ON THE COVER

MRI film (detail) of a fetus with severe congenital diaphragmatic hernia definitely demonstrates liver herniation. The Center for Fetal Diagnosis and Treatment at The Children’s Hospital of Philadelphia has continuously refined this diagnostic technique, used in the routine evaluation of most fetal anomalies, for more than a decade. Page 422.

Courtesy of Holly Hedrick, MD, and colleagues, Center for Fetal Diagnosis and Treatment; Teresa Victoria, MD and Juliet G. Palinkas, B.S., Department of Radiology, Children’s Hospital of Philadelphia, Philadelphia, PA.

EDITORIALS

335 Nasal bone in screening for trisomy 21: defining hypoplasia
Jiri Sonnek
See related article, page 361

337 The evolution of cost-effective screening and prevention of cervical carcinoma: implications of the 2006 consensus guidelines and human papillomavirus vaccination
Bradley J. Monk; Thomas J. Herzog
See related articles, pages 340 and 346

REVIEWS

ONCOLOGY

340 2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ
Thomas C. Wright Jr; L. Stewart Massad; Charles J. Dunton; Mark Spitzer; Edward J. Wilkinson; Diane Solomon; for the 2006 American Society for Colposcopy and Cervical Pathology–sponsored Consensus Conference
See related editorial, page 337, and related article, page 346

These evidenced-based consensus guidelines reflect recent changes in our understanding of how to manage women with cervical intraepithelial neoplasia and adenocarcinoma in situ of the cervix.

346 2006 consensus guidelines for the management of women with abnormal cervical cancer screening tests
Thomas C. Wright, Jr; L. Stewart Massad; Charles J. Dunton; Mark Spitzer; Edward J. Wilkinson; Diane Solomon; for the 2006 American Society for Colposcopy and Cervical Pathology–sponsored Consensus Conference
See related editorial, page 337, and related article, page 340

These evidence-based consensus guidelines are designed to assist clinicians of all subspecialties in managing women with abnormal cervical cancer screening tests.

The article summaries that appear in the Journal are abbreviated versions of Research and Clinical Opinion articles published in full on the Journal’s website at www.AJOG.org. While the great majority of these summaries are written by Journal staff and approved by the bylined authors and Journal editors, some summaries are written by the original authors and prepared for publication by Journal staff.
Clinical Opinion

Oncology

356 Risk assessment to guide the prevention of cervical cancer
Philip E. Castle; Mario Sideri; Jose Jeronimo; Diane Solomon; Mark Schiffman
A risk model for prevention of cervical cancer will provide the framework for rational algorithms that can incorporate rapidly evolving methods of screening and diagnosis.

Research

Oncology

359 High-risk cervical epithelial neoplasia grade 1 treated by loop electrosurgical excision: follow-up and value of HPV testing
Immaculada Alonso; Aureli Torné; Luis M. Puig-Tintoré; Roser Esteve; Llorenc Quinto; Sonia García; Elias Campo; Jaume Pahisa; Jaume Ordi
See Journal Club, page 433
High pretreatment high-risk human papillomavirus loads should be considered a risk factor for developing residual/recurrent disease. Posttreatment Hybrid Capture 2 has an extremely high sensitivity for detecting recurrences.

Selected Papers from the 27th Annual Scientific Meeting of the Society for Maternal–Fetal Medicine

361 Defining nasal bone hypoplasia in second-trimester Down syndrome screening: does the use of multiples of the median improve screening efficacy?
Anthony O. Odibo; Harish M. Sehdev; David M. Stamilio; Alison Cahill; Linda Dunn; George A. Macones
The use of nasal bone gestational age–specific multiples of the median resulted in improved second-trimester Down syndrome screening efficiency.
See related editorial, page 335
EDITORS’ COMMENTARY: The optimal use of nasal bone measurements in estimating the likelihood of Down syndrome has been a controversial issue. This analysis suggests that optimal specificity can be achieved by the use of multiples of the median rather than a ratio of biparietal diameter to nasal bone length. We asked Dr Jiri Sonek, the author of the original article on nasal bone measurement, to comment.

