode laser energy is preferentially absorbed by fetal blood in the targeted chorionic plate vessels. The generated heat not only induces local thermocoagulation, but the laser impact also subsequently produces coagulation necrosis of the vessels and the surrounding placental tissue. Ideally, the vessels feeding the cotyledon of interest are ablated; therefore, the tissue changes could result from both direct thermal injury and ischemic necrosis of the entire cotyledon. We hypothesized that this placental trauma would release cell-free fetal DNA into the maternal circulation.

Despite many proposed clinical applications for the quantitation of circulating fetal DNA, its tissue origin is still undefined. The placenta and fetal hematopoietic cells have been theorized to be the major sources of fetal DNA in maternal plasma and serum. In this study, we measured fetal DNA levels in the plasma of pregnant women before and sequentially after fetoscopic selective laser coagulation of the connecting vessels in cases of severe midgestational TTTS. We hypothesized that if the placenta is a tissue source of circulating fetal DNA, an elevation of fetal DNA levels should be observed, and if so, would be related to the extent of placental injury. This may provide indirect evidence that cell-free fetal nucleic acids in the maternal circulation derive from the placenta, especially after iatrogenic trophoblastic damage.

Material and methods

This study was approved by the institutional review boards at Tufts-New England Medical Center (Tufts-NEMC), Boston, Mass; University Hospital ‘Gasthuisberg’, Leuven Belgium, and Vall d’Hebron Hospital, Barcelona Spain. Women whose pregnancies were complicated by severe midgestational TTTS were recruited into this study between January 2002 and July 2003 as a nested subset of the ongoing observational Eurofoetus study for the treatment of TTTS (http://www.eurofoetus.org). The current standard inclusion criteria for fetoscopic laser therapy in Europe are based on the sonographic findings of oligo-polyhydramnios sequence. The exclusion criteria for enrollment were as follows: multifetal pregnancies of higher order than twins, ruptured membranes, established labor, or any other obstetric problem mandating immediate delivery. Patients were counseled about the nature and currently reported results of the therapy and its potential side effects, as well as the purpose of this study. Written informed consent was obtained. Fetal gender was ascertained by sonography and/or genetic amniocentesis, and was confirmed at delivery.

Maternal venous blood samples of 5 mL were drawn before the procedure, and then sequentially at 30 minutes, 60 minutes, 24 hours, and 48 hours after the laser impact. Blood samples were centrifuged at 4,500 rpm for 10 minutes to remove any residual cells. The supernatant of 400 μL was then used for DNA extraction via the QIAamp blood kit (QIAGEN Inc, Valencia, Calif) by using the Blood and Body Fluid Spin Protocol as described by the manufacturer. The extracted DNA was then eluted into a final volume of 50 μL.

Fetoscopic selective laser ablation of placental vascular anastomoses

Under appropriate local or regional anesthesia, and with real-time ultrasound guidance, the polyhydramniotic sac was entered percutaneously with a 3.0-mm trocar and cannula. A 2.0-mm fetoscope (Karl Storz, Tuttlingen, Germany) was introduced, and the placenta was inspected to identify the feto-fetal vascular anastomoses. All the recognized intertwin vessels on the chorionic plate were coagulated with a 30 to 45 W Diode laser (Dornier, Muenchen, Germany). Amnioinfusion with Hartmann solution warmed to 38°C was performed as needed to facilitate the procedure, followed by drainage of an excessive fluid at the end of the operation.

DNA extraction and real-time PCR amplification

Maternal plasma of 500 μL was centrifuged at 11,500g for 10 minutes to remove any residual cells. The supernatant of 400 μL was then used for DNA extraction via the QIАamp blood kit (QIAGEN Inc, Valencia, Calif) by using the Blood and Body Fluid Spin Protocol as described by the manufacturer. The extracted DNA was then eluted into a final volume of 50 μL.

The concentration of fetal DNA in maternal plasma was determined by real-time PCR amplification with the use of a Perkin-Elmer Applied Biosystems 7700
Sequence Detector (Applied Biosystems, Foster City, Calif). The DYS-1 sequence on the Y chromosome was used in this study to detect and quantify male DNA. The DYS-1 primers (forward: 5'-TCCTGCTTATCCAAATTCCACAT-3', reverse: 5'-ACTTCCCTCTGACATTACCTGATAATTG-3') are derived from the Y chromosome specific sequence p49a. The fluorogenic DYS-1 probe (5'-FAM-AAGTCGCCACTGGATATCAGTTCCCTGT-TAMRA-3') was used and the PCR amplification was performed as described previously with fetal DNA levels expressed in genome equivalents per milliliter (GE/mL). For each sample, the housekeeping β-globin gene sequence was amplified to estimate the total amount of DNA in the sample.

Statistical analyses

Descriptive statistics, including medians and 25th and 75th percentile ranges, were generated for all studied variables. The sequential alterations of plasma fetal DNA levels were expressed as a percent change from baseline levels. Multiple linear regression models were estimated to assess the effect of gestational age, operation time, amount of amniotic fluid drained at the end of the procedure, the amniotic fluid drained/gestational age ratio, and the number of chorionic vessels coagulated on the alteration of plasma fetal DNA levels at each time point considered separately. The total numbers of all the chorionic vessels ablated, as well as the numbers of arterial-venous (in either direction) anastomoses coagulated, were analyzed accordingly, as the latter are thought to be the prerequisite of TTTS and supply the whole cotyledon. The total number of chorionic vessels ablated included the arterial-venous, arterial-arterial, and venous-venous anastomoses. With the use of nonparametric analysis of variance (Kruskal-Wallis test), the baseline fetal DNA levels and the alterations of plasma fetal DNA in patients who had early and late in utero fetal death (IUFD) of at least 1 twin were compared with those of the patients who delivered 2 liveborn infants. Early and late IUFD were defined as IUFD that occurred within and later than 24 hours after the surgery, respectively. All statistical analyses were performed with SAS/STAT software (SAS Institute, Inc, Cary, NC). Statistical significance was assigned when the P value was less than .05.

Results

Thirty-four pregnant women with severe midgestational TTTS were enrolled. The median (25th, 75th percentiles) gestational age at the time of operation was 20.8 weeks (19.9, 22.7). The β-globin gene sequence was amplified in every plasma sample, indicating the presence of cell-free DNA in the sample. Fetal gender was identified with 100% accuracy by the blinded fetal DNA analysis of maternal plasma with 30 sets of male and 4 sets of female twins. The median (25th, 75th percentiles) plasma fetal DNA levels were 51.1 GE/mL (26.4, 62.7) before the procedure, 45.4 GE/mL (16.1, 81.3) at 30 minutes, 56.8 GE/mL (24.8, 85.2) at 60 minutes, 140.2 GE/mL (82.8, 219.2) at 24 hours, and 112.9 GE/mL (64.8, 200.8) at 48 hours after the procedure. The median (25th, 75th percentiles) elevations of plasma fetal DNA levels compared with the baseline were 0.8% (2.21, 28.24) at 30 minutes (P = .32), 15.8% (13.7, 70.8) at 60 minutes (P = .1), 179.5% (85, 323.2) at 24 hours (P = .003), and 172.9% (62.1, 240.1) at 48 hours (P = .003) after the first laser impact, as shown in Figure 1.
Descriptive statistics of the clinical covariables are summarized in Table I. No significant correlation was observed between the sequential alterations of plasma fetal DNA at each time point after the procedure and the gestational age, the amount of amniotic fluid drained at the end of the procedure, the amniotic fluid drained/gestational age ratio, and the number of ablated arterial-venous anastomoses, as summarized in Table II. Longer operation time and higher total number of chorionic vessels ablated were associated with a more pronounced elevation of fetal DNA levels at 24 hours after laser impact ($P = .01$ and $.04$, respectively). With the use of Spearman’s analysis, there was a strong correlation between the operation time and the number of vessels coagulated ($R = 0.602$, $P = .001$).

Among patients who carried male fetuses, there were 9 patients who had IUFD of 1 or both twins after the laser therapy. Four IUFD occurred within 24 hours, and 5 occurred more than 48 hours after the procedure. Among the late IUFD cases, fetal demise was detected at 3 days, 2 weeks, 3 weeks, 6 weeks, and 18 weeks the surgery, respectively. The median (25th, 75th percentiles) baseline fetal DNA levels in the patients who delivered 2 liveborn infants and those who eventually had early or late IUFD were 52 (26, 58), 68.9 (56.3, 85.9), and 27.3 (7.1, 38) GE/mL, respectively, which were not significantly different ($P = .21$). The elevation of 24-hour postprocedural fetal DNA levels was more pronounced in all patients who had IUFD compared with those who delivered 2 liveborn infants ($P = .04$). After early and late IUFD data were analyzed separately, the results suggest that the elevations of plasma fetal DNA levels after the operation in the patients who had early IUFD were higher than those who delivered 2 liveborn infants and those who had late IUFD. This difference, however, did not reach statistical significance, as demonstrated in Table III.

### Table I Clinical characteristics of the pregnant women with severe midgestational TTTS enrolled in this study

<table>
<thead>
<tr>
<th>Covariables</th>
<th>Median</th>
<th>25th, 75th percentiles</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>29.5</td>
<td>27, 32</td>
</tr>
<tr>
<td>Gestational age (wk)</td>
<td>20.8</td>
<td>19.9, 22.7</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>50</td>
<td>39, 60</td>
</tr>
<tr>
<td>Amount of amniotic fluid drained at the end of</td>
<td>1,527.5</td>
<td>1,100, 2,245</td>
</tr>
<tr>
<td>the operation (mL)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ratio between the amount of amniotic fluid</td>
<td>77.2</td>
<td>53.9, 109.1</td>
</tr>
<tr>
<td>drained and the gestational age (mL/wk)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Number of total chorionic anastomoses coagulated</td>
<td>7</td>
<td>5, 12</td>
</tr>
<tr>
<td>Number of arterial-venous chorionic anastomoses coagulated</td>
<td>5</td>
<td>3, 7</td>
</tr>
</tbody>
</table>

Comment

Surgical interruption of the placental vascular connections ideally transforms a monochorionic into a dichorionic placenta, and may discontinue the intertwin transfusion process. However, laser energy could directly injure, or cause ischemia to the trophoblastic cells. These damaged cells could consequently release their nuclear contents into the maternal circulation. The current study demonstrated the persistent elevation of plasma fetal DNA levels after laser impact for up to 48 hours. We also observed an association between the elevation of 24-hour postprocedural plasma fetal DNA levels and a longer operation time, the total number of chorionic vessels ablated, and subsequent IUFD of at least 1 twin.

Immediate tissue effects from laser have been described previously. Limited coagulation necrosis from thermal injury is found around the impact site, followed by complete cotyledon infarction caused by the arrest of blood flow in the placental subunit. In theory, ongoing damage of the cotyledon, as a result of partial placental infarction, placental apoptosis and/or necrosis, may continuously release fetal DNA. The number of chorionic vessels coagulated could reflect the volume of ischemic cotyledon. The association between the number of vessels coagulated and the elevation of postprocedural fetal DNA levels, along with the observed persistent elevation for up to 48 hours suggests that circulating fetal DNA could derive from placental injury. Longer operation time was associated with more chorionic vessels coagulated, and thus may not independently affect the elevation of plasma fetal DNA levels.

Alternatively, circulating fetal nucleic acids may derive from fetal hematopoietic cells. DePaepe et al examined 5 placentas within a month of the laser ablation procedure, and demonstrated gross and microscopic evidence of vascular collapse with associated focal subchorionic hemorrhage. It is possible that fetomaternal transfusion from this surgery could contribute to the fetal DNA pool. However, with a half-life of 16.3 to 30 minutes, fetal DNA is rapidly cleared from maternal circulation. Therefore, the observed gradual and persistent rise of postoperative fetal DNA levels suggest that the hemorrhage itself may not significantly contribute to this elevation. It is possible that in some cases, the hemorrhage may have occurred as a confined hematoma, and thus could have released the DNA over time. Analysis of circulating placental and hematopoietic messenger RNA transcripts could help to determine the tissue source of the circulating fetal nucleic acids.

Fetal demise of 1 or both twins after the procedure is not uncommon. With the use of sonographic appearance and Doppler studies, fetal outcome after this surgery can be predicted. We observed a remarkable elevation of circulating fetal DNA levels at 24 hours
indicates that circulating fetal nucleic acids could derive from damage to the trophoblast. This study also suggests the potential of plasma fetal DNA measurement as an additional prognostic factor for fetal loss after the laser procedure. Although our data suggests that after laser ablation injury to the placenta can release DNA into the circulation, the tissue origin of fetal nucleic acids in normal pregnancy is yet to be defined. Our future studies will include the analysis of placental and hematopoietic gene expression levels in sequential maternal plasma samples, which may help to better determine the relative contribution of the trophoblasts and the fetal blood cells to the pool of fetal nucleic acids in maternal circulation.16,20

**Table II**  Multiple linear regression analyses of the relationship between clinical characteristics and the sequential alteration of postprocedural plasma fetal DNA levels. Longer operation time and higher total number of chorionic vessels ablated were associated with increased fetal DNA levels at 24 hours after laser impact (R is regression coefficient)

<table>
<thead>
<tr>
<th>Covariables</th>
<th>Sequential alterations of plasma fetal DNA levels</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Maternal age</td>
<td>R = −2.08, P = .73</td>
</tr>
<tr>
<td>Gestational age</td>
<td>R = −29.57, P = .31</td>
</tr>
<tr>
<td>Operation time</td>
<td>R = 3.83, P = .14</td>
</tr>
<tr>
<td>Amount of amniotic fluid drained at the end of the operation</td>
<td>R = .33, P = .43</td>
</tr>
<tr>
<td>Ratio between the amount of amniotic fluid drained and the gestational age</td>
<td>R = −6.86, P = .43</td>
</tr>
<tr>
<td>Number of total chorionic anastomoses coagulated</td>
<td>R = −17.77, P = .13</td>
</tr>
<tr>
<td>Number of arterial-venous chorionic anastomoses coagulated</td>
<td>R = 8.9, P = .54</td>
</tr>
</tbody>
</table>

**Table III**  Sequential alterations of plasma fetal DNA levels from the baseline after laser therapy in women who eventually delivered 2 liveborn infants and who eventually experienced a demise of 1 or both twins. Early and late IUFD were fetal demises that occurred within and later than 24 hours after laser treatment, respectively. Although the elevations of postlaser plasma fetal DNA levels in the patients who had early IUFD were higher than those who delivered 2 liveborn infants and who had late IUFD, the difference did not reach statistical significance, presumably because of lack of power

<table>
<thead>
<tr>
<th>Pregnancy outcomes</th>
<th>Median (25th, 75th percentiles) sequential alteration of plasma fetal DNA levels (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>30 min</td>
</tr>
<tr>
<td>Delivered 2 liveborn infants (n = 21)</td>
<td>−5.4 (−24.8, 5.1)</td>
</tr>
<tr>
<td>Early IUFD (n = 4)</td>
<td>19.5 (−2.9, 149.2)</td>
</tr>
<tr>
<td>Late IUFD (n = 5)</td>
<td>34.4 (−50.5, 54.7)</td>
</tr>
<tr>
<td>P-value</td>
<td>.32</td>
</tr>
</tbody>
</table>

In conclusion, this study demonstrated a persistent elevation of fetal DNA levels in maternal plasma after laser coagulation of placental vessels. This suggests the ongoing release of DNA from ischemic vili, and thus indicates that circulating fetal nucleic acids could derive after the operation in the patients who eventually experienced the demise of 1 or both fetuses. Therefore, postprocedural alteration of fetal DNA levels may be an additional predictor of prognosis after the laser therapy in these high-risk patients. Our data suggested the remarkably higher postprocedural fetal DNA in the patients who eventually experienced early IUFD (Table III), although it did not reach statistical significance, presumably because of the lack of statistical power. The preexisting placental abnormalities may be responsible for this elevation. Alternatively, it may be resulted from a larger area of placental damage after laser surgery in the morbidly sick fetuses. A future study to elucidate this issue is desirable.

Acknowledgments

The other members of the Euro Twin 2 Twin group (Dr Ville, Dr Hecher, Dr Nicolaides, Mr Barki, Mr Denk, Dr Vlietinck, Dr Van Gemert and Mrs. Jackson)
are acknowledged for their efforts to set up the Euro Twin 2 Twin-project.

References


Maternal and fetal amino acid concentrations in normal pregnancies and in pregnancies with gestational diabetes mellitus

Irene Cetin, MD,a,* Maria S. Nobile de Santis, MD,a Emanuela Taricco, MD,a Tatjana Radaelli, MD,a Cecilia Teng, PhD,b Stefania Ronzoni, MD,a Elena Spada, PhD,c Silvano Milani, PhD,c Giorgio Pardi, MDa

Objective: This study was undertaken to compare amino acid concentrations in normal pregnancies and pregnancies with gestational diabetes (GDM), a condition associated with altered fetal growth.

Study design: Maternal and fetal amino acids were evaluated by high-performance liquid chromatograph at the time of delivery in 16 normal and 17 GDM pregnancies. Fetal weights were not different, but placental weights were significantly higher and fetal/placental weight ratios were significantly lower in GDM compared with normal.

Results: Ornithine was significantly increased in GDM mothers. In umbilical vein and artery of GDM significant increases were observed for valine, methionine, phenylalanine, isoleucine, leucine, ornithine, glutamate, proline, and alanine, whereas glutamine was significantly decreased.

Conclusion: Placental amino acid exchange is altered in GDM pregnancies. Moreover, the changes observed for glutamine and glutamate in the umbilical samples suggest that in GDM the fetal hepatic production of glutamate is increased, likely as a consequence of the endocrine changes in the fetal compartment.

KEY WORDS
Pregnancy
Gestational diabetes
Amino acids
Placenta
Fetus

Gestational diabetes mellitus (GDM) is a disease that complicates 3% to 5% of pregnancies and is associated with fetal overgrowth and significant increases of obesity and type 2 diabetes in the offspring.1 In the predisposed mother, decreases in maternal insulin sensitivity lead to increased glucose and nutrient supply to the fetoplacental unit with advancing gestation.2 In the fetus, the increased fat mass3 results from the combined effects of this excess of nutrients and the permissive environment of fetal hyperinsulinemia.4 Increased placental weights and placental ratios (placental weight to birth weight ratio)5 have been documented in pregnancies complicated by
GDM, with maternal insulin sensitivity negatively related to placental weight. However, the excess of intrauterine growth is not clearly correlated with maternal hyperglycemia and higher placental weights and lower fetal/placental weight ratios are observed even in the presence of optimal maternal glycemic control throughout the third trimester. In this situation, average fetal weights within the normal ranges are associated with increased fetal fat mass and with increased leptin levels.

Amino acids represent, along with glucose and lactate, major nutrients during intrauterine life, used both for protein synthesis and oxidation. Amino acid concentrations are higher in the fetal than in the maternal compartment, and amino acid transport systems have been described on both microvillous and basal membranes of the trophoblast. Moreover, a number of studies both in humans and in sheep have provided evidence for significant amino acid metabolism within the placenta, as recently reviewed. The placenta not only concentrates amino acids toward the fetus but is also involved in protein synthesis, oxidation, transamination, and synthesis of some nonessential amino acids. Interorgan cycling between fetal liver and placenta has been suggested for some nonessential amino acids so that fetal glutamine (Gln) and glycine (Gly) are metabolized in the fetal liver and released to the placenta as glutamate (Glu) and serine (Ser), respectively.

Studies in type 1 diabetic mothers have reported higher concentrations of most amino acids both in early and late gestation, suggesting that, together with maternal hyperglycemia, also increased amino acid availability could stimulate fetal hyperinsulinemia and have an important role in the development of fetal macrosomia. However, no data are available on the relationship between amino acid concentrations in the fetal and in the maternal compartment of pregnancies complicated by gestational diabetes diagnosed and treated according to current clinical protocols, associated with normal birth weights but increased fetal fat mass and lower fetal/placental weight ratios. Increased placental mass and/or alterations in placental amino acid transport systems could be associated with changes in fetal amino acid availability, leading to fetal hyperinsulinemia and increased fetal fat mass. The metabolic and hormonal differences in normal birth weight fetuses born from GDM pregnancies could carry short- and long-term consequences.

The objective of this investigation was therefore to study amino acid concentrations in mothers and fetuses of pregnancies associated with GDM under strict maternal glycemic control.

Material and methods

The studies were performed in the Department of Obstetrics and Gynecology of the DMCO San Paolo Hospital, Milano, Italy. The San Paolo Institute Board approved the protocol of the study. Informed consent was obtained from all pregnant women.

Subjects

Thirty-three pregnant women were studied at the time of delivery between 37 and 41 weeks of gestation. Gestational age was calculated from the last menstrual period and confirmed by routine ultrasonography at 20 weeks’ gestation. Sixteen patients had normal pregnancies and delivered appropriate for gestational age (AGA) infants, according to Italian birth weight–gestational age standards. In these patients, GDM was excluded by an oral glucose challenge test performed between 24 and 28 weeks, according to our routine clinical protocol.

In 17 pregnant women GDM was diagnosed between 28 and 32 weeks of gestation in the presence of a 100 g oral glucose tolerance test with 2 or more values over the ranges established by Carpenter and Coustan (plasma glucose: 1 hour <180 mg/dL; 2 hours <155 mg/dL; 3 hours <140 mg/dL). GDM patients were free from any other maternal disease. The body mass index (BMI, kg/m²) was 28.0 ± 1.6 in GDM pregnancies, not significantly different from the control group (24.5 ± 2.2). No differences with regard to parity were observed between the 2 groups. After diagnosis, all GDM patients began home blood glucose monitoring with a reflectance meter while undertaking an appropriate diet calculated as:

$$Kcal/die = X (Kcal) \times \{ IW (Kg) + [0.225 (Kg) \times ga (weeks)] \}$$

Where X is 30 to 35 Kcal if pregravid BMI is within 19.6 and 24, 35 to 40 Kcal if BMI is below 19.6, and 25 to 30 Kcal if BMI over 24 and IW (ideal weight) is 20.8 $\times$ height² (m²) (ga is gestational age in weeks).

According to blood glucose values measured over 1 week, treatment was as follows: (1) only diet when average fasting and preprandial glucose less than 90 mg/dL and postprandial (after 2 hours) glucose less than 120 mg/dL (n = 13) and (2) diet plus insulin with average glucose values exceeding 90 mg/dL fasting and 120 mg/dL postprandially (n = 4). GDM pregnant women were monitored every 2 weeks and average maternal glycemia at the time of the last control was within the values described previously in all cases. In GDM pregnancies, fetal growth was monitored by ultrasound performed at the time of diagnosis and repeated at 36 to 38 weeks, as part of our routine clinical protocol.

In both normal and GDM pregnancies, cesarean section was performed for elective indications, such as breech presentation and repeat cesarean section. None of the women was in labor.

Maternal and fetal blood samples were obtained at the time of elective cesarean section in normal and GDM pregnancies. Umbilical arterial and venous blood
samples were collected from a doubly clamped segment of the cord.

**Biochemical analyses**

Fetal and maternal blood samples were collected into heparinized 2.5-mL syringes and immediately sealed and stored on ice. Plasma for amino acid analyses was obtained by centrifugation at 3000 revolutions per minute (rpm) for 10 minutes at −4°C and frozen at −70°C until the time of analysis. Plasma was quickly thawed and deproteinized with a solution of 10% sulfosalicylic acid with norleucine added as an internal standard and buffered with lithium hydroxide to pH 2.2. Samples were centrifuged at 14,000 rpm for 10 minutes and the supernatant fraction was filtered throughout a Millipore (Millipore Corp, Bedford, Mass) filter and loaded into a Dionex (Dionex Corp, Sunnyvale, Calif) high-performance liquid chromatograph with refrigerated automated sampler.

The samples were analysed by cation exchange column with 3 buffers changed by gradient isothermally. Ninhydrin was used as color reagent, and a dual-wavelength spectrophotometer with 440-nm and 570-nm wavelengths was used for concentration determinations. The column, buffers, and ninhydrin reagent were purchased from Pickering Laboratory (Mountain View, Calif). All the instruments operation and data processing were controlled by the Dionex AI-450 software (Dionex Corp). Samples from each study were analyzed on a single column in the same run, with a variance of ±2%.

**Calculations**

The umbilical coefficient of extraction was calculated as: umbilical venoarterial difference/umbilical arterial concentration.
Statistical analysis

Amino acid concentrations have been log-transformed with the aim of stabilizing variance and approximating Gaussian distribution. To process data with multivariate techniques, which require complete records, auxiliary values were substituted for missing values (43/1782) by resorting to an expectation-maximization procedure, based on the assumption of multivariate Gaussian distribution conditional on the group of pregnancies (normal or GDM). The differences between normal and GDM pregnancies in maternal and fetal amino acid concentrations were tested with univariate unpaired Student *t* tests. Because the series consisted of only 33 pregnancies, but there were 54 variables to process (18 amino acids by 3 samples of plasma), the multivariate analysis was limited to 9 amino acids. As a rule of thumb, we selected those amino acids that resulted to be significant at least once (.01 level) or twice (.05 level) at univariate analysis. The overall significance of differences between normal and GDM pregnancies in the amino acid profile was tested by Hotelling *T*². Adjusted *P*-values for single tests, ensuring a .05 overall risk of type I error were computed according to James. Multivariate analyses were performed with SAS procedures GLM (SAS, Cary, NC).

Results

Table I presents gestational age, fetal and placental weights, and the fetal/placental weight ratio at delivery in the 2 groups. Gestational age and fetal weights were not significantly different in GDM compared with normal pregnancies, whereas placental weights were significantly higher and fetal/placental weight ratios were significantly lower in GDM pregnancies. Maternal plasma amino acid concentrations in the 2 groups of pregnancies are presented in Table II. Compared with normal mothers, GDM mothers show ornithine concentrations higher by about 70% (*P* = .0001). The difference was confirmed to be significant when the conservative *P*-value adjusted for multiple endpoints was adopted. No other differences emerged between the 2 groups. No significant correlations were observed between maternal plasma amino acid concentrations and fetal weights.

Umbilical venous plasma concentrations were significantly higher for methionine (Met), isoleucine (Ile), leucine (Leu), phenylalanine (Phe), ornithine (Orn), Glu, proline (Pro), and alanine (Ala), whereas Gln was significantly lower in GDM compared with normal pregnancies (Table III). Multivariate analysis confirmed the difference between the 2 groups (*P* = .0050) in the amino acid profile, particularly for Met, Phe, Orn, Glu,
and Pro, which resulted in being significant even with the use of the adjusted $P$-value.

Umbilical arterial plasma concentrations were significantly higher for valine (Val), Met, Ile, Leu, Orn, Glu, Ala, and Pro, whereas Gln was significantly lower in GDM compared with normal pregnancies (Table IV). Multivariate analysis confirmed the difference between the 2 groups ($P = .0022$) in the amino acid profile, particularly for Met, Orn, Glu, Ala, and Pro, which resulted in being significant even with the use of the adjusted $P$-value. No significant correlations were observed between umbilical venous plasma amino acid concentrations and fetal weights in GDM patients.

In GDM fetuses, a significant negative correlation was observed between plasma concentrations of Glu and Gln in both umbilical arterial and venous samples (Figure).

Umbilical venoarterial plasma amino acid differences were not significantly different from zero or were they significantly different in GDM compared with normal pregnancies, but the umbilical coefficient of extraction (umbilical venoarterial difference/umbilical arterial concentration) was significantly lower for Ala and Pro in GDM compared with normal pregnancies.

The correlations between umbilical venous and maternal plasma amino acid concentrations in normal and GDM pregnancies are presented in Table V. Significant correlations were observed for most essential and many nonessential amino acids in both groups of pregnancies.

### Comment

To our knowledge, this is the first report about fetal and maternal amino acid concentrations in pregnancies associated with GDM. In our GDM population with well-controlled maternal glycemia and normal birth weight, significant increases were observed for a number of essential and nonessential amino acids in umbilical venous plasma, paralleled by significant increases in umbilical arterial concentrations. These increases were related to differences in the maternal amino acid levels only for Orn, that is significantly higher in mothers with GDM. This finding is at variance from what previously reported in type 1 diabetic pregnant women by Kalkhoff et al.$^{17}$, i.e., higher maternal concentrations for Ser, threonine (Thr), histidine (His), Pro, lysine (Lys), and arginine (Arg) in late pregnancy. However, it is important to underline the differences between the 2 studies. Our study considered pregnancies with GDM with strict maternal glycemic control giving birth at term to AGA infants, whereas the study by Kalkhoff et al.$^{17}$ investigated type 1 diabetic pregnant women with significantly

### Table IV  Umbilical arterial plasma amino acid concentrations in N and GDM pregnancies

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>N Mean* ± SE(μmol/L)</th>
<th>GDM Mean* ± SE(μmol/L)</th>
<th>GDM/N ratio (95% CL)</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Essential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lys</td>
<td>316.6 ± 12.1</td>
<td>336.4 ± 12.5</td>
<td>1.06 (0.95-1.18)</td>
<td>.2648</td>
</tr>
<tr>
<td>His</td>
<td>107.7 ± 4.1</td>
<td>112.1 ± 4.1</td>
<td>1.04 (0.94-1.16)</td>
<td>.4531</td>
</tr>
<tr>
<td>Thr</td>
<td>257.6 ± 19.3</td>
<td>271.5 ± 19.8</td>
<td>1.05 (0.85-1.30)</td>
<td>.6177</td>
</tr>
<tr>
<td>Val</td>
<td>200.1 ± 7.2</td>
<td>225.7 ± 7.8</td>
<td>1.11 (1.00-1.23)</td>
<td>.0418$^+$</td>
</tr>
<tr>
<td>Met</td>
<td>24.2 ± 1.1</td>
<td>29.7 ± 1.3</td>
<td>1.23 (1.08-1.39)</td>
<td>.0022$^+$</td>
</tr>
<tr>
<td>Ile</td>
<td>55.6 ± 2.6</td>
<td>66.4 ± 3.1</td>
<td>1.19 (1.04-1.37)</td>
<td>.0115$^+$</td>
</tr>
<tr>
<td>Leu</td>
<td>104.7 ± 4.4</td>
<td>120.5 ± 4.9</td>
<td>1.15 (1.02-1.30)</td>
<td>.0220$^+$</td>
</tr>
<tr>
<td>Phe</td>
<td>63.1 ± 2.1</td>
<td>69.2 ± 2.3</td>
<td>1.10 (1.00-1.21)</td>
<td>.0588</td>
</tr>
<tr>
<td>Nonessential</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Orn</td>
<td>58.9 ± 3.3</td>
<td>84.0 ± 4.6</td>
<td>1.43 (1.22-1.67)</td>
<td>.0001$^+$</td>
</tr>
<tr>
<td>Arg</td>
<td>100.8 ± 10.4</td>
<td>79.2 ± 7.9</td>
<td>0.79 (0.59-1.05)</td>
<td>.1025</td>
</tr>
<tr>
<td>Tau</td>
<td>100.8 ± 12.4</td>
<td>135.5 ± 16.2</td>
<td>1.34 (0.95-1.91)</td>
<td>.0946</td>
</tr>
<tr>
<td>Ser</td>
<td>144.4 ± 3.9</td>
<td>150.6 ± 4.0</td>
<td>1.04 (0.97-1.13)</td>
<td>.2721</td>
</tr>
<tr>
<td>Glu</td>
<td>36.6 ± 6.8</td>
<td>110.0 ± 19.7</td>
<td>3.00 (1.78-5.08)</td>
<td>.0002$^+$</td>
</tr>
<tr>
<td>Gln</td>
<td>482.4 ± 44.8</td>
<td>368.3 ± 33.2</td>
<td>0.76 (0.59-0.99)</td>
<td>.0454$^+$</td>
</tr>
<tr>
<td>Gly</td>
<td>217.2 ± 8.9</td>
<td>229.4 ± 9.1</td>
<td>1.06 (0.94-1.19)</td>
<td>.3464</td>
</tr>
<tr>
<td>Ala</td>
<td>238.5 ± 15.7</td>
<td>332.6 ± 21.2</td>
<td>1.39 (1.16-1.68)</td>
<td>.0010$^+$</td>
</tr>
<tr>
<td>Tyr</td>
<td>59.3 ± 2.6</td>
<td>64.4 ± 2.7</td>
<td>1.09 (0.96-1.23)</td>
<td>.1825</td>
</tr>
<tr>
<td>Pro</td>
<td>141.7 ± 8.8</td>
<td>186.1 ± 11.2</td>
<td>1.31 (1.10-1.57)</td>
<td>.0035$^+$</td>
</tr>
</tbody>
</table>

* Computed as back-transformation of log values.
1 $P < .05$.
2 $P < .01$.
3 $P < .001$ vs N.
higher percentage of average birth weight and lower gestational age at delivery. More recently, studies analyzing maternal protein and amino acid metabolism by stable isotope methodologies did not find significant differences in either untreated\textsuperscript{24} or treated GDM.\textsuperscript{25,26}

In our study, the increased amino acid levels observed in the umbilical, but not in the maternal circulation for Met, Ile, Leu, Phe, Ala, and Pro suggest therefore that placental amino acid exchange and/or feto/placental metabolism are altered in GDM. Interestingly, although

Figure Relationship of glutamic acid to glutamine plasma concentration (μmol/L - log-scale) in the umbilical artery (left) and in the umbilical vein (right) in normal (open circle) and in GDM (solid circle) pregnancies.

<table>
<thead>
<tr>
<th>Amino acid</th>
<th>N</th>
<th>GDM r (95% CL)</th>
<th>Probability</th>
<th>Probability</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lys</td>
<td>0.42 (-0.09-0.76)</td>
<td>0.64 (0.23-0.86)</td>
<td>.1055</td>
<td>.0043*</td>
</tr>
<tr>
<td>His</td>
<td>0.67 (0.26-0.87)</td>
<td>0.16 (−0.35-0.59)</td>
<td>.0037*</td>
<td>.5471</td>
</tr>
<tr>
<td>Thr</td>
<td>0.92 (0.78-0.97)</td>
<td>0.90 (0.75-0.97)</td>
<td>.0001</td>
<td>.0001†</td>
</tr>
<tr>
<td>Val</td>
<td>0.72 (0.34-0.89)</td>
<td>0.75 (0.43-0.91)</td>
<td>.0011†</td>
<td>.0002†</td>
</tr>
<tr>
<td>Met</td>
<td>0.65 (0.22-0.86)</td>
<td>0.35 (−0.16-0.71)</td>
<td>.0057*</td>
<td>.1725</td>
</tr>
<tr>
<td>Ile</td>
<td>0.77 (0.44-0.92)</td>
<td>0.76 (0.44-0.91)</td>
<td>.0003†</td>
<td>.0002†</td>
</tr>
<tr>
<td>Leu</td>
<td>0.71 (0.34-0.89)</td>
<td>0.80 (0.52-0.92)</td>
<td>.0013*</td>
<td>.0001†</td>
</tr>
<tr>
<td>Phe</td>
<td>0.26 (−0.27-0.67)</td>
<td>0.01 (−0.47-0.49)</td>
<td>.3285</td>
<td>.9571</td>
</tr>
<tr>
<td>Orn</td>
<td>0.49 (−0.01-0.79)</td>
<td>0.02 (−0.47-0.50)</td>
<td>.0535</td>
<td>.9417</td>
</tr>
<tr>
<td>Arg</td>
<td>0.55 (0.08-0.82)</td>
<td>0.41 (−0.08-0.75)</td>
<td>.0254†</td>
<td>.0996</td>
</tr>
<tr>
<td>Tau</td>
<td>0.55 (0.07-0.82)</td>
<td>0.48 (−0.01-0.78)</td>
<td>.0269†</td>
<td>.0524</td>
</tr>
<tr>
<td>Ser</td>
<td>0.47 (−0.03-0.79)</td>
<td>0.24 (−0.27-0.65)</td>
<td>.0633</td>
<td>.3629</td>
</tr>
<tr>
<td>Glu</td>
<td>0.27 (−0.26-0.68)</td>
<td>0.09 (−0.41-0.55)</td>
<td>.3161</td>
<td>.7350</td>
</tr>
<tr>
<td>Gln</td>
<td>0.30 (−0.23-0.69)</td>
<td>0.34 (−0.17-0.71)</td>
<td>.2701</td>
<td>.1852</td>
</tr>
<tr>
<td>Gly</td>
<td>0.69 (0.30-0.89)</td>
<td>0.49 (0.01-0.78)</td>
<td>.0020*</td>
<td>.0466†</td>
</tr>
<tr>
<td>Ala</td>
<td>0.90 (0.74-0.97)</td>
<td>0.64 (0.24-0.86)</td>
<td>.0001†</td>
<td>.0042*</td>
</tr>
<tr>
<td>Tyr</td>
<td>0.80 (0.50-0.93)</td>
<td>0.32 (−0.18-0.70)</td>
<td>.0001†</td>
<td>.2076</td>
</tr>
<tr>
<td>Pro</td>
<td>0.34 (−0.19-0.71)</td>
<td>0.72 (0.37-0.89)</td>
<td>.2024</td>
<td>.0006†</td>
</tr>
</tbody>
</table>

* P < .01.
† P < .001
* P < .05 vs N.
these amino acids did not show differences in the maternal concentrations, for most of them, as expected, a significant relationship was observed between umbilical venous and maternal levels. On the contrary, ornithine levels were significantly increased in both maternal and umbilical circulation of GDM pregnancies, although their levels were not significantly correlated.

Considering the higher placental weights observed in this, as well as in previous studies, a significant role for changes in placental transport and metabolism can be hypothesized in GDM. Placental transport of amino acids in diabetic pregnancy has been studied in vitro, yielding conflicting results. Recently, system A activity was reported to be significantly increased in the microvillous, but not in the basal membrane of placentas from diabetic pregnancies independently of fetal growth.27

Although increased placental weights and possible increases in placental transporter activities could be responsible for increased maternal to fetal amino acid placental transport, the differences observed for Glu and Gln seem also to indicate that fetal and placental metabolism are altered in GDM pregnancies. In the absence of changes in the mother, in our study, Glu was significantly higher in both umbilical artery and vein of GDM fetuses, whereas Gln was significantly lower.

In the pregnant sheep, significant uptake of Glu has been demonstrated by the fetal liver, together with a significant release of Glu, which is then taken up by the placenta, suggesting glutamine-glutamate exchange between the fetal liver and the placenta.28 The fetal liver is the primary site of Gln-to-Glu conversion, with approximately 45% of Gln hepatic uptake converted to Glu. In our study, the negative correlation observed between Glu and Gln in the fetal compartment of GDM pregnancies, together with the increased concentrations of Glu are suggestive for an increased hepatic Gln-to-Glu conversion. Many possible explanations can be put forward to explain this hypothesis: first, the endocrine changes typical of GDM; second, an increase in fetal liver mass and blood flow.

We have previously reported significantly lower umbilical venous and fetal-maternal concentration differences for a number of essential and nonessential amino acids in intrauterine growth retarded pregnancies.29 Kinetic studies with stable isotopes indicated that these differences could be due to an increase in protein breakdown in the fetal/placental compartment and/or to a decrease in the transplacental transfer rate.30 It is interesting to note that the opposite findings in our GDM cases are observed in the presence of birth weights within the normal ranges. Nevertheless, it has been recently shown that infants of women with GDM have increased body fat even when their weights are similar to the control population.31 However, it would be extremely valuable to investigate whether similar or larger changes in fetal amino acid concentrations are observed in GDM associated with large-for-gestational- age (LGA) fetuses. Interestingly, the differences observed in umbilical venous and arterial concentrations were not associated with significant changes in umbilical venoarterial differences. Hence, further studies are needed to understand how the increased fetal amino acid concentrations influence intrauterine growth and future life in infants of GDM mothers.

Acknowledgments

We sincerely thank Prof Frederick C. Battaglia for his useful suggestions and comments in the design of the study and in the preparation of the manuscript.

References


Soluble factors released by placental villous tissue: Interleukin-1 is a potential mediator of endothelial dysfunction

Corinne Rusterholz, PhD,a Anurag K. Gupta, MSc,a Berthold Huppertz, PhD,b Wolfgang Holzgreve, MD,a Sinuhe Hahn, PhDa,*

Laboratory for Prenatal Medicine, University Women’s Hospital/Department of Research, University of Basel, Basel, Switzerland, a Department of Anatomy II, University Hospital, University of Technology Aachen, Aachen, Germany b

Received for publication April 2, 2004; revised July 15, 2004; accepted August 23, 2004

KEY WORDS
Placental factors
Endothelial activation
Interleukin
Inflammatory response

Objective: The purpose of this study was to analyze the potential of placental-conditioned medium to activate endothelial cells in vitro and to identify the placental factors that mediate this effect.