363 Placental angiogenesis markers sFlt-1 and PI GF: response to cigarette smoke
Ramkrishna Mehendale; Judith Hibbard; Asgerally Fazleabas; Richard Leach
Cigarette smoke extract reduces placental secretion of sFlt-1, which suggests a mechanism of reduction in the risk of preeclampsia among smokers.
EDITORS’ COMMENTARY: This interesting study may begin to explain the reduced incidence of preeclampsia that is observed in women who smoke.

(continued on page 9A)
Self-reported cognitive functioning in formerly eclamptic women
Annet M. Aukes; Ineke Wessel; Albertien M. Dubois; Jan G. Aarnoudse; Gerda G. Zeeman
Women who have had eclampsia report significantly more cognitive failures, compared with healthy parous control subjects, which suggests that the posterior reversible encephalopathy syndrome might have long-term consequences.

EDITORS’ COMMENTARY: Aukes et al describe long term neurological cognitive deficiencies in women who experienced eclamptic seizures at the time of delivery. Additional retrospective data from larger series and prospective studies are warranted.

Clinical trial of interconceptional antibiotics to prevent preterm birth: subgroup analyses and possible adverse antibiotic–microbial interaction
Alan T. N. Tita; Suzanne P. Cliver; Alice R. Goepfert; Michael Conner; Robert L. Goldenberg; John C. Hauth; William W. Andrews
Specific endometrial microbes may modify the effect of interconceptional antibiotics, and increase pregnancy loss and preterm birth.

EDITORS’ COMMENTARY: This thought-provoking manuscript challenges the concept that endometrial colonization, defined as a positive culture or plasma cell endometritis, is associated with adverse pregnancy outcome.

The association of crown-rump length discordance in twin gestations with adverse perinatal outcomes
Judy Tai; William A. Grobman
Differences in first trimester crown-rump length of >85th percentile are associated with several measures of adverse perinatal outcome. The hypothesis that is generated by this analysis is that differences in the growth of twins that have long been associated with adverse outcomes often have origins in the first trimester. Prospective studies to confirm this observation and to identify causes are needed.

EDITORS’ COMMENTARY: The hypothesis generated by this analysis is that differences in growth of twins that have long been associated with adverse outcomes often have their origins in the first trimester. Prospective studies to confirm this observation and to identify etiologies are needed.

Fetal cord blood mononuclear cells that are collected at term from HIV-1 infected women harbor transcriptionally active integrated proviral DNA
Jane E. Ellis; Gregory A. Hair; Michael K. Lindsay; Aftab A. Ansari; J. Bruce Sundstrom
Circulating fetal mononuclear cells that harbor actively replicating human immunodeficiency virus are present at term in some fetuses from pregnant women who are infected with human immunodeficiency virus and who receive highly active antiretroviral therapy and undergo elective cesarean delivery.

First-trimester risk assessment for Trisomies 21 and 18 in twin pregnancy
Stephen T. Chasen; Sriram C. Perni; Robin B. Kalish; Frank A. Chervenak
Use of maternal age, nuchal translucency, and biochemistry provides excellent first-trimester risk assessment in twins.
Changes in prepregnancy body mass index between pregnancies and risk of primary cesarean delivery
Darios Getahun; Lillian M. Kaminsky; Denise A. Elsasser; Russell S. Kirby; Cande V. Ananth; Anthony M. Vintzileos
Changes in prepregnancy body mass index between the first 2 pregnancies are associated with increased risk of primary cesarean delivery for most indications.

Prepregnancy body mass index and the length of gestation at term
Naomi E. Stotland; A. Eugene Washington; Aaron B. Caughey
Higher body mass index is associated with an increased risk of prolonged gestation in a cohort of women who deliver at ≥37 weeks’ gestation.

Pulmonary arteriole muscularization in lambs with diaphragmatic hernia after combined tracheal occlusion/glucocorticoid therapy
Marcus Davey; Shincy Shegu; Enrico Danzer; Eduardo Ruchelli; Scott Adzick; Alan Flake; Holly L. Hedrick
Prenatal exposure to glucocorticoids, shortly before birth, reduced medial hypertrophy of pulmonary arterioles in lambs with diaphragmatic hernia after tracheal occlusion therapy.