Study design: Placental-conditioned medium was generated by the culturing of normal term placental villous explants for up to 48 hours. Human umbilical vein endothelial cells were exposed to the conditioned media, and cellular proliferation, viability, and activation were investigated.

Results: The proliferation of endothelial cells that were exposed to 20% placental-conditioned medium was reduced by 25%, but their survival was not compromised. Conditioned medium also up-regulated the expression of E-selectin and stimulated the release of soluble intercellular adhesion molecule-1 and the secretion of interleukin-6. Treatment with interleukin-1 receptor antagonist, but not with an anti-tumor necrosis factor-α neutralizing antibody, blocked the release of soluble intercellular adhesion molecule-1 and interleukin-6.

Conclusion: Placentally derived interleukin-1 may be 1 of the potential mediators of the maternal inflammatory response that is observed in late pregnancy.

Pregnancy is an exceptional physiologic condition to which the maternal body must adapt. The survival of the fetal allograft imposes on the maternal immune system to maintain a state of relative immunosuppression. In the third trimester, pregnancy is also associated with features that suggest a state of maternal inflammation. The coagulation system is activated; the vascular endothelium shows increased permeability, and changes in cells of the innate immune system are detected. Pre-eclampsia, a multisystem disorder of the second half of pregnancy that is characterized by systemic endothelial cell dysfunction and overt maternal inflammation, has been proposed to be at the extreme end of the wide range of maternal inflammatory responses that are induced by pregnancy itself.

Inflammatory leukocytes in normal pregnancy present several characteristics that are indicative of their
activation. Not only do pregnant women have increased numbers of monocytes and granulocytes compared with nonpregnant women, but also both cell types express higher levels of several adhesion molecules and exhibit increased basal intracellular reactive oxygen species. The origin of the inflammatory response that develops in normal pregnancy towards term is likely to involve the placenta. Recent data have indicated that neutrophils were stimulated to produce superoxide and increase their capacity to bind endothelial cells in vitro by placental-conditioned medium that is generated from normal microvillous tissue.

In addition to their role in regulating vascular function, cells of the vascular endothelium are important components in inflammatory processes, by producing chemokines and cytokines and by up-regulating their surface expression of adhesion molecules that are required for leukocyte-endothelial interactions. Because they are in direct contact with the bloodstream, they are potential targets to soluble factors and placental debris that are released by the placenta into the maternal circulation.

In the present study, we analyzed the potential of soluble factors that are released from the placenta to activate endothelial cells in vitro. Villous explants from normal term placentae were cultured in conditions that maintain overall tissue integrity and allow turnover of the syncytiotrophoblast, which includes cytotrophoblast fusion and the shedding of apoptotic syncytiotrophoblast knots. We demonstrate that placental-conditioned medium alters endothelial cell proliferation, but not viability, and stimulates the production of E-selectin, soluble intercellular adhesion molecule (sICAM)-1, and interleukin (IL)-6 by the endothelial cells in vitro. We identify IL-1 as a possible mediator of this endothelial cell activation.

Material and methods

Placental explants

This study was approved by the Institutional Review Board of Basel, Switzerland; written informed consent was requested before tissue collection. Placentae were obtained after normal delivery or elective cesarean delivery from uncomplicated term pregnancies. Villous tissue was dissected into small pieces and extensively rinsed in sterile phosphate buffered saline solution (PBS). Explants of 40 to 60 mg each were individually deposited in 6 replicates into 2-mL Dulbecco modified Eagle’s medium:F-12 nutrient mixture (1:1; Invitrogen, Grand Island, NY) supplemented with 10% heat-inactivated fetal calf serum (BioConcept, Allschwill, Switzerland), 1 × antimycotic/antibiotics solution (Invitrogen), 25 IU/mL heparin (Roche Diagnostics, Mannheim, Germany) and 50 U/mL aprotinin (Fluka, Buchs, Switzerland). The explants were incubated for 40 to 48 hours at 37°C in humidified air containing 5% carbon dioxide. Placental-conditioned medium was then centrifuged at 500 g to discard large debris and stored at −20°C. Unconditioned medium consisted of freshly prepared placental culture medium.

Endothelial cell proliferation and apoptosis

HUVECs were seeded onto gelatin-coated 96-well plates (15 × 10^3 per well) in human endothelial-serum free medium that was supplemented with human recombinant basic fibroblast growth factor and 10 ng/ml epidermal growth factor, according to the manufacturer’s recommendations (Invitrogen) and cryopreserved. Experiments were performed with cells from passage 3 to 7. Four HUVEC preparations were used, and experiments were repeated on at least 2 preparations to ensure that the cellular response was not biased by individual genetic and/or physiologic variability. In each experiment, duplicates or triplicates were analyzed.

Endothelial cell activation

Confluent HUVECs were incubated with a range (3%-30%) of placental-conditioned medium for 6 or 24 hours.
in 96-well plates, as indicated, and analyzed for the expression of E-selectin or for the secretion of sICAM-1 and IL-6, respectively. Unconditioned medium served as control. In some experiments, HUVECs were grown in 24-well plates, and the conditioned medium was added in an insert Transwell with a membrane pore size of 0.02 μm (Nunc A/S, Roskilde, Denmark). Anti-tumor necrosis factor-α (TNF-α) neutralizing goat antibody (2 μg/mL; R&D Systems, Abingdon, United Kingdom) or recombinant human IL-1 receptor antagonist (IL-1ra; 0.012-0.5 μg/mL; R&D Systems) were incubated with the conditioned medium for 1 hour at 37°C before addition to the HUVECs. Human recombinant TNF-α (10 ng/mL; R&D Systems) was used when indicated.

Enzyme-linked immunosorbent assays

To measure membrane expression of E-selectin, HUVECs were fixed for 15 minutes in 2% paraformaldehyde (Sigma Chemical Company), then washed twice with PBS that contained 1.5 mmol/L MgCl₂ and 2 mmol/L 2-mercaptoethanol (Sigma Chemical Company). Endogenous peroxidase activity was neutralized by 20 minutes of incubation in 0.1% NaN₃-0.3% H₂O₂ in PBS. Mouse anti-human CD62E antibody (2 μg/mL; BBIG-E4(5D11); R&D Systems) was incubated with cells for 2 hours. After being washed, horseradish peroxidase-conjugated anti-mouse immunoglobulin G antibody (BD Biosciences Pharmingen, San Diego, Calif) was added for 1 hour. Color development of the 2, 2'-azino-bis-(3-ethylbenzothiazoline-6-sulfonic acid), diammonium salt (ABTS) chromogen (Zymed Laboratories Inc, San Francisco, Calif) was measured in an enzyme-linked immunosorbent assay (ELISA) reader (Vmax; Molecular Devices, Sunnyvale, Calif). The absorbance of the negative controls where the primary antibody has been omitted was deduced from the absorbance of the samples. IL-6 and sICAM-1 were quantified by sandwich ELISA (R&D Systems) by curve-fitting to a standard using the SoftmaxPro software (Molecular Devices). Because placental explants also release detectable amounts of sICAM-1 and IL-6, these levels were subtracted from those of HUVEC-treated cultures. TNF-α, IL-1α, and IL-1β were quantified with commercial ELISA (Endogen, Perbio Science, Lausanne, Switzerland).

Statistics

The data are presented as mean ± standard error. Statistical analysis was done with SigmaStat software (SPSS Inc, Chicago, Ill). Paired or unpaired t-tests were performed as appropriate. A Mann-Whitney rank sum test was used when the normality test failed. Statistical significance was set at a probability value of <.05.

Results

HUVECs that were incubated in placental-conditioned medium showed reduced proliferation, compared with
cells that were incubated in unconditioned medium (Figure 1, A). The proliferation was reduced by more than one-half in 100% conditioned medium and by one-fourth in 20% conditioned medium. The inhibition of proliferation was not a consequence of an increase in cellular apoptosis. Confluent HUVECs that were exposed to placental-conditioned medium did not show any sign of cell death, as evidenced by the lack of apoptotic DNA fragmentation and the absence of caspases activation (Figure 1, B). Furthermore, HUVECs that were exposed to conditioned medium had similar metabolic activity, as compared with control cells. The percentage of mitochondrial dehydrogenase activity that was relative to that of the control was 88% ± 17% (not significant; n = 5 samples) and 105% ± 12% (not significant; n = 5 samples) in endothelial cells that were cultured in 30% and 10% conditioned medium, respectively.

To determine whether the reduced proliferation could reflect a state of cellular activation, we analyzed HUVECs that were incubated in 20% conditioned medium for the expression of several activation markers. Increased membrane expression of the adhesion molecule E-selectin was detectable as soon as 6 hours after the addition of placental factors. The increase in optical density, as measured by ELISA, was 3.5-fold (Table). Placental factors also stimulated the release of the soluble form of ICAM-1 by 14.5-fold over that of control cultures and triggered the production of elevated amounts of IL-6 (Table).

Syncytiotrophoblast microvillous fragments (STBM) are microparticles that are shed by the syncytiotro-
phoblast and affect endothelial cell proliferation in vitro. We analyzed the contribution of placental particulate matter in HUVEC activation by inserting a porous membrane between the cell layer and the conditioned medium, which should hinder the transfer of these particles. The degree of sICAM-1 or IL-6 production by the HUVEC cultures was not different in the presence or in the absence of the membrane (Figure 2).

Villous explants produce many inflammatory cytokines, among which IL-1 and TNF-α are known activators of endothelial cells. In the culture conditions, we used placental explants (n = 6 explants) that produced the following quantities of cytokines: 306 ± 122 pg/mL TNF-α; 89 ± 31 pg/mL IL-1α, and 546 ± 217 pg/mL IL-1β. To investigate the role of TNF-α in HUVEC response, we used an anti–TNF-α–neutralizing antibody. The release of sICAM-1 (Figure 3) and IL-6 (data not shown) by HUVEC cultures on exposure to placental medium was attenuated only minimally in the presence of the neutralizing antibody; HUVEC activation by recombinant TNF-α was inhibited totally by the neutralizing antibody (Figure 3). Likewise, we investigated the role of IL-1α and IL-1β by interfering with their binding to the IL-1 receptor using the receptor antagonist IL-1ra. Interestingly, IL-1ra efficiently blocked HUVEC activation by the conditioned medium (Figure 4, A). This inhibitory effect was specific and dose dependent (Figure 4, B).

**Comment**

We show here that placental factors that are generated in vitro from term placental villous explants are not toxic to cultured HUVECs because the cells remain viable and retain normal mitochondrial metabolism. Significantly, placental factors reduce HUVEC cell proliferation and trigger a cellular inflammatory response. It is likely that the inhibition of HUVEC cell proliferation is a consequence of their activation. Indeed, it has been shown that the stimulation of HUVECs with the inflammatory cytokines TNF-α and interferon-γ synergistically induce cell cycle arrest. Studies are in progress to investigate whether HUVEC cell activation and the inhibition of proliferation are linked events.

The activation of HUVECs was evaluated by 2 parameters: the up-regulated expression of the adhesion molecules E-selectin and ICAM-1 (the latter was determined by a measure of its soluble form that was released by activated cells) and the production of IL-6.
These markers of endothelial cell activation were chosen because they have been reported to be present in increased levels in the circulation of women with preeclampsia, which is a state of overt inflammation.\textsuperscript{15-17} Their release into the blood stream is thought to reflect endothelial dysfunction in vivo. E-selectin and ICAM-1 are involved in leukocyte-endothelial cell interactions. IL-6 is a pleiotropic cytokine with immuno-modulatory and inflammatory properties. Interestingly, plasma from normal pregnant women stimulates IL-6 production by cultured HUVECs, albeit to a lesser degree than pre-eclamptic plasma.\textsuperscript{16}

TNF-\(\alpha\) and IL-1 are cytokines that are known to induce a functional program that is related to thrombosis and inflammation in endothelial cells.\textsuperscript{18} Both cytokines are expressed by trophoblasts and macrophages within term placenta, and their secretion by cultured villous explants has been documented.\textsuperscript{13,19,20} However, their role on maternal cell function remains elusive. The response of HUVECs to placental factors was only marginally reduced by anti–TNF-\(\alpha\) antibody. In contrast, it was abolished by an IL-1ra. This result is of considerable interest in regard to the finding that, in the development of in vitro models of placental injury, the production of both IL-1\(\alpha\) and IL-1\(\beta\) by placental villous explants was shown to be greatly dependent on environmental oxygen tension.\textsuperscript{13} Alterations in placental oxygen supplies are proposed to be at the source of placental injury in preeclampsia.\textsuperscript{4,21}

TNF-\(\alpha\) does not appear to play a major role under the conditions that we have used. This is in contrast to a recent study that investigated the effect of soluble factors that were generated by villous explants that were subjected to hypoxia/reoxygenation and identified TNF-\(\alpha\) as a mediator of endothelial activation.\textsuperscript{22} However, this study used other conditions to culture placental explants and also demonstrated that additional factors were involved in HUVEC activation, because the induction of E-selectin expression could be inhibited only partially with an anti–TNF-\(\alpha\)–neutralizing antibody. We suggest that members of the IL-1 family might be such factors.

Our results also show that, under the conditions that we have used, placentally derived particulate matter has no effect on the release of sICAM-1 and IL-6 by HUVEC cultures. This data, however, must be considered with caution, because recent findings from our laboratory indicate that isolated STBMs that are derived from cultured placental villi are able to attenuate HUVEC cell proliferation in a dose-dependent manner.\textsuperscript{23} Furthermore, we have preliminary results that suggest that high amounts of isolated STBMs may stimulate the production of IL-6 by HUVEC cultures (C. Rusterholz, unpublished data). Further work will be required to clarify the role of these microparticles on endothelial cell inflammation.

In conclusion, our data confirm the previous suggestion that inflammatory cytokines that are produced by the placenta might participate in the development of the maternal inflammatory response to pregnancy that is observed in vivo.\textsuperscript{20} In particular, our data suggest that placentally derived IL-1 might be 1 of these mediators by stimulating maternal endothelial cell activation. Activated endothelial cells might then interact with inflammatory immune cells and platelets to produce the hemostatic changes of late pregnancy. It is clear, however, that the maternal response to pregnancy is multifactorial. In particular, endothelial dysfunction in pregnancy-related diseases (such as preeclampsia) is likely to include angiogenic imbalance and oxidative stress.\textsuperscript{24,25} In this context, the role of IL-1 in preeclampsia remains to be assessed.

Acknowledgments

We thank Mrs A. Gülsolmaz and the medical staff of the Women Hospital of Basel for the help in collecting placentae; Mrs N. Chiodetti for excellent technical assistance; Ms S. Hristoskova for introducing us to the HUVEC culture system, and Dr A. Schoeberlein for her valuable help with statistical analysis.

References


Effects of vasoactive agents on intracellular calcium and force in myometrial and subcutaneous resistance arteries isolated from preeclamptic, pregnant, and nonpregnant woman

Ruwan C. Wimalasundera, MRCOG,a,b,* Simon A. McG. Thom, FRCP,a Lesley Regan, FRCOG,b Alun D. Hughes, PhDa

Clinical Pharmacology, NHLI Division,a and Obstetrics & Gynaecology,b Faculty of Medicine, Imperial College London, St Mary’s Hospital, London, United Kingdom

Received for publication May 8, 2004; revised July 12, 2004; accepted July 20, 2004

Objective: Preeclampsia is a common and serious complication of pregnancy, characterized by maternal hypertension and proteinuria, placental insufficiency, and fetal growth restriction. The purpose of this study was to investigate whether intracellular Ca$^{2+}$ ([Ca$^{2+}$]) and contractile responses of vascular smooth muscle to vasoactive agents are altered in preeclampsia compared with normal pregnancy and the nonpregnant state.

Study design: Subcutaneous and myometrial resistance arteries from women who had preeclampsia, normal pregnancy, and nonpregnant women were obtained at the time of cesarean section or hysterectomy. Arteries were mounted on an isometric myograph and loaded with the Ca$^{2+}$ indicator, fura-2AM, to permit simultaneous measurement of force and [Ca$^{2+}$]. Responses to endothelium-dependent relaxants (acetylcholine and substance P) and vasoconstrictors (depolarizing potassium solution, phenylephrine, and angiotensin II) were examined.

Results: The fall in [Ca$^{2+}$] and relaxation in response to acetylcholine was significantly inhibited in both myometrial and subcutaneous arteries from preeclamptic women compared with arteries from nonpregnant or normal pregnant women. However, responses to substance P did not differ between the 3 groups. There were no significant differences in [Ca$^{2+}$] or force responses to high potassium, phenylephrine, or angiotensin II in myometrial and subcutaneous resistance vessels in women with preeclampsia compared with normal pregnant women. However, force, but not [Ca$^{2+}$] responses to angiotensin II, in subcutaneous vessels from normal pregnant and preeclamptic women were reduced compared with subcutaneous arteries from nonpregnant women, indicating that pregnancy is associated with a reduction in Ca$^{2+}$ sensitization in this tissue. A similar effect was not seen in myometrial arteries.
Materials and methods

Materials

Physiological saline solution (PSS) comprised NaCl 118 mmol/L, KCl 4.69 mmol/L, MgSO4.7H2O 1.18 mmol/L, KH2PO4 1.18 mmol/L, NaHCO3 25 mmol/L, glucose 5.5 mmol/L, CaCl2.6H2O 2.5 mmol/L, Na2EDTA 0.03 mmol/L, and EDTA 0.026 mmol/L. Fura-2AM in dry DMSO (Molecular Probes, Eugene, Ore) was dissolved in PSS containing 0.002% (v/v) pluronic F-127, 0.1% (v/v) cremophor EL, and 1 mg/mL bovine serum albumin. Stock solutions of all other drugs were made up in distilled water and, unless otherwise stated, all drugs and chemicals were from Sigma (St Louis, Mo).

Preeclampsia is a major cause of maternal and perinatal mortality and morbidity that is characterized by a rise in maternal blood pressure and proteinuria in the second half of pregnancy; the rise in blood pressure is due to an increase in peripheral vascular resistance. Preeclampsia is a multisystem disorder resulting in generalized organ hypoperfusion, including placental insufficiency, activation of the coagulation cascade, and loss of fluid from the intravascular compartment. Some reports have suggested that endothelial dysfunction is an important component of the pathology underlying preeclampsia.1,2 Although endothelial dysfunction may contribute to preeclampsia, peripheral vascular resistance is determined by vascular smooth muscle tone. The contractile tone of vascular smooth muscle depends on the concentration of intracellular calcium ([Ca2+]i) and the sensitivity of the contractile apparatus to [Ca2+].3 Contractile stimuli increase [Ca2+]i by stimulating influx of Ca2+ through Ca2+ channels, and releasing Ca2+ from intracellular storage sites associated with the sarcoplasmic reticulum (SR).4 Vasoconstrictors can also increase the sensitivity of the contractile apparatus to changes in [Ca2+]i by inhibiting myosin phosphatase via rho kinase or other pathways.5 The regulation of [Ca2+]i, and sensitivity to [Ca2+]i, in preeclampsia is therefore likely to be important to understanding the increased peripheral vascular resistance seen in preeclampsia.

The aim of this study was to investigate the simultaneous changes in force and [Ca2+]i in isolated resistance vessels from women with preeclampsia, uncomplicated normal pregnancies, and nonpregnant women in response to vasoconstrictor agents and endothelium-dependent vasodilator agents. In order to compare changes in the maternal systemic and local uterine circulations, subcutaneous and myometrial resistance arteries were studied from the same patients.

Conclusion: Endothelial function is altered in preeclampsia, with loss of effect of acetylcholine, but not substance P. Vasoconstrictor reactivity is not increased in preeclampsia compared with uncomplicated normal pregnancy, and this is unlikely to be an explanation for the increased peripheral vascular resistance seen in preeclampsia.

Subjects

The study was carried out in accordance with the Helsinki Declaration of the World Medical Association (1989), approved by the ethics committee at St Mary’s Hospital, London, and all subjects gave informed consent. Three groups of primiparous women were recruited: women with preeclampsia (PET) who needed an emergency cesarean section (n = 11), women with uncomplicated singleton pregnancies (PC) undergoing elective cesarean sections (n = 14), nonpregnant women (NP) having elective hysterectomies (n = 9).

Preeclampsia was diagnosed on the basis of the criteria suggested by Higgins and de Swiet.5 Namely, blood pressure <140/90 mm Hg before 20 weeks of gestation, increasing to >90 mm Hg diastolic after 20 weeks of gestation on 2 occasions at least 4 hours apart, or to >100 mm Hg on 1 occasion, with associated proteinuria of >0.3 g/24 hours or 2+ proteinuria on dipstick testing developing de novo after 20 weeks of gestation.

Normal pregnant control subjects were women who had uncomplicated singleton pregnancies with normal blood pressure and no proteinuria throughout the pregnancy. Nonpregnant subjects were healthy, premenopausal women with normal blood pressures undergoing a hysterectomy for a nonmalignant indication. The groups were matched as far as possible for maternal age and gestation.

Women with a BMI of >32 or other chronic medical conditions were excluded from the study. Women treated with calcium antagonist drugs before delivery were excluded from the preeclamptic group. Women with multiple pregnancies, polyhydramnios, or an anterior placenta previa were also excluded because these conditions may confound myometrial sampling. Two women in the preeclamptic group were receiving α-methyldopa before delivery, and 2 of the nonpregnant group were receiving gonadotrophin-releasing hormone analogs, and 1 had a levonorgestrel intrauterine system before surgery. None of the normal pregnant women were on any medication.
At the time of cesarean section, once the baby had been safely delivered, a sample of the myometrium (approximately 2 cm \( \times \) 2 cm \( \times \) 1 cm) from the upper border of the lower segment uterine incision was excised and stored in cold PSS. An equivalent-sized sample of subcutaneous fat from the margin of the Pfannenstiel incision was also excised. Similarly, at the time of elective hysterectomy, subcutaneous fat samples and myometrial samples from the lower part of the uterus closest to the cervix were taken. After collection of the samples, resistance vessels (150-500 \( \mu \)m in diameter) were dissected from the surrounding tissue and used within 24 hours of collection.

Arteries were mounted in an isometric myograph containing PSS at 37°C and bubbled with 95% O\(_2\) and 5% CO\(_2\). Arteries were allowed to equilibrate for 1 hour, and then set at a ‘normalized’ internal circumference 0.9 \( \times \) L100 estimated to be 0.9 times the circumference they would maintain if relaxed and exposed to 100 mm Hg transmural pressure. This was calculated for each individual vessel on the basis of the passive length-tension characteristics of the artery and the Laplace relationship. All experiments were started by repetitively stimulating vessels for 2 minutes with a depolarizing potassium solution (KPSS) until reproducible contractions were elicited. KPSS comprised PSS with equimolar substitution of NaCl (118 mmol/L) by KCl.

Arteries were then loaded with the fluorescent indicator, fura-2AM (6 \( \mu \)mol/L) for 2 hours at 37°C. After loading, vessels were thoroughly washed to remove free fura-2AM, and allowed to equilibrate in PSS for 30 minutes before fluorescence measurements were made. Fluorescence was measured using a Deltascan spectrofluorimeter (Photon Technology International, Inc, South Brunswick, NJ) connected to an Axiovert 35 fluorescence microscope (Carl Zeiss, Oberkochen, Germany) using only quartz objectives. \([\text{Ca}^{2+}]_i\) was assessed on the basis of the ratio of fluorescence emission measured at 510 nm evoked by excitation at 340 and 380 nm after subtraction of background fluorescence (F340/380). Emission signals and force were measured simultaneously at 4 Hz, and acquired on-line using an A/D interface (Photon Technology International, Inc) connected to a PC. Data were stored and later analyzed off-line using commercially available software (Photon Technology International, Inc). In view of difficulties in calibrating fluorescence intensity to absolute \([\text{Ca}^{2+}]_i\) in the cytoplasm, changes in \([\text{Ca}^{2+}]_i\), are presented as F340/380 or normalized as % change in peak F340/380 signal induced by depolarization with KPSS, as previously described.

### Table I  Details of patient’s age, parity (P = primiparous, M = multiparous), gestation, blood pressure (BP) before 20 weeks, at 20 weeks, and preoperation birth weight of infants and normalized arterial diameter (L100)

<table>
<thead>
<tr>
<th></th>
<th>NP (n = 9)</th>
<th>PC (n = 14)</th>
<th>PET (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>43 (36–48)</td>
<td>34 (22–40)</td>
<td>32.5 (24–42)</td>
</tr>
<tr>
<td>Parity</td>
<td>P = 4, M = 5</td>
<td>P = 14</td>
<td>P = 11</td>
</tr>
<tr>
<td>Gestation (wk)</td>
<td>–</td>
<td>39 (38–39)</td>
<td>34.5 (24–39)*</td>
</tr>
<tr>
<td>BP (mm Hg) at booking</td>
<td>–</td>
<td>108 ( \pm ) 7/58 ( \pm ) 15</td>
<td>115 ( \pm ) 5/60 ( \pm ) 2</td>
</tr>
<tr>
<td>BP (mm Hg) at 20 weeks</td>
<td>–</td>
<td>99 ( \pm ) 5/60 ( \pm ) 4</td>
<td>114 ( \pm ) 5/68 ( \pm ) 2</td>
</tr>
<tr>
<td>BP (mm Hg) preop</td>
<td>121 ( \pm ) 1/70 ( \pm ) 5</td>
<td>109 ( \pm ) 8/68 ( \pm ) 7</td>
<td>148 ( \pm ) 6/101 ( \pm ) 4</td>
</tr>
</tbody>
</table>
| Birth weight (kg)    | –          | 3.5 (2.5–3.8) | 2.35(0.58–3.8)* 
| L100 subcutaneous (\( \mu \)m) | 330 \( \pm \) 22 | 290 \( \pm \) 23 | 381 \( \pm \) 29 |
| L100 myometrial (\( \mu \)m) | 422 \( \pm \) 19 | 380 \( \pm \) 68 | 436 \( \pm \) 30 |

Results are presented as the median (range) or mean \( \pm \) SEM of n observations. Preop indicates either before cesarean section or hysterectomy.
* \( P < .05 \) comparing PC with PET by an unpaired Student t test.
† \( P < .05 \) comparing PET with NP and PC by ANOVA and LSD test.

![Figure 1](image-url)  **Effect of acetylcholine (ACH, 10 \( \mu \)mol/L) and substance P (Subst P, 10 nmol/L) on intracellular \([\text{Ca}^{2+}]_i\) and force following precontraction with phenylephrine (10 \( \mu \)mol/L) in subcutaneous (Subcut) and myometrial arteries. Data are mean \( \pm \) SEM of 9 to 14 arteries.  **\( **P < .01 \) by ANOVA and LSD test.**
Study protocols

After loading with fura-2AM arteries were precontracted with a near maximal concentration of phenylephrine (PE, 10 μmol/L) and responses to the endothelium-dependent relaxants substance P (Subst P, 100 nmol/L) and acetylcholine (ACH, 10 μmol/L) examined. After washout, cumulative concentration response curves were constructed to angiotensin II (Ang II, 100 pmol/L to 100 nmol/L) and PE (1 nmol/L to 10 μmol/L).

Data analysis

Vasodilator responses were calculated as % reduction in [Ca^{2+}]_i or tone induced by PE (10 μmol/L), [Ca^{2+}]_i and force responses induced by vasoconstrictors were expressed as % maximum response to KPSS measured immediately before each cumulative concentration response. Concentration response data were fitted to a logistic function by nonlinear regression using GraphPad Prism 3.1 (GraphPad Software, Inc, Institute for Scientific Information, San Diego, Calif) and log EC_50 determined. The maximum responses to an agonist (Emax) were determined as the maximum measured responses, rather than the fitted maximum. All data are presented as medians (range) or mean ± SEM of n observations. There were no significant differences between groups by ANOVA.

Results

The characteristics of the study groups are shown in Table I. Unsurprisingly, the preeclamptic group had higher blood pressures, a lower gestation at delivery, and a lower birth weight. Women in the nonpregnant group tended to be older than pregnant women with or without preeclampsia.

Table II  Resting fluorescence ratio (F340/380), and responses to high potassium (KPSS) in subcutaneous and myometrial arteries from nonpregnant (NP), normal pregnant (PC), and preeclamptic (PET) women

<table>
<thead>
<tr>
<th></th>
<th>NP (n = 9)</th>
<th>PC (n = 14)</th>
<th>PET (n = 11)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subcutaneous arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting F340/380</td>
<td>0.56 ± 0.03</td>
<td>0.61 ± 0.03</td>
<td>0.52 ± 0.02</td>
</tr>
<tr>
<td>F340/380 response to KPSS</td>
<td>0.07 ± 0.007</td>
<td>0.05 ± 0.007</td>
<td>0.03 ± 0.004</td>
</tr>
<tr>
<td>Force response to KPSS (N/m)</td>
<td>1.6 ± 0.3</td>
<td>1.35 ± 0.5</td>
<td>2.2 ± 0.4</td>
</tr>
<tr>
<td>Myometrial arteries</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Resting F340/380</td>
<td>0.60 ± 0.04</td>
<td>0.60 ± 0.03</td>
<td>0.56 ± 0.04</td>
</tr>
<tr>
<td>F340/380 response to KPSS</td>
<td>0.054 ± 0.02</td>
<td>0.052 ± 0.01</td>
<td>0.046 ± 0.01</td>
</tr>
<tr>
<td>Force response to KPSS (N/m)</td>
<td>3.75 ± 0.7</td>
<td>2.9 ± 0.05</td>
<td>2.5 ± 0.05</td>
</tr>
</tbody>
</table>

The normalized vessel diameters were similar in all patient groups for both the subcutaneous and myometrial arteries (Table I), but given the relatively large variances, this observation does not exclude there being small differences in diameters between groups.

Effect of vasodilators

The fall in [Ca^{2+}]_i and relaxation in response to ACH (10 μmol/L) was reduced in both subcutaneous and myometrial vessels from preeclamptic women compared with the other 2 groups (Figure 1). There were no significant differences in response between the NP and PC groups. In contrast, there were no significant differences in response to Subst P (100 nmol/L) in subcutaneous or myometrial vessels between the 3 patient groups either in terms of the fall in [Ca^{2+}]_i or relaxation (Figure 1).

Effect of vasoconstrictors

Arteries in the isometric myograph had no active tone at rest, and there were no differences in basal [Ca^{2+}]_i, based on F340/380, [Ca^{2+}]_i responses to KPSS, or force responses to KPSS in the myometrial or subcutaneous arteries from the 3 patient groups (Table II). [Ca^{2+}]_i or force responses to PE also did not differ significantly for either subcutaneous or myometrial arteries when the 3 patient groups were compared. (Figure 2). Arteries from preeclamptic and normal pregnant women had a significantly lower Emax to Ang II than the vessels from nonpregnant women (P < .01 by ANOVA and LSD), and the log EC_50 for Ang II was greater in the vessels from nonpregnant women than those of preeclamptic or normal pregnant women (NP = −8.00 ± 0.16, PC = −7.7 ± 0.1, PET = −7.6 ± 0.12; P < .05 by ANOVA and LSD), but there was no significant difference in [Ca^{2+}]_i response induced by Ang II in subcutaneous vessels. Responses in arteries from normal pregnant women tended to be greater in the myometrial
vessels, but differences between groups were not statistically significant (Figure 3).

In order to better understand the basis of the difference in contractile response to Ang II in subcutaneous arteries changes in \([Ca^{2+}]_i\) were related to the change in force. The relationship between force and \([Ca^{2+}]_i\), appeared linear after activation with Ang II. In subcutaneous arteries the relationship in arteries from NP differed significantly from the other groups \((P < .05\) by ANCOVA), but there was no significant difference in sensitivity to \([Ca^{2+}]_i\) between PC and PET in

Figure 2  Force and intracellular calcium responses to phenylephrine (PE, 1 nmol/L to 10 μmol/L) in (A) subcutaneous and (B) myometrial arteries from nonpregnant (NP), normal pregnant (PC), and preeclamptic (PET) women. Data are mean ± SEM of 9 to 14 arteries.
subcutaneous arteries or between any group in myometrial vessels (Figure 4).

Comment

This study examined \([Ca^{2+}]_i\) and force responses to a range of vasoactive agents of myometrial and subcutaneous resistance arteries from nonpregnant women with women with normal pregnancy or preeclampsia. This is the first study to compare the vascular reactivity of different vascular beds taken from the same subject in preeclampsia. Previous studies have looked at vascular reactivity responses in preeclampsia in resistance vessels from a single tissue bed and extrapolated the findings to all systemic vessels.

Preeclampsia was associated with an impairment of the ability of ACH to cause a fall in \([Ca^{2+}]_i\), and relaxation in subcutaneous or myometrial arteries precontracted with phenylephrine. In contrast, \([Ca^{2+}]_i\) and force responses to Subst P, another endothelium-dependent vasodilator, were unaffected by preeclampsia. Changes in \([Ca^{2+}]_i\) in response to endothelium-dependent vasodilators have not been examined previously in the context of preeclampsia, but impaired endothelium-dependent relaxation to ACH was initially reported by McCarthy et al.

**Figure 3** Force and intracellular calcium concentration response curve for angiotensin II (Ang II, 100 pmol/L to 100 nmol/L) in (A) subcutaneous and (B) myometrial arteries from nonpregnant (NP), normal pregnant (PC), and preeclamptic (PET) women. Data are mean ± SEM of 9 to 14 arteries.
in subcutaneous resistance vessels from preeclamptic women. Because this impairment was present in our study in both myometrial and subcutaneous vessels, it is suggestive of a widespread depression of endothelium-dependent relaxation to ACH in preeclampsia. However, because endothelium-dependent relaxation to Subst P was unaffected, it seems that endothelial dysfunction may be limited to a specific agonist or agonists. Such a phenomenon may help to explain previously conflicting evidence regarding endothelium-dependent relaxation in preeclampsia. Knock et al. reported an impairment of endothelium-dependent relaxation to both ACH and bradykinin in subcutaneous resistance vessels from preeclamptic women. Pascoal et al. reported loss of relaxation to ACH, but not to bradykinin, in omental resistance arteries, while Suzuki et al. reported loss of relaxation to both bradykinin and Subst P in omental resistance arteries. In contrast, Kenny et al. reported that relaxation to bradykinin was similar in small myometrial arteries from normal pregnant, nonpregnant women, and women with preeclampsia, although the relative importance of NO and EDHF in bradykinin responses differed between groups. More recently, VanWijk et al. reported that endothelium-dependent relaxation to ACH was preserved in subcutaneous resistance vessels taken from preeclamptic women and studied in a perfusion myograph. It seems likely that, rather than being associated with a generalized impairment of endothelial function, preeclampsia has more complex effects on the endothelium. Whether the differences between agonists relate to the nature of the relaxant released by the endothelium in response to the agonist (eg, NO, EDHF, CNP, etc), or some other aspect of endothelial function remains to be established.

Responses to vasoconstrictors were not affected by preeclampsia. It was not possible to match gestational age between normal pregnancies and those with preeclampsia, but because no differences were seen between these groups, it seems unlikely that this had a major influence on our observations. In our study, we found no significant differences in the basal [Ca^{2+}] levels or resting tone between the 3 patient groups, and responses to a depolarizing potassium solution or phenylephrine did not differ significantly in either subcutaneous and myometrial arteries in nonpregnant, normal pregnant, or preeclamptic women. Another study recently reported similar findings in subcutaneous resistance vessels in a perfusion myograph, but did find increased sensitivity of contraction to changes [Ca^{2+}] in subcutaneous resistance vessels from preeclamptic women when arteries were exposed to changes in perfusion pressure. Discrepancies between our data and that of VanWijk et al. may be related to the recognized differences in vascular responses when similar vessels are compared between the isometric and isobaric myographs. Earlier studies reported that responses to norepinephrine, a nonselective α-adrenoceptor agonist, were unchanged in omental and subcutaneous resistance arteries in preeclampsia. But another study reported an increased response to norepinephrine in omental resistance vessels in preeclampsia compared with normal pregnancy, and increased responses to a depolarizing potassium solution have also been observed.

We did observe that subcutaneous arteries from nonpregnant women were significantly more responsive to Ang II than vessels from normal pregnant women or women with preeclampsia. This increase in response to Ang II was shown to be due to increased sensitivity of contraction to changes [Ca^{2+}] induced by Ang II in arteries from nonpregnant women. From the concentration response data it appears that responses to Ang II may not have achieved their full maximum, and it is possible that use of higher concentrations of agonist would have yielded additional useful information.

![Figure 4](https://example.com/figure4.png)
Ang II is known to play a critical role in the regulation of blood pressure and renal sodium handling. Ang II acting on AT1 receptors causes vasoconstriction, growth of blood vessels, and increased deposition of extracellular matrix.\(^\text{16}\) Ang II also stimulates or inhibits vascular smooth muscle cell apoptosis depending on the subtype of angiotensin receptor present,\(^\text{19}\) and acts as a pro-inflammatory agent.\(^\text{20}\) In the early 1960s Abdul-Karim,\(^\text{21}\) and later, Chesley et al\(^\text{22}\) suggested that the pressor response to an in vivo infusion of angiotensin II was suppressed in normal pregnancy compared with the nonpregnant state. This was later supported by in vitro studies on omental resistance arteries by Aalkjaer et al.\(^\text{16}\) These studies suggested that the suppression of response to Ang II was lost in vessels from preeclamptic women, whose responses were similar to the nonpregnant state. The authors therefore surmised that the increased response to Ang II might contribute to the increased peripheral resistance seen in preeclampsia.

Our data confirm that responses to Ang II in subcutaneous vessels are suppressed both in terms of \([\text{Ca}^{2+}]\), and force in normal pregnancy compared with the nonpregnant state. However, in contrast to previous observations in omental arteries, there is a similar suppression of response to Ang II in subcutaneous arteries from preeclamptic women compared with vessels from nonpregnant women. Moreover, there was no evidence that responses to Ang II in myometrial vessels were reduced in pregnant women; indeed, if anything, responses to Ang II were increased in the myometrial arteries from normal pregnant women. These observations extend earlier studies by Allen et al, who reported no qualitative difference in response to Ang II in myometrial vessels from preeclamptic, normal pregnant, and nonpregnant women.\(^\text{23}\) Our data indicate, therefore, that resistance arteries from different vascular beds show different reactivity to vasoactive substances, and may respond differently to pregnancy, and this needs to be accounted for when interpreting changes in vascular reactivity in preeclampsia.

In summary, endothelial function is altered in preeclampsia with loss of effect of acetylcholine, but not substance P. Vasoconstrictor reactivity is unaffected by preeclampsia compared with uncomplicated normal pregnancy, and this is unlikely to be an explanation for the increased peripheral vascular resistance seen in this condition.

**References**

Temporal changes in drug metabolism (CYP1A2, CYP2D6 and CYP3A Activity) during pregnancy

Timothy S. Tracy, PhD,a,* Raman Venkataramanan, PhD,b Douglas D. Glover, MD,c Steve N. Caritis, MD,d for the National Institute for Child Health and Human Development Network of Maternal-Fetal-Medicine Units

Department of Experimental and Clinical Pharmacology and Center of Excellence in Women's Health, University of Minnesota, Minneapolis, Minn,a Departments of Pharmaceutical Sciences and Pathology, University of Pittsburgh, Pittsburgh, Pa,b Department of Obstetrics and Gynecology, West Virginia University, Morgantown, WV,c Department of Obstetrics, Gynecology and Reproductive Sciences, Magee Women's Hospital, Pittsburgh, Pa,d

Received for publication April 2, 2004; revised July 23, 2004; accepted August 23, 2004

KEY WORDS
Pregnancy
Drug metabolism
Cytochrome P450

Objective: The purpose of this study was to determine whether drug metabolism (CYP1A2, CYP2D6 and CYP3A) activity varies in the pregnant state compared with the nonpregnant state.

Study design: Subjects were studied at 14 to 18 weeks of gestation, 24 to 28 weeks of gestation, and 36 to 40 weeks of gestation and again at 6 to 8 weeks after the delivery. Twenty-five subjects completed all 4 study periods and had evaluable data. Salivary caffeine clearance was used as a measure of CYP1A2 activity; dextromethorphan O- and N-demethylation were used to assess CYP2D6 and CYP3A activity, respectively.

Results: CYP1A2 activity was significantly reduced at all periods of the pregnancy as compared with the postpartum period during the first (−32.8% ± 22.8%), second (−48.1% ± 27%), and third periods (−65.2% ± 15.3%), respectively. In contrast, CYP2D6 activity was increased significantly throughout the pregnancy (25.6% ± 58.3% at 14-18 weeks of gestation, 34.8% ± 41.4% at 24-28 weeks of gestation, and 47.8% ± 24.7% at 36-40 weeks of gestation) as compared with the postpartum period. CYP3A activity was consistently, significantly increased (35%-38%) during all stages of the pregnancy.