A cost decision analysis of 4 tocolytic drugs
Edward Hayes; Leslie Moroz; Laura Pizzi; Jason Baxter
Decision analysis revealed that nifedipine and indomethacin are superior tocolytics, based on cost.

Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption
Cande V. Ananth; Morgan R. Peltier; Celeste De Marco; Denise A. Elsasser; Darios Getahun; Rima Rozen; John C. Smulian; for the New Jersey–Placental Abruption Study Investigators
In this case-controlled study, 677C→T and 1298A→C polymorphisms in methylenetetrahydrofolate reductase were not associated with increased risk of placental abruption.

A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization
Michael Gavino; Eileen Wang
Rapid real-time polymerase chain reaction testing for group B streptococcus in a labor and delivery unit is feasible and appears to be a sensitive assay.

Duration of antimicrobial prophylaxis for group B streptococcus in patients with preterm premature rupture of membranes who are not in labor
Jesus R. Alvarez; Shauna F. Williams; Vijaya L. Ganesh; Joseph J. Apuzzio
Genital tract carriage of group B streptococcus can be eradicated after three days of antimicrobial prophylaxis in gravida with preterm premature rupture of membranes.
392 The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease
Jack Rychik; Zhiyun Tian; Michael Bebbington; Feng Xu; Margaret McCann; Stephanie Mann; R. Douglas Wilson; Mark P. Johnson
We describe the spectrum of cardiovascular abnormalities seen in twin-twin transfusion syndrome and propose a detailed cardiovascular scoring system for the assessment of disease severity.

394 Evolving trends in 2000 cases of multifetal pregnancy reduction: a single-center experience
Joanne Stone; Victoria Belogolovkin; Andrea Matho; Richard L. Berkowitz; Erin Moshier; Keith Eddleman
Trends over time were analyzed by starting and finishing numbers, reductions to a singleton pregnancy, use of chorionic villus sampling, and monochorionicity in 2000 patients who underwent multifetal pregnancy reduction.

396 A prospective, randomized, multicenter trial of amnioreduction vs selective fetoscopic laser photocoagulation for the treatment of severe twin-twin transfusion syndrome
Timothy M. Crombleholme; David Shera; Hanmin Lee; Mark Johnson; Mary D’Alton; Flint Porter; Jacquelyn Chyu; Richard Silver; Alfred Abuhamad; George Saade; Laurence Shields; David Kauffman; Joanne Stone; Craig T. Albanese; Ray Bahado-Singh; Robert H. Ball; Larissa Bilaniuk; Beverly Coleman; Diana Farmer; Vickie Feldstein; Michael R. Harrison; Holly Hedrick; Jeffrey Livingston; Robert P. Lorenz; David A. Miller; Mary E. Norton; William J. Polzin; Julian N. Robinson; Jack Rychik; Per L. Sandberg; Istvan Seri; Erin Simon; Lynn L. Simpson; Larisa Yedigarova; R. Douglas Wilson; Bruce Young
This prospective randomized trial comparing survival following serial amnioreduction vs selective fetoscopic laser photocoagulation for severe twin-twin transfusion syndrome was inconclusive in determining the superior treatment modality.

400 Do vaginal birth after cesarean outcomes differ based on hospital setting?
Emily A. DeFranco; Roxane Rampersad; Kristin L. Atkins; Anthony O. Odibo; Erika J. Stevens; Jeffrey F. Peipert; David M. Stamilio; George A. Macones
Vaginal birth after cesarean (VBAC) is attempted more commonly in university hospitals and hospitals with obstetrics-gynecology residency programs, but VBAC outcomes are similar, regardless of hospital setting.
Case-control comparison of cesarean hysterectomy with and without prophylactic placement of intravascular balloon catheters for placenta accreta
Vineet Shrivastava; Michael Nageotte; Carol Major; Michael Haydon; Deborah Wing
Prophylactic placement and deployment of intravascular balloon catheters failed to improve outcomes for women with placenta accreta undergoing cesarean hysterectomy. Three catheter-related complications are described.