Conclusion: Opposing changes in drug metabolism occur during pregnancy, with CYP1A2 activity decreased and CYP2D6 and CYP3A activities increased. The direction of dosing adjustments during pregnancy will depend on the drug and the enzyme that is responsible for its metabolism.

© 2005 Elsevier Inc. All rights reserved.

Pregnancy is a dynamic state during which a number of physiologic and biochemical changes occur. Furthermore, the degree to which these changes occur may be affected by the stage of pregnancy. It is well established that changes in plasma volume, glomerular filtration rate, and body water occur during pregnancy and tend
to deviate more from the nonpregnant state as the pregnancy progresses. Although the magnitude of changes in these commonly monitored biochemical and physiologic parameters has been well established, much less is known about changes during pregnancy that may affect the pharmacokinetics and pharmacodynamics of drug therapy.

Despite efforts to minimize the consumption of drugs (both prescription and over-the-counter) and xenobiotics during pregnancy, it is commonly necessary to prescribe medications to assure the continued health of both mother and fetus. Substantial concern is given to choosing drugs that will minimize the risk/benefit ratio for the maternofoetal unit. Apart from toxicologic issues, one must also be cognizant of the optimal dose of drug to be administered to the pregnant woman, also to optimize therapy while minimizing the risk of toxicity. However, limited information exists as to the pharmacokinetics or physiologic parameters has been well established, changes in these commonly monitored biochemical determinations more difficult. The cytochrome P450 (CYP) system is the predominant oxidative enzyme system that is involved in human drug metabolism and plays a substantial role in the biotransformation of many drugs that might be administered during pregnancy. Phenotypic trait measures that are based on several in vivo probes for various CYP-mediated metabolisms have been developed, but some present particular problems in pregnant women because of concerns of fetal toxicity. Caffeine metabolism has been validated as an in vivo probe for CYP1A2 activity and does not present substantial risks when administered at typical doses to pregnant women. Likewise, the formation of dextromethorphan from the antitussive dextromethorphan has been commonly used as a marker of CYP2D6 and CYP3A activity, and the administration of this agent is also with minimal risk to the pregnant woman and fetus. CYP3A activity refers to the collective activity of a subfamily of enzymes, CYP3A4, CYP3A5, and CYP3A7.

Because limited information is available as to changes in drug metabolizing enzyme activity throughout the course of pregnancy, the objective of the current study was to assess changes in CYP1A2, CYP2D6, and CYP3A activity longitudinally during the first through the third trimesters of pregnancy and at 6 to 8 weeks after delivery. This was accomplished with caffeine metabolism as a marker of CYP1A2 metabolism, dextromethorphan O-demethylation as a marker of CYP2D6 activity, and dextromethorphan N-demethylation as a marker of CYP3A activity in pregnant subjects.

Material and methods

Subjects

Thirty-five pregnant women were recruited from the clinics at Magee Women’s Hospital and West Virginia University. Pregnant women between the ages of 18 and 35 years were recruited before the 14th week of pregnancy. Informed consent was obtained from all the subjects, and the study protocol was approved by the Institutional Review Boards of Magee Women’s Hospital and West Virginia University.

The subjects who received drugs that were known to induce or inhibit hepatic drug metabolism were excluded from the study, along with smokers. Smoking status and alcohol consumption was assessed by interview.

Study design

Subjects were studied 4 times: at 14 to 18 weeks of gestation, at 24 to 28 weeks of gestation, at 36 to 40 weeks gestation, and at 6 to 8 weeks after delivery. These time periods were chosen to coincide with normal prenatal visits when blood may be drawn for multiple marker screening or glucose challenge testing. Subjects were asked to fast overnight and to abstain from caffeine-containing products for 48 hours before each study period. Subjects were admitted to the General Clinical Research Center of the University of Pittsburgh (n = 30/35 total studied) on the morning of the study. Subjects who were recruited from the West Virginia University clinics (5 of the 35 subjects included in the final analysis) completed the entire study on an outpatient basis following the same protocol, with the exception of not receiving the acetaminophen probe substrate. On initiation of the study, subjects were instructed to void their bladders and to collect a urine sample and a saliva sample (approximately 5-10 mL) for predose analysis (ie, time zero). Collection of saliva was facilitated by chewing on paraffin film. Subjects received 100 mg of caffeine (Vivarin tablets), 15 mg of dextromethorphan (Benylin cough syrup), and 500 mg of acetaminophen (Tylenol Extra Strength) orally along with 250 mL of water. Data from the administration of acetaminophen (n = 20) will be provided in another report. An analysis of these data revealed no differences in the activities of each CYP for those subjects who received acetaminophen and those subject who did not. Additional saliva samples were collected at 1, 4, 8, 12, and 24 hours after drug administration. Although the 1- and 24-hour samples were collected at the General Clinical Research Center (except as noted for the 5 subjects from the West Virginia Institute of Rehabilitation and Research, Dallas, Texas), the samples were collected at the General Clinical Research Center of the University of Pittsburgh (n = 30/35 total studied) on the morning of the study. Subjects who were recruited from the West Virginia University clinics completed the entire study on an outpatient basis following the same protocol, with the exception of not receiving the acetaminophen probe substrate. On initiation of the study, subjects were instructed to void their bladders and to collect a urine sample and a saliva sample (approximately 5-10 mL) for predose analysis (ie, time zero). Collection of saliva was facilitated by chewing on paraffin film. Subjects received 100 mg of caffeine (Vivarin tablets), 15 mg of dextromethorphan (Benylin cough syrup), and 500 mg of acetaminophen (Tylenol Extra Strength) orally along with 250 mL of water. Data from the administration of acetaminophen (n = 20) will be provided in another report. An analysis of these data revealed no differences in the activities of each CYP for those subjects who received acetaminophen and those subject who did not. Additional saliva samples were collected at 1, 4, 8, 12, and 24 hours after drug administration. Although the 1- and 24-hour samples were collected at the General Clinical Research Center (except as noted for the 5 subjects from the West Virginia University clinics).
clinics), the 4-, 8-, and 12-hour samples were collected at the patient’s home. Subjects were asked to fill in the time of saliva collection in a record. A complete 24-hour urine sample from the time of drug administration was also collected in all subjects, and the samples were kept refrigerated. Subjects were asked to drop off the saliva and urine containers the next day. In addition, during each of the 4 study periods, a single blood sample (5 mL) was collected to evaluate liver function tests (aspartate aminotransferase, alanine aminotransferase, total bilirubin), kidney function (24-hour serum creatinine), serum estrogen, and serum progesterone.

Drug/metabolite assays

Saliva caffeine concentrations were measured by high-performance liquid chromatography, according to the methods of Frye et al.8 with the slight modification that a high-performance liquid chromatography column (PE-Brownlee C18 5 μm, 4.6 × 100 mm; Perkin-Elmer, Torrance, Calif) was used. Urinary concentrations of dextromethorphan and dextrorphan were determined by high-performance liquid chromatography with fluorescent detection. Sample preparation and extraction was carried out according to the methods of Ducharme et al9; and chromatographic separation was carried out according to the methods of Straka et al.10

Data analysis

The apparent oral clearance of caffeine was calculated as \( \text{Dose}/\text{AUC}_{0-\infty} \), where AUC is the area under the salivary caffeine concentration versus time profile from time zero to infinity, as determined by noncompartmental methods (WinNonlin; Pharsight Corp, Mountain View, Calif). This apparent oral clearance of caffeine was used as the phenotypic trait measure for CYP1A2 activity.6 The dextromethorphan/dextrorphan urinary ratio that was identified in the 24-hour urine sample was used as a measure of CYP2D6 activity, and the dextromethorphan/3-hydroxyymorphinan ratio in this same sample was used as a measure of CYP3A activity.7 Statistical analysis was carried out with Kaleidagraph software (Synergy Software, Reading, Pa). The data were found not to be normally distributed; therefore, a log transformation was performed before statistical analysis. Repeated measures analysis of variance with multiple comparisons was performed to test for statistical significance for a comparison of the gestational periods with the postpartum period. The Wilcoxon signed rank test was used to test whether the median percent change in activity that was determined in all subjects at each gestational period was statistically different from zero (no change). To control for these multiple comparisons, statistical significance was set at a probability value of <.016 (0.05/3 comparisons).

Results

Thirty-five subjects were entered into the study protocol. Two subjects were determined to be CYP2D6 poor metabolizers (dextromethorphan/dextrorphan urine ratio, > 0.3) and were not included in the analysis. Twenty-eight extensive metabolizer subjects completed all 4 study periods and thus had longitudinally evaluable data. Of these, 3 subjects had incomplete urine collections during at least 1 of the 4 periods that resulted in CYP2D6 and CYP3A activity ratios > 5 standard deviations away from the mean and were considered outlier values and were excluded from the final analysis. A total of 25 extensive metabolizer subjects were included in the final data analysis. No adverse events that were attributable to drug administration were noted in any of the subjects.

The apparent oral clearance of caffeine decreased throughout the course of pregnancy, as compared with the nonpregnant state (6 weeks after delivery; Figure 1, A). A statistically significant reduction in the apparent oral caffeine clearance was noted at all 3 study periods of the pregnancy as compared with the postpartum period. To better assess the magnitude of changes within individual subjects, percent differences from the postpartum value were calculated for each gestational period; these results are presented in Figure 1, B. Again, the percent change from baseline (after delivery) was statistically different from baseline at each study period. Interestingly, no differences were noted in the volume of distribution of caffeine among the various gestational periods, which suggests that volume changes did not play a role (data not shown). These findings suggest that CYP1A2 activity is decreased throughout the course of pregnancy, with the greatest decrease occurring during the third trimester.

The dextromethorphan/dextrorphan urinary ratio was used as a measure of CYP2D6 activity and was reduced significantly at all periods of the pregnancy, as compared with the postpartum state (Figure 2, A). Because a reduction in the dextromethorphan/dextrorphan urinary ratio (ie, less parent drug and more metabolite recovered in the urine) is reflective of an increase in CYP2D6 activity, it appears that the activity of this isoform is increased during pregnancy and that CYP2D6 activity increases temporally as the pregnancy progresses. Percent changes in CYP2D6 activity at each period of the pregnancy as compared with the nonpregnant state were calculated for each individual and the mean (± SD) changes are given in Figure 2, B. Again, there was a statistically significant increase in the percent change of CYP2D6 activity over baseline values at all 3 study periods. Clearly, not only CYP2D6 activity increases with increasing gestational age, but also the variability in the percent change among subjects decreases.
Finally, CYP3A activity was monitored as the change in the dextromethorphan/3-hydroxymorphinan ratio in urine (decreased ratio indicates increased metabolic activity). A statistically significant reduction in the mean dextromethorphan/3-hydroxymorphinan ratio across all subjects was noted at each period (Figure 3, A), with the reduction being relatively constant across the 3 periods.

Again, analysis of individual percent changes within subjects demonstrated that CYP3A activity was increased approximately 35% to 38%, regardless of the period assessed (Figure 3, B) and these differences were statistically significant.

Data were analyzed as to whether a relationship exists between hormone levels (serum estrogen and serum...
progesterone) and either CYP1A2 or CYP3A activity. No correlation was noted with estrogen or progesterone serum levels and either of the CYP activities.

Comment

Pregnancy is a dynamic state in which many physiologic and metabolic functions are altered. Unfortunately, there is a paucity of data regarding changes in drug metabolism throughout the course of pregnancy and appropriate dosing strategies based on observed changes. This study used 2 marker substrates (caffeine and dextromethorphan) for common drug metabolizing enzymes to assess changes in enzymatic activity during the early second (14-18 weeks of gestation), late second/early third (24-28 weeks of gestation), and third trimesters (36-40 weeks of gestation), as compared with the 6- to 8-week postpartum state. CYP1A2 activity (caffeine clearance) was reduced significantly during each of the 3 trimesters of pregnancy. Conversely, CYP2D6 activity (dextromethorphan O-demethylation) and CYP3A activity (dextromethorphan N-demethylation) were increased significantly during all periods of the pregnant state as compared with the postpartum period. Thus, it appears that one cannot predict universally that the metabolism of all drugs will be increased or decreased during pregnancy. Furthermore, to make rational dosing adjustment predictions, one must consider the individual enzymes that are responsible for a drug’s metabolism because the clearance of some drugs will be decreased; the clearance of other drugs may be increased during the course of the pregnancy, and the degree of change may not remain constant throughout the pregnancy.

CYP1A2 has been shown to be important in the metabolism of several drugs that are administered during pregnancy for co-existing conditions that include the agents fluvoxamine, imipramine, olanzapine, and zileuton. Although limited work has been performed on pregnancy-related changes in the metabolism of drugs that are metabolized by CYP1A2, there have been studies that assessed the metabolism of caffeine in pregnant women. Brazier et al.11 studied the pharmacokinetics of caffeine during late pregnancy and again 4 days after delivery. Most subjects were in the 37th to 38th week of gestation when studied in the pregnant state. These authors found a 65% reduction in caffeine clearance in women during late pregnancy, and again 4 days after delivery. Most subjects were in the 37th to 38th week of gestation when studied in the pregnant state. These authors found a 65% reduction in caffeine clearance in women during late pregnancy, as compared with the immediate postpartum state. Using the AAMU + 1X + 1U/17U (5-acetylamino-6-amino-3-methyluracil + 1-methylxanthine + 1-methyluric acid/1,7-dimethyluric acid) ratio as a measure of CYP1A2 activity, Tsutsumi et al.12 assessed changes in CYP1A2 activity during early, middle, and late pregnancy. These authors found reductions in the mean metabolic ratios of CYP1A2 activity of 35%, 50%, and 52% for each of the 3 periods, respectively, as compared with the postpartum state (approximately 4 weeks after delivery).

Findings from both the aforementioned studies agree well with the results of the present study in which the mean reductions in caffeine clearance of 40%, 37%, and 68% for the first, second, and third trimesters,
respectively, as compared with the postpartum period. Interestingly, when the percent changes within individuals were computed, a more graded response was noted, in that the percent decrease compared with the postpartum period for the first trimester was 33%, for the second trimester was 48%, and for the third trimester was 65%. This suggests that mean phenotypic trait measures may sometimes mask longitudinal changes in individual subjects.

CYP2D6 metabolizes several drugs that are used clinically in the care of obstetric patients, which include the antidepressants amitriptyline, paroxetine, and fluoxetine and the narcotic analgesic codeine. The metabolism of citalopram (CYP2C19, CYP2D6, and CYP3A4 substrate) has been reported to be increased during pregnancy. In particular, the further N-demethylation of the desmethylcitalopram metabolite (thought to be primarily CYP2D6 mediated) was increased 23% to 54%, depending on the stage of pregnancy. These same authors also evaluated the N-demethylation of fluoxetine to norfluoxetine in late pregnancy compared with the postpartum period and found the ratio of maternal norfluoxetine/fluoxetine plasma concentrations to be 2.4-fold higher during late pregnancy as compared with the postpartum state. These findings of increases in CYP2D6 activity during late pregnancy are similar to the findings of Hogstedt et al, who also found the clearance of the CYP2D6 substrate metoprolol to be substantially increased. Using the CYP2D6 probe, dextromethorphan, Wadelius et al reported the induction of CYP2D6 (as measured by the dextromethorphan/dextrorphan ratio) in late pregnancy; however, they did not study early pregnancy. The current results extend these studies and demonstrate that the “induction” of CYP2D6 during pregnancy occurs in a temporal fashion. Although CYP2D6-mediated metabolism is increased as early as the late first/early second trimester, it continues to increase throughout the course of pregnancy. The largest percent increase in CYP2D6 activity occurred in the third trimester (approximately 50%). The observed percent increase from approximately 25% during the first trimester to approximately 50% at the third trimester is remarkably congruent with the findings of the N-demethylation of desmethylcitalopram during pregnancy, as stated earlier.

The increase of CYP2D6 activity throughout the course of pregnancy is particularly interesting, considering the generally held notion that CYP2D6 is not inducible, at least by xenobiotics. The most obvious potential inducing substance would be the elevated endogenous hormones (e.g., estrogen and progesterone) that occur during pregnancy. However, there are no data to suggest that these hormones can induce CYP2D6 activity, which suggests that other endogenous compounds may be responsible for the observed induction in CYP2D6.

CYP3A activity was also increased throughout pregnancy, but to a more modest degree than was observed for CYP2D6. Also, in contrast with CYP2D6, the increase in CYP3A activity was constant at all trimesters of pregnancy (approximately 30%). These findings are of clinical importance in obstetric patients because CYP3A metabolizes several compounds that are used clinically in the care of pregnant women (e.g., the antiviral compounds like ritonavir, calcium channel blockers such as nifedipine, antibiotics such as erythromycin and azithromycin and the narcotic methadone). Barton et al reported that the clearance of the CYP3A substrate nifedipine was higher during the immediate postpartum period as compared with the clearance that was observed previously in nonpregnant women. Studying the pharmacokinetics of indinavir during the second and third trimesters, Kosel et al reported that the area under the concentration-time curve of indinavir was decreased in the second and third trimesters as compared with the postpartum period. Furthermore, in this same study, it was reported that the urinary 6β-hydroxycortisol to cortisol ratio, a surrogate marker of CYP3A activity, was increased during pregnancy, which suggests an induction of CYP3A activity. Wadelius et al, who used the dextromethorphan/3-hydroxymorphinan ratio, found modest changes in CYP3A activity when it was assessed in late pregnancy compared with the postpartum state. Again, it is unclear what might mediate this increase in CYP3A activity during pregnancy. Although in vitro work has suggested that progesterone may activate (non-induction) CYP3A activity, clinical studies of the effect of menstrual cycle phase or menopausal state have found no effect of these hormonal changes on CYP3A activity. However, fetal CYP3A activity has been noted and may play a role in this increased activity.

In summary, it appears that CYP1A2 activity is decreased throughout pregnancy with progressive decreases that correlate with increasing gestation. In contrast, CYP2D6 and CYP3A activities are increased during pregnancy, although to different degrees and in differing temporal fashions, with the CYP3A effect being relatively constant throughout the pregnancy. Because of these differences, it appears that all 3 isoforms that were studied (CYP1A2, CYP2D6, and CYP3A) may be regulated differentially during pregnancy. Thus, it appears that modifications in dosing of drugs that are metabolized by these enzymes potentially should be modified in pregnant subjects and that alterations in dose may be needed as the pregnancy progresses.

Acknowledgments

We thank Ms Blanche Rybeck for her expert technical assistance, Mr Dan Besspiata for his analysis of the
urine and saliva samples, and Dr Richard Brundage for statistical assistance.

References

Abortion education in medical schools: A national survey

Eve Espey, MD, MPH,* Tony Ogburn, MD, Alice Chavez, MD, Clifford Qualls, PhD, Mario Leyba

Department of Obstetrics and Gynecology, University of New Mexico, Albuquerque, NM

**Objective:** This survey was performed to examine the inclusion and extent of abortion education in US medical schools.

**Study design:** A 3-item confidential survey requesting information about abortion education throughout the 4 years of medical school was mailed to the OB-GYN clerkship directors of the 126 accredited US medical schools.

**Results:** Seventy-eight surveys were returned, for a response rate of 62%. Overall, 17% of clerkship directors reported no formal education about abortion either in the preclinical or clinical years. In the third-year OB-GYN rotation, 23% reported no formal education, whereas 32% offered a lecture specifically about abortion. While 45% of third-year rotations offered a clinical experience, participation was generally low. About half of schools offered a fourth-year reproductive health elective, but few students participated.

**Conclusion:** Abortion education is limited in US medical schools. As an integral part of women’s reproductive health services, abortion education deserves a place in the curricula of all medical schools.

—

Abortion is one of the most common procedures women undergo in the US. It is estimated that 43% of women have had an abortion by age 45.1 Abortion care education is therefore generally accepted as an integral part of OB-GYN health curricula. The Council on Residency Education in Obstetrics and Gynecology (CREOG) includes objectives for residency training in abortion and its complications in the Core Curriculum in Obstetrics and Gynecology.2 The OB-GYN Residency Review Committee requires that training and education about abortion be offered to all OB-GYN residents.3 The Association of Professors of Gynecology and Obstetrics (APGO) has included medical student objectives about abortion in their Core women’s health curriculum since the 7th edition in 1997; these objectives, while not requirements, serve as guidelines to assist clerkship directors in developing curricula.4 Although information has been published on medical students’ attitudes toward abortion5-7 and individual schools’ programs for teaching reproductive health,8,9 the extent to which abortion education occurs in US medical schools remains unclear. The purpose of this study was to determine the inclusion and extent of abortion education in US medical schools.

**Material and methods**

We developed a 3-item confidential survey to determine the presence of abortion education and its extent in the preclinical and clinical years of medical school. The first item related to education in preclinical years, the second item to education in the third-year clerkship, and the third item to fourth-year reproductive health electives.
For the preclinical and clerkship year(s), the survey item read: “Do students at your medical school have any formal abortion education?” Respondents were given choices to mark, including 1) Lectures with abortion as a primary focus, 2) Lectures on another topic in which abortion is mentioned, 3) Small group sessions/tutorials, 4) Clinical experience observing or participating in surgical/medical abortion services, and 5) Don’t know. If respondents indicated that a clinical experience was offered, they were asked if the experience was formally integrated into the curriculum (eg, a half-day experience set up at a Planned Parenthood abortion site), or if students had to arrange involvement in the experience themselves. Additionally, respondents were asked what percentage of students participated in the experience. Respondents could also choose an “other” category and write comments. For the fourth-year electives, the survey item read, “Is a fourth-year elective offered that provides a clinical abortion education experience?” Respondents for those institutions that offered such a fourth-year elective were asked to estimate the percentage of students per year who participate. Demographic information was not collected because of the sensitive nature of the survey.

Clerkship directors for the OB-GYN rotations of the 126 US medical schools were identified by calling the offices of OB-GYN department chairs. Surveys were sent by e-mail to each of the clerkship directors in February of 2003. Surveys were returned by fax, and the institution was identified on the first page of the fax. Each survey was then coded with a number corresponding to a list of the individual institutions, and maintained in a locked cabinet. One of the authors entered the data (AC) into an Epi Info program and was unaware of the identity of the institution when entering the data. Nonrespondents were identified and contacted a second time with an e-mail reminder and, if no response, with a single telephone call. All contacts were made by May of 2004. Responses were analyzed in the aggregate without personal identifiers. The study received approval through the University of New Mexico Human Research Review Committee.

### Table I

Components of abortion education in the preclinical years and in the third-year OB-GYN clerkship*

<table>
<thead>
<tr>
<th>Education components</th>
<th>Preclinical years</th>
<th>Third-year OB-GYN clerkship</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lecture on abortion</td>
<td>15 (19%)</td>
<td>25 (32%)</td>
</tr>
<tr>
<td>Lecture, abortion mentioned</td>
<td>17 (22%)</td>
<td>29 (37%)</td>
</tr>
<tr>
<td>Small group</td>
<td>4 (5%)</td>
<td>9 (12%)</td>
</tr>
<tr>
<td>Clinical experience</td>
<td>5 (6%)</td>
<td>35 (45%)</td>
</tr>
<tr>
<td>No formal education</td>
<td>34 (44%)</td>
<td>18 (23%)</td>
</tr>
<tr>
<td>Don’t know</td>
<td>18 (23%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Numbers in columns do not sum to 100% because some programs included more than one education component.

### Table II

Effect of curricular integration on student participation in the third-year clinical abortion experience (n = 27*)

<table>
<thead>
<tr>
<th>Level of student participation</th>
<th>Experience integrated into curriculum</th>
<th>Experience not integrated into curriculum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Most</td>
<td>8 (30%)</td>
<td>0</td>
</tr>
<tr>
<td>Half</td>
<td>3 (11%)</td>
<td>0</td>
</tr>
<tr>
<td>Few</td>
<td>10 (37%)</td>
<td>2 (7%)</td>
</tr>
<tr>
<td>None</td>
<td>0</td>
<td>4 (15%)</td>
</tr>
</tbody>
</table>

Fisher exact test, P < .001.

* Missing values (n = 8) were excluded.

### Results

We received 78 completed surveys, for a response rate of 62%. Two clerkship directors indicated that they were unwilling to complete the survey, but did not explain why. Comparisons between education in the preclinical and third-year OB-GYN clerkship demonstrate more abortion education in the latter (Table I).

Eighteen (23%) clerkship directors reported they did not know if any education about abortion was included in the preclinical years. Thirty-four (44%) indicated that no formal education occurred. Only 19% reported a lecture specifically about abortion, and only 11% had a small group discussion of abortion and/or a clinical experience in abortion care.

Predictably, all clerkship directors were aware of the third-year curriculum (Table I). Twenty-five percent reported no formal education about abortion in the OB-GYN clerkship. Less than half (45%) of clerkships offered a clinical experience in abortion care for students rotating in the third year.

Of the 35 medical schools that offered a clinical experience, 74% of the experiences were at least partially integrated into the curriculum. We defined “integrated” as an experience that students were alerted to in advance, most often at the clerkship orientation, either verbally or in writing. “Non-integrated” experiences were those where students who expressed an interest to the clerkship director could take the initiative to arrange their own experience. Participation in a clinical abortion care experience was variable (Table II). In programs where the clinical experience was integrated into the curriculum, more students were likely to participate (P < .001).

Medical schools that offered abortion education in the preclinical years were more likely to offer education in the third-year clerkship (P = .002). Overall, 17% of medical schools reported no formal abortion education in either the preclinical or the third-year clerkship.

Thirty-one (52%) of all medical schools that responded offered a reproductive health elective in the fourth year; however, 92% of programs reported that 10% or fewer students in the class participated in these electives.
Comment

We found that abortion education is limited in US medical schools. The topic is not covered at all in 17% of schools, and coverage may be minimal at other schools. Although we counted “Lecture on another topic in which abortion is mentioned” as education about abortion, the true value of such lectures may be limited. Only 19% of clerkship directors reported a lecture specifically about abortion in the preclinical years. Additionally, we found it concerning that almost a quarter of clerkship directors reported that they did not know whether any abortion education took place in the preclinical years.

Thirty two percent of OB-GYN clerkships include a lecture specifically about abortion. While lectures are useful and commonly used, a clinical experience may be preferable. Emmons et al9 examined learning preferences among a heterogeneous group of trainees including medical students, residents, and advanced practice clinicians. All of these learners preferred a clinical activity to either a small group conference or a large group activity, such as a lecture. Similarly, those who had more clinical exposure were more likely to feel competent practicing a wider range of clinical skills.

We found that 35 (44%) had a clinical experience available for students. Those clerkships with an experience formally integrated into the clerkship had more participants than those that left it up to the student to request that an experience in abortion care be arranged. Also, our impression, based on comments provided by a number of respondents, was that clerkships in which a “champion” actively promoted the clinical experience had more student participants than those that did not. The champion was typically a faculty member who provides abortion services and was responsible for abortion education in the clerkship curriculum. We speculate that students respond favorably to the concept of education about abortion when abortion is presented as an integral, mainstream part of women’s health services.

When a clinical experience was available and was integrated into the curriculum, the format was most often a half-day experience or a 1-week elective. At the University of New Mexico, we offer both a half-day experience at Planned Parenthood (70% of students participate) and a 1-week reproductive health experience to replace a week of gynecology (20% of students participate). From the standpoint of scheduling, a half-day experience may allow more students to participate. Some clerkship directors at programs lacking a clinical experience in abortion care felt that, were such an experience offered, participation would be minimal. As demonstrated in Table II, we found that many students elected to participate when the abortion care experience was available and integrated into the structure of the clerkship.

It is encouraging that half the schools surveyed offered a fourth-year reproductive health elective. While these electives are valuable in reaching the small number of students who are particularly motivated to gain expertise in reproductive health, they do not fulfill the need for the kind of general education that reaches all students in the preclinical years or clinical clerkships.

Several schools mentioned that the topic of abortion is only covered in an ethics course. Although the ethical dimensions of abortion are important, other aspects of abortion—its public health significance, pre- and post-procedure care—are equally if not more important. Focusing solely on the ethical dimension of abortion leaves important aspects uncovered. As we move toward evidence-based medicine and strive to include population health issues in student education, these perspectives should be emphasized.

Our study has several limitations. We chose to survey directors of OB-GYN clerkships. Although some abortion education may be given in other rotations, such as Family Medicine, we chose OB-GYN clerkship directors nevertheless because 1) these individuals were likely to be responsible for core objectives in women’s health, and 2) OB-GYN residencies are required to offer abortion training to their residents. Similarly, because we did not survey curriculum leaders of the preclinical years, our data concerning abortion education during these years may contain inaccuracies. The lack of knowledge of clerkship directors about the preclinical curriculum is, however, in itself, disturbing. The accrediting body for medical schools, the Liaison Committee on Medical Education (LCME) has as a standard that medical school curricula be “coherent and coordinated.”10 In order to achieve an integrated 4-year medical school curriculum, a knowledge of the content of the preclinical and clinical curriculum is critical. In the area of women’s health, communication between those who direct preclinical courses and the OB-GYN clerkship directors could help ensure comprehensive coverage of important issues and avoid unplanned repetitions. A gap in knowledge that exists for abortion education likely has its counterpart in other important components of the curriculum.

Another limitation of the study is that 38% of clerkship directors did not respond to the survey. If the nonrespondents’ institutions had different educational experiences from those who completed the survey, our results may be biased. Because no identifiers were attached to responses at analysis, we could not compare differences between respondents and nonrespondents. We speculate, however, that those clerkship directors who did not respond were more likely not to have included abortion education because educators are often eager to share perceived valuable components of the curriculum.

The only previously published study of abortion education in medical school reports a clinical experience in abortion care at a single institution. This investigation
into medical students’ attitudes about a clinical experience in abortion care revealed that a substantial number of student participants in abortion care experiences became more supportive of women’s access to abortion.\textsuperscript{11} A compelling reason for encouraging student participation in an abortion care experience is the propensity for such an experience to change students’ attitudes.

We conclude that abortion education is deficient in US medical schools. Control over family size is vital for women’s physical, social, and economic well being. Comprehensive knowledge of family planning, including abortion, is a central component of women’s health. While many physicians choose not to offer abortion services in their practices, even so, they should understand abortion procedures and complications because of the high prevalence of abortion. The controversial nature of abortion creates an even greater imperative for a rational, evidence-based, and public health-oriented discussion of this topic in the curriculum of all US medical schools.

References

10. Liaison Committee on Medical Education. Functions and structure of a medical school May 2000.
Attitudes of faculty and students toward case-based learning in the third-year obstetrics and gynecology clerkship

Wendy F. Hansen, MD,a,* Kristi J. Ferguson, PhD,b Christopher S. Sipe, MD,a Joel Sorosky, MDa

Department of Obstetrics and Gynecology, Carver College of Medicine, Iowa City, Iowa,a and Department of Community and Behavioral Health, University of Iowa College of Public Health, Iowa City, Iowab

Accepted for publication April 15, 2004; revised September 26, 2004; accepted October 6, 2004

KEY WORDS
Problem-based learning
Education
Medical Educational measurement

Objective: This study was undertaken to compare the attitudes of faculty and medical students toward case-based learning and lecture format during the obstetrics and gynecology clerkship.

Study design: For this prospective comparative study, student presentations were alternately assigned to traditional lecture- or case-based format every 6 weeks. Presentations were made to other students and a single faculty. A total of 31 faculty members, 30 student presenters, and 122 student participants completed evaluations. Teaching methods were compared.

Results: Faculty members favored lecture format over case-based learning for “attentiveness and interaction of the group” (3.9 vs 4.5, \( P < .018 \)) and for “meeting the objectives” (3.7 vs 4.5, \( P < .002 \)). Student participants favored case-based learning in “understanding the relationship between knowledge and clinical practice” (4.34 vs 4.06, \( P < .05 \)) and “enjoyed” (4.34 vs 3.90, \( P < .008 \)). Student presenters showed no differences between groups.

Conclusion: Faculty favored lecture format whereas student participants favored a case-based presentation. Student presenters were comfortable with both formats.

© 2005 Elsevier Inc. All rights reserved.

Problem-based learning (PBL), first introduced in 1969 as an alternative to the traditional lecture series, is an educational method based on adult learning theory. At its center is a carefully constructed case that acts as the focal point for students to learn problem solving skills and acquire knowledge about both the basic and clinical sciences. In PBL, the student is an active participant identifying their own learning needs and strategies in a small group format with a faculty tutor. Its long-term goals are to promote critical thinking skills and to acquire knowledge that is better retained, usable in a clinical context, and integrated from many disciplines.

PBL was first introduced at McMaster University in Canada. Southern Illinois University and University of New Mexico were among the first medical schools in the United States to implement a PBL curriculum. In 1985

* Reprint requests: Wendy F. Hansen, MD, Division of Maternal-Fetal Medicine, Department of Obstetrics and Gynecology, University of Kentucky, 800 Rose St, Lexington, KY 40536.
E-mail: wfhans2@email.uky.edu

0002-9378/S - see front matter © 2005 Elsevier Inc. All rights reserved.
Harvard Medical School field tested the “New Pathway Curriculum,” a PBL model. Since then, it has gained widespread acceptance and has become integrated in some form into the first 2 years of medical education in the United States and abroad.

Case-based learning (CBL) is a variant of PBL and involves a case vignette that is designed to reflect the educational objectives of a particular topic. Medical students at the University of Iowa participated in a hybrid of PBL and traditional curriculum during their first 2 years. However, PBL had not yet been adopted in the obstetric and gynecology third-year clerkship.

Given the small group nature of our 6-week clerkship, the familiarity with PBL by our students, and the long-established traditional student presentation as 5% of the overall clinical clerkship grade, the University of Iowa seemed particularly well suited to study the introduction of CBL into the third-year clinical clerkship.

The purpose of our study was to evaluate attitudes of the faculty, the students as presenters, and the students as participants to CBL during the 6-week obstetrics and gynecology clerkship.

Design

The study is a prospective comparative evaluation of student and faculty attitudes toward CBL and lecture format as presented by third-year medical students to other third-year students with a single faculty member present.

The University of Iowa Institutional Review Board determined that the study did not require review.

In preparation for the study, 13 obstetric topics and 18 gynecology topics were identified. Learning objectives were then written for each topic. Both topics and learning objectives were extracted from the Women’s Health Care Competencies for Undergraduate Medical Education.

Next, clinical cases were written for each topic. All cases were written by 2 of the authors, (W.H. authored for obstetrics and C.S. authored for gynecology).

Assignment to CBL or lecture format was systematic. Each 6-week clerkship was designated either CBL or lecture and formats alternated every 6 weeks. Assignment to an individual topic was self-selected from the 13 obstetric and 18 gynecology topics identified earlier.

Students were given either a case with the learning objectives or a topic (no case) with the learning objectives. They received no special instructions. The presentation counted for 5% of their grade.

Three evaluation tools were created.

1. For students presenting in either CBL or lecture format, an evaluation tool consisting of 10 items using a 5-point scale (1-unsatisfactory and 5-superior) was developed. Students responsible for presenting that day were asked to complete the evaluation immediately after their presentation.

2. For student participants (those in the audience), an evaluation tool consisting of 9 items using the same 5-point scale was developed. Students were asked to complete the evaluation tool immediately after listening/participating in another students’ presentation (peer evaluation). There were generally 3 to 5 students present at each session.

3. For faculty, an evaluation tool consisting of 4 items using the same 5-point scale was developed. Faculty members were asked to complete this evaluation immediately after the students’ presentations. The faculty members were responsible for completing a separate evaluation form for grading. Students and faculty alike were made aware that the evaluation tools were for an educational research project and would not be used for grading purposes. Completed evaluations were entered into an Access database. The 2 groups: CBL and lecture format were then compared.

Statistics

Statistical analysis was performed with SAS 8.2 (SAS Institute, Cary, NC). Student t test was used for comparison of means and \(\chi^2\) for categorical variables. Alpha coefficients were calculated for each evaluation tool.

Results

Thirty-one faculty members, 30 student presenters, and 122 student participants completed the evaluations.

Student presenter

Of the 30 student presenters, 19 presented with a case and objectives (CBL) and 11 presented with a topic and objectives (lecture format). There was no statistical difference between groups in the 9-item evaluation (Table I). There was a tendency for item 4 “stimulated my interest in learning” to favor CBL \((P > .056)\). Both groups used similar resources in presenting and spent between 3 to 4 hours in preparation.

Student participant

A total of 122 student participants, 90 with CBL and 32 with lecture format, completed evaluations. Of the 9 items, 2 reached statistical significance (Table II). Students participating in CBL were “better able to understand the relationship between knowledge and
clinical practice” (4.34 vs 4.06, *P* < .05) when compared with the lecture format and “enjoyed” CBL more than the lecture format (4.34 vs 3.90, *P* < .008).

### Faculty

A total of 31 faculty members, 19 observed CBL and 12 observed lecture format, completed evaluations. Of the four items, 2 reached statistical significance (Table III). Faculty members favored lecture format over CBL for “attentiveness and interaction of the group toward the leader” (3.9 vs 4.5, *P* < .018) and for “meeting the APGO objectives” (3.7 vs 4.5, *P* < .002).

### Internal reliability

Alpha reliability coefficients (Cronbach’s alpha) were calculated for each of the evaluation tools. For the student observers, the 9-question scale had an alpha coefficient of .87. For the student presenters, the 9-question scale had an alpha coefficient of .84. For the faculty, the 4-item scale had an alpha coefficient of .70.

### Conclusions

Integration of PBL into medical student education has met with some controversy. The small group nature of PBL requires far more resources than its traditional counterpart “lecture” and requires different skills from the teacher/tutor. Although it is clear that PBL is more enjoyable and promotes satisfaction among students, it is not clear that it is superior to traditional didactic teaching sessions on traditional measures of knowledge, such as national licensing examinations, on measures of clinical reasoning or diagnostic ability.4 Our results support previous findings that CBL in general is more enjoyable and that students are better able to see the connection between knowledge and clinical practice.

In contrast to the students, the faculty favored traditional lecture format. This is not surprising given that lecture was the mainstay of the clerkship for many years and for the majority of faculty, the main educational method used throughout their own medical school experience. It is their comfort zone. Lecture allows the faculty to reach their teaching goals in an organized and methodical way, ensuring that each participant receives the proscribed information that achieves the learning objectives. Discussions are by nature less directly organized and predictable. It may be more difficult to assure that each participant receives the learning objectives. CBL activities are also more vulnerable to the personalities of the individuals involved and can often be skewed by more aggressive or inquisitive participants. Although a lecture format ensures that each member has received learning objectives, it does not ensure that they have learned it.

Is student satisfaction and/or enjoyment a worthwhile goal to measure or is the pure goal transference of knowledge? Is this enjoyment simply a reflection of a more relaxed environment that CBL promotes? In CBL, students are actively engaged in their learning. The goals of teaching are many and layered. If satisfaction stimulates interest and interest stimulates further

---

### Table I Attitudes of students as presenters

<table>
<thead>
<tr>
<th></th>
<th>CBL (mean)</th>
<th>Lecture (mean)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Relationship between studies and practice</td>
<td>4.253</td>
<td>4.000</td>
<td>.129</td>
</tr>
<tr>
<td>Interdependence of facts, concepts, and principles</td>
<td>4.476</td>
<td>4.000</td>
<td>.138</td>
</tr>
<tr>
<td>Skills enhanced in formulating/testing hypotheses and drawing conclusions</td>
<td>4.142</td>
<td>3.714</td>
<td>.295</td>
</tr>
<tr>
<td>Stimulated interest</td>
<td>4.571</td>
<td>4.000</td>
<td>.056</td>
</tr>
<tr>
<td>Helped learners gain better understanding</td>
<td>4.181</td>
<td>4.000</td>
<td>.582</td>
</tr>
<tr>
<td>Comfortable with format</td>
<td>4.381</td>
<td>3.857</td>
<td>.159</td>
</tr>
<tr>
<td>Confident in accomplishing goals</td>
<td>4.454</td>
<td>4.285</td>
<td>.577</td>
</tr>
<tr>
<td>Beneficial learning experience</td>
<td>4.476</td>
<td>4.142</td>
<td>.309</td>
</tr>
<tr>
<td>Preparation time (h)</td>
<td>3.636</td>
<td>3.571</td>
<td>.767</td>
</tr>
</tbody>
</table>

### Table II Attitudes of students as participants

<table>
<thead>
<tr>
<th></th>
<th>CBL (mean)</th>
<th>Lecture (mean)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Knowledge and practice</td>
<td>4.344</td>
<td>4.064</td>
<td>.050</td>
</tr>
<tr>
<td>Basic science and practice</td>
<td>4.287</td>
<td>4.032</td>
<td>.116</td>
</tr>
<tr>
<td>Learning objectives reflected</td>
<td>4.616</td>
<td>4.709</td>
<td>.476</td>
</tr>
<tr>
<td>Logical and organized</td>
<td>4.593</td>
<td>4.677</td>
<td>.523</td>
</tr>
<tr>
<td>Interest exhibited</td>
<td>4.395</td>
<td>4.562</td>
<td>.265</td>
</tr>
<tr>
<td>Instructional aids</td>
<td>4.209</td>
<td>4.200</td>
<td>.957</td>
</tr>
<tr>
<td>Receptive and responsive</td>
<td>4.487</td>
<td>4.586</td>
<td>.451</td>
</tr>
<tr>
<td>Beneficial learning experience</td>
<td>4.418</td>
<td>4.187</td>
<td>.110</td>
</tr>
<tr>
<td>Method of teaching enjoyed</td>
<td>4.344</td>
<td>3.906</td>
<td>.008</td>
</tr>
</tbody>
</table>

* Significant < .05.

### Table III Attitudes of faculty

<table>
<thead>
<tr>
<th></th>
<th>CBL (mean)</th>
<th>Lecture (mean)</th>
<th><em>P</em> value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group attentiveness/interaction</td>
<td>3.947</td>
<td>4.583</td>
<td>.018</td>
</tr>
<tr>
<td>Beneficial learning session</td>
<td>4.421</td>
<td>4.583</td>
<td>.524</td>
</tr>
<tr>
<td>APGO objectives met</td>
<td>3.789</td>
<td>4.583</td>
<td>.002</td>
</tr>
<tr>
<td>Enjoyable session</td>
<td>4.263</td>
<td>4.583</td>
<td>.198</td>
</tr>
</tbody>
</table>

* Significant < .05.
study and retention, then it may be an indirect indicator of teaching success. If transference of knowledge was our only goal, students could just read a textbook.

Our CBL model was quite different than the comprehensive PBL model of the first 2 years of medical education. First, the student, not a faculty member, was the tutor and the expert lead discussant and their approach to a case was highly individual.

Learning strategies were not part of the process given the limited time. Rather the case was the story and the focal point for which the objectives could be met. This was often the first time a student was asked to teach with a case (no other instructions were given). Student participants were not graded and not necessarily prepared or expected to participate for grading purposes. Lastly, many of the faculty members were not experienced with the methodology of PBL.

Although written comments were not asked for on the evaluation, many unsolicited peer comments were offered. Interestingly, a few students observed that their presentation was actually a combination of both lecture and CBL. When an attempt to gather participation faltered, many students fell back to lecture mode.

Potential weaknesses of the study are the small numbers at a single institution, the nonvalidated cases written by neophyte authors, and the 3 new evaluation tools. Alpha coefficients for the 2 student evaluation tools (.87 for student participants and .84 for student presenters) are well within the acceptable range for reliability. Although the faculty evaluation alpha coefficient is a little low (.70), it is reasonable for a 4-item scale and should improve as we revise the scale in future administrations of the questionnaires.

The difference in numbers of presenters and participants between CBL and lecture format are a reflection of the of the study period that included 3 clerkships with only 1 being assigned lecture format, whereas 2 were assigned CBL format. The difference does not reflect any choice by students and therefore should not introduce bias.

A strength of our study is that we found differences with relatively low numbers. Although the actual differences are small and some may argue are not practically different, the results suggest the need for further study. Integration of CBL into the clinical clerkship could be potentially improved with formal instruction to student presenters, education of faculty and defined expectations of student participants.

Most of our scores averaged over 4.0 for both CBL and lecture format, raising several possibilities. It may reflect the superior nature of both formats. Grade inflation may be a factor; however, our students are generally quite honest in their evaluations at the end of the clerkship (not grade inflation). Although this evaluation was stated repeatedly to be anonymous and for research only, it is possible they saw it as a reflection on their peers and did not want to be too critical. If this is the case, then both CBL and lecture format would be equally affected.

CBL in a clinical clerkship offers several potential long term benefits. First, it teaches our students to be teachers by modeling behavior that they have observed in the first 2 years of medical school. It is student oriented as opposed to faculty oriented. It introduces faculty to a different model of teaching, helping to bridge the gap between then and now.

Integration of CBL into the formal didactics of the obstetrics and gynecology clerkship improves student satisfaction. Student presenters were equally comfortable with CBL and lecture format.

We thank both the students and faculty at the University of Iowa who willingly participated and supported this study by completing 1 more evaluation in the midst of a busy day.

References

IMAGING

Uterine tissue development in healthy women during the normal menstrual cycle and investigations with magnetic resonance imaging

Caroline L. Hoad, PhD,a Nick J. Raine-Fenning, MD,b Jonathan Fulford, PhD,a Bruce K. Campbell, PhD,b Ian R. Johnson, MD,b Penelope A. Gowland, PhDa,*

Sir Peter Mansfield Magnetic Resonance Centre, School of Physics and Astronomy,a and Academic Division of Reproductive Medicine, School of Human Development,b University of Nottingham, Nottingham, UK

Received for publication April 21, 2004; revised June 28, 2004; accepted July 19, 2004

Objective: High-resolution magnetic resonance imaging (MRI) was used to monitor both uterine endometrial and junctional zone morphometry during the normal menstrual cycle.

Study design: Twenty-four healthy, ovulating women were studied during a single menstrual cycle. Three scans were performed to prospectively coincide with the follicular, periovulatory, and luteal phases of the cycle.

Results: MRI data showed a significant increase in endometrial and junctional zone volume, between the follicular and periovulatory phases, with a significant decrease in endometrial volume observed from the periovulatory to luteal phases. The regularity index, which is a novel subjective assessment of junctional zone structure, varied significantly and demonstrated a less regular junctional zone in the luteal phase.

Conclusion: This study has quantified the normal developmental changes of uterine tissue during the menstrual cycle with MRI. Junctional zone data from MRI may play a major role in future studies that investigate menstrual disorders, subfertility, and pathologic changes.

Ultrasound endometrial thickness measurements are used widely to determine endometrial receptivity and to detect potential pathologic conditions in gynecology.1,2 Such measurements have reasonable negative predictive value, in that malignant change and conception are unlikely with an endometrial bilayer of <5 mm,3,4 but disease and pregnancy still occur in patients with thin endometria.5,6 However, morphometry alone has limited value in the prediction of endometrial function and disease. Dynamic changes in endometrial growth could be monitored over the menstrual cycle to address this problem. With the use of currently existing imaging techniques such as transvaginal 3-dimensional ultrasound scanning7 and magnetic resonance imaging8...
(MRI), dynamic changes in thickness and multidimensional information (eg, volume) can be determined.

MRI is now used widely to image pelvic pathologic condition and has been shown to be superior to ultrasound scanning in the staging of some uterine cancers. However its role in other areas of gynecology is yet to be realized. MRI is inherently 3-dimensional in nature and, with correct sequences, can define clearly the endometrial, myometrial, and junctional zone regions. It is noninvasive; therefore, serial measurements during the menstrual cycle may be conducted. Several studies have already looked at changes in endometrial and junctional zone thickness in both normal and hormonally modified menstrual cycles. These studies examined small groups of women (<10 women in a group) and were not always controlled for the exact date of ovulation. The aims of this current study were to use MRI to monitor both endometrial and junctional zone morphometry during the normal menstrual cycle and to examine whether volumetric analysis provides improved information on tissue development compared with thickness measurements. These measurements form part of a wider study to evaluate the roles of quantitative MRI and 3-dimensional ultrasound in gynecology.

Material and methods

Experimental design

This study was a longitudinal, observational study. Subjects were asked to call on the first day of menstruation (day 1) to book their first appointments for ultrasound scans (day 3) and MRI (between days 5 and 7 of the menstrual cycle). Blood samples were taken, and 3-dimensional ultrasound was performed on alternate days from day 3 until ovulation was detected ultrasonographically by the collapse of the dominant follicle and then every 4 days until the next menstrual period. Ovulation was confirmed initially ultrasonographically and subsequently was verified by observation of a surge in plasma concentrations of luteinizing hormone. Depending on the length of their typical menstrual cycle, a second MRI scan was booked to coincide with the periovulatory period (day 13-15 for a 28-day cycle); however, if ultrasound scans showed the dominant follicle to be developing either more quickly or slowly than this, then the scan was rebooked accordingly. A final MRI scan was undertaken at approximately 6 to 8 days after the periovulatory scan. The dates of each scan were then reclassified to ± days from ovulation, which was defined as day 0. Data were grouped according to the phase of the cycle: follicular phase, 12-5 days before ovulation; periovulatory phase, ±3 days from ovulation; luteal phase, 4-10 days after ovulation.

Volunteer selection

Twenty-four women with regular ovulatory cycles and no history of infertility, miscarriage, or endometriosis formed the study group. These women were of reproductive age and were not using hormonal or intrauterine devices as contraception. This study was approved by the Local Hospital Ethics Committee. All volunteers were interviewed by a clinician (N.J.R.-F.) to determine their eligibility, to explain the study, and to obtain written consent before entering the study.

Data acquisition

A 0.5 T scanner with a Marconi (Surrey Medical Imaging Systems, UK) console was used for the MRI scan. Volunteers were allowed to lie either prone or supine in the scanner with their arms placed by their head to avoid motion artifacts from them. A set of 20 echo planar images (8-mm slice thickness) was used to determine the approximate central slice of the uterus. Twelve slices were acquired with the following sequence parameters: slice thickness, 7 mm; interslice separation, 0.5 mm; repetition time, 4000 msec; interecho time, 25 msec; effective echo time, 125 msec; echo train length, 8, in plane resolution 1.17 mm × 1.17 mm, 256 × 192 image matrix.

Data analysis

Volume and thickness measurements were carried out by 1 observer (C.L.H.) to reduce variability. Figure 1 shows typical, central sagittal slice images through the uterus at various time points over the menstrual cycle. Endometrial and junctional zone thickness measurements were carried out on the central slice using Analyze software (Biomedical Imaging Resource, Mayo Foundation, Rochester, Minn). Endometrial thickness was measured at the maximum width of the tissue region, perpendicular to the long axis of the uterus. In previous MRI studies of endometrial thickness, no indication was given of the variability of an individual’s measurements, whereas for this study 5 measurements were averaged for each central slice image. Junctional zone thickness was measured as the average of 5 thickness measurements equally spread around the uterus, perpendicular to the visible endometrial/junctional zone intersection on the central slice image. Volumes of the endometrium and junctional zone were also calculated with the Analyze region of interest program, with which each uterine tissue volume was identified on each slice. Total volumes were then calculated from all the slices. As with the thickness measurements, the average of 5 drawings of the region of interest was calculated for each set of data.
While the morphometric parameters were being calculated, it was noted that the junctional zone appearance varied between subjects and varied during the cycle, for individual subjects, in terms of both position around the endometrium and regularity of the zone’s shape. Because no references in the literature could be found that described the regularity or placement of the junctional zone around the endometrium, a subjective measure of the junctional zone regularity and geometric center was devised and implemented on the central sagittal image. Two observers (C.L.H. and J.F.) independently graded this slice between 1 and 5 for 2 categories. The first category was “regularity” (how smooth the junctional zone edge was with the myometrium; Figure 2, A), with 1 being very irregular, 5 being very smooth and regular, and 3 having some irregularity. The second category was “geometry” (how centralized was the endometrium within the junctional zone; Figure 2, B), with 1 being completely non-central, 5 being completely central, and 3 being slightly off-center.

A comparison was made between endometrial volume measurements that were carried out using MRI and those measurements that were determined from 3-dimensional ultrasound scans of the same patients. Where possible, the measurements for both techniques were carried out on the same day; however, if this did not occur, ultrasound measurements were averaged from days either side of the MRI data. The ultrasound volumes were measured with the VOCAL-imaging program (Kretz Technik, Zipf, Austria). VOCAL allows the user to define the volume of interest manually with a standard computer mouse as the dataset is rotated about a central axis. Reliability and validity of this method are outlined in more detail in the literature.

### Statistical analysis

The morphometric data were tested for normality with the Shapiro Wilk’s test, and the statistical significance did not identify definitely the distributions as normal. Paired Wilcoxon signed ranks tests, in SPSS software (version 11; SPSS Inc, Chicago, Ill), were used to determine the changes between the different phases of the menstrual cycle (follicular to periovulatory, periovulatory to luteal, and follicular to luteal were compared). Agreement between the MRI and ultrasound measurements of endometrial volume was carried out with the Bland and Altman technique for the assessment of agreement between the 2 methods of clinical measurement, where the difference between the MRI and ultrasound measurements is plotted against the average of the 2 measurements. The interobserver variability for the subjective junctional zone measurements was determined by the calculation of the weighted kappa from the subjective data.

### Results

Of the 24 women who were scanned, 1 data set was lost during the study because poor quality images were obtained consistently throughout the menstrual cycle, which resulted from very noisy data on 1 occasion and radiofrequency artifacts running through the center of the uterus on the other 2 occasions. The final study group therefore was comprised of 23 volunteers with a mean age of 31 years (range, 20-42 years). The mean duration of the menstrual cycle for all 23 women who were included in the study was 29 days (range, 26-33 days) and was divided relatively equally between follicular (mean, 15 days; range, 12-22 days) and luteal (mean, 14 days; range, 10-16 days) phases. Of those 23 patients, 22 patients were scanned on 3 occasions during their menstrual cycle, and 1 patient was scanned on just 2 occasions. However, 6 volunteers had their scan days reclassified after confirmation of ovulation by serologic evaluation, with 4 days from the beginning of the follicular phase not used (because they were outside the range −12 to −5 days from ovulation); therefore, the total number of scans in each phase of the cycle were as follows: 23 follicular, 21 periovulatory, 18 luteal. The junctional zone was not visible on 5 data sets. This was due to 1 of 2 reasons: either the myometrium was very dark and a separate darker junctional zone layer was not detectable, or no dark region between the endometrium and myometrium was visible on the images, despite
a moderately high signal intensity of the myometrium. This further reduced the grouped junctional zone data to 21 for the follicular phase, 20 for the periovulatory phase, and 16 for the luteal phase.

Morphometry

Figures 3 and 4 show median tissue volumes and thickness that were measured over all volunteers, with data at each particular phase of the cycle. The intraobserver standard deviation for volume and thickness measurements was <10% of the calculated subject mean. Paired Wilcoxon signed ranks tests showed significant increases between the follicular phase and periovulatory phase, in endometrial volume ($P < .001$; $n = 21$), thickness ($P < .001$; $n = 21$) and junctional zone volume ($P = .004$; $n = 18$). Endometrial volume significantly decreased from the periovulatory phase to the luteal phase ($P = .011$; $n = 17$) for the paired data. Luteal phase volume and thickness were significantly greater than the follicular volume and thickness for the endometrium ($P = .001$; $n = 18$), and junctional zone volume ($P = .048$; $n = 14$). Junctional zone thickness showed no variation over the menstrual cycle.

The comparison of the MRI and 3-dimensional ultrasound endometrial volume measurements is shown in Figure 5. There was reasonable agreement between the 2 techniques over the first one half of the cycle until ovulation, with a mean difference of $0.25 \pm 1 \text{ cm}^3$ (MRI/ultrasound). Larger discrepancies were found in the second one half of the cycle after ovulation, with ultrasound predominantly measuring a larger endometrial volume than MRI (mean difference, $-0.61 \pm 1.3 \text{ cm}^3$). It was noted that the differences between the 2 methods increased with the average measurement.

Subjective measurements of junctional zone

Subjective assessment of the junctional zone shape can be found in the Table. The regularity index decreased from both the follicular and periovulatory phases to the luteal phase (follicular: $P = 0.034$, $n = 15$; periovulatory: $P = .013$, $n = 15$, paired Wilcoxon signed ranks test). No change was seen in the geometry index over the menstrual cycle. The average value for this index was 3. The interobserver agreement was fair for the regularity index ($k = 0.39$) and moderate for the geometry index ($k = 0.42$).

Comment

This is the largest MRI study to date to measure uterine tissue development in healthy women over the normal menstrual cycle and that, in particular, focused on measurements of the junctional zone and the endometrium. All data were analyzed with respect to the actual dates of ovulation, which were determined ultrasonographically, and were confirmed by serologic evaluation. This classification of measurements has been reported only once previously for MRI studies. Because ovarian hormones determine uterine development, it is important that different length cycles are corrected according to ovulation.

An increase in the endometrial volume and thickness and junctional zone volume was seen between the early follicular and ovulation phases. These results agree with previously published MRI thickness data and histologic data, in which growth was restricted to the follicular phase of the menstrual cycle. Median endometrial volume and thickness that were measured by MRI decreased slightly during the luteal phase of the cycle, although they remained statistically greater than the follicular phase measurements. During the luteal phase of the cycle, progesterone induces a secretory change in the endometrium, which results in tortuous coiling of the epithelial glands, which could result in compaction of the tissue and a decrease in endometrial
volume. This result disagrees with previous studies that used standard T2-weighted spin echo sequences, which showed a continued increase in endometrial thickness during the luteal phase of the cycle but were based on much smaller subject numbers.

A limitation of this study is the low number of MRI scans that were carried out per person over the menstrual cycle and hence the large grouping of days for the phases of the cycle. This may have flattened out any smaller variations of these measurements with time, although none were observed with ultrasound scanning, which was carried over more frequently.

Uterine tissue volume is expected to be a more sensitive measure of development than tissue thickness, because it takes into account the complete uterine geometry and these data suggest that volume shows greater discriminatory potential. This is illustrated by the junctional zone data, in which no variation of thickness was seen over the menstrual cycle, whereas junctional zone volume significantly increased between the follicular and periovulatory phases. This study was the first to measure total junctional zone volume from MRI data; consequently, there are no data with which to compare this result. A previous MRI study showed a slight increase in junctional zone thickness; however, no statistical analysis was carried out on the data because of small sample size. The junctional zone is irregular; therefore, it is difficult to get a reliable representation of the complete junctional zone thickness because this may vary substantially from 1 point to another. Furthermore, because the junctional zone lies around the endometrium, its volume could increase as the endometrium develops, without its thickness increasing.

The changes to the regularity index over the menstrual cycle, which were assessed by the novel subjective method described in this article, indicate that this region becomes more irregular in the luteal phase of the cycle and that this may be related to uterine contractions. It is well known that peristaltic contractions occur and change in nature over the course of the cycle and with hormonal state. More recently changes in the thickness of the junctional zone and myometrium, which are seen on serial T2-weighted MRI images, have been attributed to uterine contractions; these changes were observed predominantly during the luteal phase of the cycle. It should be noted, however, that the regularity effect that is reported here is not attributed to motion artifacts on the images. The origin of the junctional zone that was observed with MRI has been a matter of some debate. It was suggested initially that it arose from a change in blood flow or in blood vessels at the boundary between the myometrium and endometrium. However, it has been shown, with the use of hysterectomy samples, that the junctional zone lies underneath the arcuate vessels. In relatively slow, conventional MRI sequences, the signals may be affected by peristalsis, which includes the effect of changing blood volume because of muscular contractions, although the low signal intensity of the junctional zone is maintained on hysterectomy sections. It now seems most likely that the junctional zone is a region of compact smooth muscle bundles with reduced water content, decreased extracellular space, and more densely packed muscle cells that give rise to the characteristic MRI short T2 and T1 and general low signal appearance of this region.

A comparison of the 3-dimensional ultrasound and MRI data of the patients in this study showed that generally there was reasonable agreement between the 2 techniques, with a standard deviation of the differences between the techniques of approximately 1 cm³. Ultrasound scans tended to measure larger volumes of endometrium compared with MRI (mean differences of MRI minus ultrasound scans were negative), and the largest discrepancies in these measurements were in the luteal phase of the cycle. Wiczynk et al found a continued increase in endometrial thickness over the menstrual cycle and showed good agreement between MRI and ultrasound scanning during the first half of the cycle (MRI thickness slightly larger than ultrasound thickness), with

<table>
<thead>
<tr>
<th>Phase of cycle</th>
<th>Regularity index *</th>
<th>Geometry index</th>
</tr>
</thead>
<tbody>
<tr>
<td>Follicular</td>
<td>3.4 ± 0.2</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Periovulatory</td>
<td>3.5 ± 0.2</td>
<td>3.3 ± 0.3</td>
</tr>
<tr>
<td>Luteal</td>
<td>2.9 ± 0.2</td>
<td>2.9 ± 0.3</td>
</tr>
</tbody>
</table>

Values are mean ± SEM.

* How smooth is the junctional zone edge with the myometrium? (1, irregular; 5 smooth).

† How centralized is the endometrium within the junctional zone? (1, non-central; 5, central).

† Significant decrease in index from follicular (P = .034; n = 15) and periovulatory (P = .013; n = 15) to luteal phase.
greater differences seen in the luteal phase (MRI measuring a lower endometrial thickness than did ultrasound scanning). The most likely cause of the differences between ultrasound scan and MRI is discrepancies in the delineation of the zones of the uterus, which has been described previously by Mitchell et al., who also showed that measured endometrial thickness was lower in MRI compared with ultrasound scanning. The differences in the measurements in the luteal phase of the cycle may be due to the different definitions of boundaries of the junctional zone that were used for the 2 techniques. The contrast for the MRI data is relatively constant throughout the cycle, with a bright endometrium, very dark junctional zone, and medium intensity myometrium, whereas ultrasound contrast in the uterus varies over the cycle and tends to detect a much thinner region that is defined as the junctional zone. In addition, the luteal phase endometrium is less clear on ultrasound scans, especially the endocervical junction. Because of the constant contrast between the different uterine tissues over the menstrual cycle, MRI may provide a more reliable method of for the study of junctional zone development during the menstrual cycle. Quantitative MRI techniques can measure the nuclear magnetic resonance relaxation times of the tissues and the water self-diffusion coefficient in the tissue (related to tissue structure) and the tissue perfusion. Therefore, MRI also offers the possibility of studying changes tissue perfusion and changes related to tissue structure in the different regions of the uterus throughout the menstrual cycle.

In conclusion, this study has quantified the changes in uterine tissue throughout the normal menstrual cycle and observed growth in both the endometrial and junctional zones between the follicular and periovulatory phase and decreases during the luteal phase. The regularity of the junctional zone varied during the menstrual cycle as was assessed by a novel subjective method. The clear delineation of the junctional zone on MRI during all phases of the menstrual cycle will allow this imaging modality to play a more major role in future investigations of gynecologic problems, such as menstrual disorders, subfertility, and pathologic changes.

We thank Jeanette Clewes and Nigel Kendall for their assistance in measuring and analyzing the 3-dimensional ultrasound volume data and Paul Clark and Ron Coxon for technical assistance with the magnetic resonance scanner.

References


LETTERS TO THE EDITORS

Who should be the guardians of women’s “sacred space”? 

To the Editors: We are surprised by the view expressed by Wall and Brown regarding vaginal examinations by medical students without the patients’ explicit knowledge or consent.1 They appear to argue that a “key concept in determining what is and what is not proper in this setting lies in the concept of the surgeon as a fiduciary of the patients’ trust.” They also describe the trusted surgeon as a gate-keeper for permission “to enter the sacred space in which the surgical operation will be carried out.” Although their arguments that a medical student might touch a patient and be of assistance to her when holding a retractor, it is an entirely different matter to do a vaginal examination that is of no benefit to the patient. It is not up to the surgeon to decide what is “sacred” for a patient; it is up to the patient, who might have a very different view of her genitals being viewed, a retractor being held, and fingers being inserted into the vagina. The concept of a medical student being a member of the team holds while the medical student benefits the patient. It falls down when they do a vaginal examination for their learning, which is not relevant to the operation. If the medical student was not there, the extra vaginal examination would not be performed. The vaginal examination cannot be relied on as the medical student is untrained. A qualified doctor who is training in, eg, inserting a dilator or IUCD, might well require an extra vaginal examination under the supervision of the senior surgeon, but this would be to ensure she or he has the position of the uterus right for safety reasons.

In the United Kingdom, the law would be quite clear that an unnecessary vaginal examination by a medical student that was not required for the benefit of the patient as part of the operation would be an assault and damages can be exemplary.2

We are entirely in agreement that we must not be complacent about teaching, but have not found higher standards of informed consent to be the cause of a decline in examinations in theater. We are witnessing a rise in refusals by awake patients in the clinics and this is why we are having to become innovative in the use of plastic models and trained lay gynecologic teaching associates.3

If gynecologists are concerned about the public loss of trust in doctors (possibly related to the sad occasional abuse of patients), they would be wise to listen respectfully to women about their concerns rather than distort notions of “implied consent” and doctors’ determination of a patients’ “sacred space.”

Susan Bewley, MD, FRCOG*
Janice Rymer, MD, MRCOG, FRANZCOG, ILTM
St Thomas Hospital
London SE1 7EH
United Kingdom
E-mail: susan.bewley@gstt.nhs.uk

References

Reply

To the Editors: Drs Bewley and Rymer seem to regard medical students as clinical parasites who suck knowledge from patients while providing absolutely nothing of value in return. We disagree. Such a perception is deeply rooted in the culture of British medical education, in which students come to the study of medicine as undergraduates in late adolescence. They are younger, less mature, less experienced, and less broadly educated than their North American counterparts. Perhaps British medical students are extraneous to the care of the patient. North American students are not.

The argument that “the vaginal examination cannot be relied upon as the medical student is untrained” misrepresents the surgical context. The purpose of the preoperative examination is not diagnostic (in the sense of deciding whether surgery is warranted or not); rather, the preoperative examination is confirmatory and tactical in nature. It is instrumental in making the final determination regarding how the operation will be carried out. The medical student is not providing information that the surgeon must somehow “rely upon” rather, the medical student is being instructed in her duties as a member of the surgical team, duties that directly benefit the patient. Ensuring that one’s surgical assistants (who may include medical students) understand how the operation is to be performed is a mandatory part of surgical practice.

Medical students who function as surgical assistants perform services of direct therapeutic benefit to patients. The surgical consent form unequivocally states that the operation will be performed by the surgeon and “such assistants as may be selected by him/her.” Medical students who function as surgical assistants in gynecologic operations should be expected to examine patients before assuming such duties.

Patients should know that students will assist in surgical operations (under close supervision) and patients should know that students may carry out physical examinations as part of their clinical duties. Patients undergoing surgery for gynecologic conditions should understand that medical students will be similarly involved in their care. However, to argue, as Drs Bewley and Rymer appear to argue, that such participation by medical students is of no benefit to patients and is therefore inappropriate strikes us as inaccurate, misguided, disappointing, and ultimately detrimental both to medical education as well as to women who develop genital disease that requires surgical care.

L. Lewis Wall, MD, DPhil*
Douglas Brown, PhD
Department of Obstetrics and Gynecology
Washington University
St. Louis, MO 63110
E-mail: walll@msnotes.wustl.edu

Oxidative stress in women with preeclampsia

To the Editors: We were interested to read the article of Moretti et al. The authors conclude that a breath test demonstrated significantly greater oxidative stress in women with preeclampsia than in women with uncomplicated pregnancy and nonpregnant control subjects.

Oxidative stress has been implicated as contributing to the pathophysiologic condition of numerous disorders. In the obstetric context, increasing evidence suggests that oxidative stress is an important factor in the pathogenesis of preeclampsia. Free radicals are produced by normal metabolic processes, and their levels are thought to increase in association with the increased metabolic activity during pregnancy. Furthermore, preeclampsia has long been known to be associated with an increase in the levels of various markers of oxidative stress and a decrease in the levels of the antioxidant components of the plasma.

Knowledge of the response of the serum antioxidant systems in preeclampsia is limited. The concentrations of the individual antioxidant plasma components can be measured separately in the laboratory, but these measurements are time-consuming, labor intensive, and costly. Because the effects of the plasma antioxidant components are additive, the measurement of the total antioxidant response (TAR) reflects the redox status of the plasma. The most widely used methods for the measurement of the plasma TAR are either colorimetric, fluorescence-based, or chemiluminescence. However, these highly sophisticated techniques are not necessarily available in the routine clinical biochemistry laboratory.
We have developed a novel assay for the determination of the plasma TAR and have used this method to evaluate the extent of oxidative stress in patients with preeclampsia, as compared with healthy, normotensive pregnant women. We found a statistically significant decrease in the plasma TAR levels in the women with preeclampsia compared to the control group, which correlated with a significant increase in serum peroxide levels. Our method has several major advantages over the techniques that currently are available. Our assay is simple, rapid, and inexpensive and easily can be fully automated, which makes it eminently suitable for use in any clinical biochemistry laboratory.

Our preliminary data suggest that the routine measurement of the TAR may provide a useful tool to aid in the assessment of the oxidative stress status of patients with preeclampsia. Furthermore, routine screening of the TAR during pregnancy may also prove extremely useful in terms of the early recognition of maternal oxidative stress, which could then be treated with a targeted therapy and a more careful follow-up of patients who are at risk of preeclampsia. Our promising novel method is eminently suitable for such routine screening.

Mehmet Harma, MD*
Muge Harma, MD
Ozcan Erel, MD
Departments of Obstetrics and Gynecology, and Biochemistry, Medical School, University of Harran, Sanliurfa, Turkey
Ankara, Turkey
E-mail: mehmetharma@superonline.com

References

Reply

To the Editors: We commend Harma et al for their important advances in developing new markers of oxidative stress. As we noted in our paper, reactive oxygen species are highly toxic; they inflict a constant barrage of oxidative damage to DNA, proteins, lipids, and other biologically important molecules. This process generates a wide variety of downstream metabolic products that are candidate biomarkers of oxidative stress.

We have developed a noninvasive breath test for markers of oxidative stress, the breath methylated alkane contour (BMAC), and we found that the BMAC was elevated significantly in women with preeclampsia. This breath test and the biomarkers that were developed by Harma et al and others may provide powerful tools for future research. Controlled prospective clinical studies could compare these different markers for their sensitivity and specificity as predictors and prognosticators of preeclampsia and to measure the effects of newer therapies, such as antioxidants.

Michael Phillips, MD*
Michael Moretti, MD
Renee N. Cataneo, MA
Joel Greenberg, BS
Menssana Research, Inc
Fort Lee, NJ 07024-6510
E-mail: mphillips@menssanareserach.com

References
Randomized study on surgical treatment for vaginal prolapse

To the Editors: In their randomized study on surgical treatment for vaginal prolapse, Maher et al. make the point that there was a significant increase risk of anterior wall and vault prolapse after sacrospinous colpopexy. They then state that there was a nonsignificant increase tendency of posterior wall prolapse after the sacrocolpopexy, which nullified the difference.

On close inspection of Table II, there were rectoceles present preoperatively in 35 of 47 women in the abdominal group, but only 11 of the rectoceles were repaired. In the vaginal group, there were 30 rectoceles present preoperatively, yet there were 44 posterior colporrhaphies performed. It appears an excess number of posterior colporrhaphies were performed in the vaginal group, whereas only one third of the recognized rectoceles were corrected in the abdominal group. This alone would explain why there were more rectoceles present postoperatively in the abdominal group. Had the rectoceles been appropriately corrected at the time of the sacrocolpopexy, it is unlikely that they could conclude that “the abdominal approach may be preferable in women with predominately anterior and vault prolapse, and the vaginal approach may be preferable in those women with predominantly posterior and vault prolapse.” Rather, I suspect they might conclude that (1) recurrent cystoceles and vault prolapse alone continue to be the main drawbacks of the vaginal approach, and (2) if rectoceles are corrected, the abdominal sacral colpopexy yields overall better lasting support.

Peter K. Thompson, MD
Woman’s Hospital of Texas
Houston, TX 77054
E-mail: pthompson@obgynassociates.com

Reference


Reply

To the Editors: Thank you for the opportunity of responding to Dr. Thompson’s letter. He suggests that if more patients underwent a posterior colporrhaphy (PC) at the time of sacral colpopexy (SC), the success rate of the sacral colpopexy would have been significantly greater resulting in different study conclusions. This statement is based on a presumption that the SC is ineffective in the surgical management of rectoceles. This is incorrect for the following reasons. First, the surgical technique of the SC as reported in the methodology describes placing the posterior arm of the mesh 8 cm along the posterior vaginal wall that would correct all but the lowest of rectoceles.1 Second, Cundiff et al.2 and Fox and Stanton3 also describe the SC with posterior mesh extension as being highly effective in the surgical management of rectoceles. Finally, on further subgroup evaluation of the SC group in our study, 3 of 11 women (27%) who underwent SC with postcolporrhaphy had recurrent grade 2 rectocele compared with 5 of 36 (14%) who underwent SC without PC ($P = .34$).

Furthermore, Dr. Thompson believes that the posterior colporrhaphy was overused in the vaginal group of the study. He rightly points out that 44 women underwent PC while only 30 had preoperative grade 2 or greater rectoceles. We used the PC to create a continuous fascia layer covering the whole posterior compartment, including enterocoele, rectocele, and perineal deficiency. Women with vault prolapse, perineal deficiency, and a grade 1 rectocele would undergo a PC. We regret that this aspect of the surgical technique was not more fully described in the methodology.

Dr. Thompson’s proposed conclusions to our study are inappropriate on the basis of an understanding of anatomy of the posterior compartment, the surgical technique used, the literature, and further subgroup analysis of our data.
Accuracy of cervical cancer staging needs improvement

To the Editors: Hoffman et al focussed on the subject of the accuracy of pelvic examination in the assessment of patients with operable cervical cancer. They demonstrated an accuracy of 52% and 45% for the estimation of the tumor diameter by office examination and examination under anesthesia, respectively. Their data are supported by a study of Baltzer et al, who investigated the accuracy of clinical staging and correlation with the histologic specimen by giant-section specimen in 1092 cervical cancer patients FIGO Ib-III. They found an overestimation of the FIGO stage in 23%, an underestimation in 16.0% and a correct correlation in 61%. Most interestingly, the overestimation rate for FIGO IIb (n = 82, stage IIb was not investigated by Hoffmann et al) regarding parametrial involvement was 67.1%. The clinical examination is still the basis for the FIGO staging of cervical cancer and the basis for therapeutic decision making for or against surgery or radiotherapy. Cervical cancer is the only gynecologic tumor that is staged clinically. The most important criterion for cervical cancer staging is the attending gynecologist’s examining finger, whereas all other gynecologic tumors are staged by histopathologic criteria. This clinical staging is also the basis for substantial studies advocating the use of radio-chemotherapy in advanced stages of cervical cancer. Hoffmann et al showed a good correlation with risk-factor detection predicting adjuvant radiochemotherapy. Nevertheless, lymph node involvement as an important prognostic factor as well as bladder or bowel infiltration cannot be accurately assessed by this approach. Hertel et al showed the weak significance of imaging techniques as CT and MRI-examination for the staging of cervical cancer compared with a laparoscopic staging. We therefore conclude that efforts should be made to introduce a minimal invasive staging for cervical cancer as it has been described by Hertel et al to change the cervical cancer staging into a histopathologic assessment. We further question if studies that are based on invasive staging and histopathologic criteria and those that are based only on preoperative clinical examination, even when they are combined with CT or MRI, are comparable.

Sven Ackermann, MD*  
Matthias W. Beckmann, MD, PhD  
Department of Obstetrics and Gynecology  
University of Erlangen-Nuremberg  
91054 Erlangen  
Germany  
E-mail: sven.ackermann@gyn.imed.uni-erlangen.de

References
Reply

To the Editors: We wish to thank Drs Ackermann and Beckmann for their interest in our article. As they point out, Baltzer et al1-3 have demonstrated the inaccuracy of pelvic examination in designating patients with cervical cancer as stage IIb. As is consistent with clinical practice in North America, these women did not undergo primary surgical resection at our institution and therefore were not included in the study.

We agree that surgical staging would be more accurate than clinical staging even if MRI examination were included. Surgical staging for cervical cancer has been studied extensively in the past and appears to benefit only a small subset of patients. With a better understanding of which patients might benefit and with the use of laparoscopy, the risks versus benefits of surgical staging for many women with cervical cancer deserves further investigation. Clinical examination will continue to play an important role in the initial triage of patients to the various options available.

Mitchel S. Hoffman, MD
Division of Gynecologic Oncology
University of South Florida College of Medicine
Department of Obstetrics and Gynecology
Tampa, FL 33606
E-mail: macevedo@hsc.usf.edu

References

Unnecessary—The mother of invention?

To the Editors: Kabir et al make a case for the clinical and public health importance of classifying cesarean deliveries as "unnecessary."1 However, their methodology has several flaws and fails to adequately address adequately data quality issues inherent in the analysis of data from US birth certificates.

Kabir et al scanned data fields on the birth certificate to determine the presence of potential indications or risk factors. If none were reported, that cesarean delivery was classified as unnecessary. Data elements included all checkboxes for maternal medical risk factors, complications of labor and delivery, and congenital anomalies of child. Birth weight outside 2500 to 4000 g and gestational age outside 38 to 41 weeks were sufficient to classify the cesarean section as necessary.

This approach assumes complete, valid, and reliable documentation of each data element on the birth certificate. It also assumes that each diagnosis or condition was diagnosed or known antenatally or intrapartum, and that the presence of each condition if known bears on clinical management decisions concerning method of delivery. Congenital anomalies are poorly documented on birth certificates, with extremely low sensitivity and only moderate positive predictive values.2 Conditions such as hemoglobinopathies, Rh sensitization, moderate/heavy meconium, and precipitous labor are not indications for cesarean delivery. The contribution of the “other” categories in these checklists is larger than for most of the specified conditions. Birth weight and gestational age contribute overwhelmingly to the index. Gestational age in and of itself is not a primary indication for cesarean section. Inclusion of births at 37 weeks in the preterm category confuses the issue, and all infants are born at less than 25,000 g (error in Table I). Reporting percentages to 2 decimal places implies too much statistical precision.

Additional data needed to determine the necessity of each cesarean delivery include: estimated fetal weight; medically or nonmedically indicated elective cesarean sections; progress of labor; timing of analgesia and use of epidurals; timing and method of augmentation of labor; and type of hospital—by ownership, status as a teaching or nonteaching hospital, and level of perinatal care. Although the new national standard certificate of live birth contains some of these data fields,3 others remain elusive, and the quality of most data elements must be viewed as questionable.4 Although we should applaud the zeal of Kabir et al, it would be best if subsequent researchers use richer, clinically based data.
sources or linked population-based data from hospital discharge and vital statistics databases.

Russell S. Kirby, PhD, MS, FACE
Department of Maternal and Child Health
School of Public Health
University of Alabama at Birmingham
Birmingham, AL 35294-0022
E-mail: rkirby@uab.edu

References


Reply

To the Editors: We appreciate the relevant comments of Dr Kirby that emphasize the limitations of the use of birth certificate data in the retrospective study design we used in the evaluation of potential indicators for cesarean section associated with method of delivery. We are fully aware of the shortcomings of such analysis and point to them in the discussion of the article. Hence, we did not “assume complete, valid, and reliable documentation of each data element on the birth certificate.” Nor did we assume “that each diagnosis or condition was diagnosed or known antenatal or intrapartum, and that the presence of each condition if known bears on clinical management decision concerning methods of delivery.” We also emphasize in the discussion that other important patient, system, and/or clinician factors that may affect the complicated decision-making process for cesarean section are unknown and not measurable in designs such as this one.

Dr Kirby rightly identifies other indicators that may not be documented on the birth certificate and several that were included as indicators for cesarean section in the classification system we used, which alone, may not justify cesarean intervention. We chose to use this methodology to enable comparison with results from previous studies by others who used this classification.

Despite these limitations, we believe our results provide a reliable yet imprecise estimate of the proportion of cesarean sections that may lack recommended criteria and therefore may be considered “unnecessary.” We agree that these criteria alone are imprecise estimates given the complex decision-making between patient and provider and other influences that determine any surgical intervention. We look forward to “richer, clinically based data sources or linked population-based data from hospital discharge and vital statistics databases” that will add to the information discerned from our study. However, far more complex and prospective, clinical study designs are needed than the proposed epidemiologic analyses suggested by Dr Kirby, to address what are considered unnecessary cesarean sections in the eyes of clinicians, patients, payers, or hospital systems.

William C. Steinmann, MD
Tulane Center for Clinical Effectiveness
New Orleans, LA 70112-2699
E-mail: wsteinm@tulane.edu
Correction

The article, “Comparing McRoberts’ and Rubin’s maneuvers for initial management of shoulder dystocia: An objective evaluation” by Gurewitsch et al, published in the January 2005 issue (volume 192, pages 153-60), contains an error. On page 157, the incorrect reference was listed as a source for Figure 6. The last sentence of the caption should read:

(Reprinted with permission from Elsevier. 25)

© 2005 Elsevier Inc. All rights reserved.