Level of glycemic control and pregnancy outcome in type 1 diabetes: a comparison between multiple daily insulin injections and continuous subcutaneous insulin infusions
Rony Chen; Avi Ben-Haroush; Alina Weissman-Brenner; Nir Melamed; Moshe Hod; Yariv Yogev
In type 1 diabetes, continuous subcutaneous insulin infusions may be associated with a higher rate of both maternal diabetic ketoacidosis and neonatal hypoglycemic events.

Neonatal outcomes in pregnancies with preeclampsia or gestational hypertension and in normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation
Mounira Habli; Richard J. Levine; Cong Qian; Baha Sibai
Hypertensive pregnancies that delivered at 35, 36, and 37 weeks of gestation had increased adverse perinatal outcomes, compared with normotensive pregnancies.

A population study of the contribution of medical comorbidity to the risk of prematurity in blacks
Deborah B. Ehrenthal; Claudine Jurkovitz; Matthew Hoffman; Charlan Kroelinger; William Weintraub
Controlling for the higher prevalence of medical comorbidities in black women did not account fully for their increased risk for prematurity and low infant birthweight.

Metabolic score as a novel approach to assessing preeclampsia risk
Rebecca M. Mazar; Sindhu K. Srinivas; Mary D. Sammel; Christina M. Andrela; Michal A. Elovitz
Metabolic score, generated from the presence of elevated body mass index, chronic hypertension, and diabetes, is independently associated with the development of preeclampsia, particularly severe disease.

Randomized comparison of intravenous terbutaline vs nitroglycerin for acute intrapartum fetal resuscitation
Kristin M. Pullen; Edward T. Riley; Sarah A. Waller; Larisa Taylor; Aaron B. Caughey; Maurice L. Druzin; Yasser Y. El-Sayed
No difference in efficacy was noted between terbutaline and nitroglycerin for acute intrapartum fetal resuscitation.

Characterization of a murine model of fetal programming of atherosclerosis
Nima Goharkhay; Elena Sbrana; Phyllis K. Gamble; Esther H. Tamayo; Ancizar Betancourt; Karina Villarreal; Gary D. V. Hankins; George R. Saade; Monica Longo
We found a significant role of fetal programming on the incidence of hypercholesterolemia and atherosclerosis in the apoprotein E-deficient mouse model.
SELECTED PAPERS FROM THE 27TH ANNUAL SCIENTIFIC MEETING OF THE SOCIETY FOR MATERNAL–FETAL MEDICINE  (continued)

418 Gender-specific effect of overexpression of sFlt-1 in pregnant mice on fetal programming of blood pressure in the offspring later in life
Fangxian Lu; Egle Bytautiene; Esther Tamayo; Phyllis Gamble; Garland D. Anderson; Gary D. V. Hankins; Monica Longo; George R. Saade
Over-expression of sFlt-1 during pregnancy causes hypertension in adult male offspring, which highlights the role of the intrauterine environment in the developmental origin of health and disease.

420 Ontogeny of endothelial nitric oxide synthase mRNA in an ovine model of fetal and placental growth restriction
Bradley T. Ziebell; Henry L. Galan; Russell V. Anthony; Timothy R. H. Regnault; Thomas A. Parker; Juan A. Arroyo
Placental eNOS mRNA concentration is altered across gestation in an ovine model of IUGR.

422 Liver position and lung-to-head ratio for prediction of extracorporeal membrane oxygenation and survival in isolated left congenital diaphragmatic hernia
Holly L. Hedrick; Enrico Danzer; Aziz Merchant; Michael W. Bebbington; Huaqing Zhao; Alan W. Flake; Mark P. Johnson; Kenneth W. Liechty; Lori J. Howell; R. Douglas Wilson; N. Scott Adzick
Liver position is the best prenatal predictor of need for extracorporeal membrane oxygenation and survival in isolated left congenital diaphragmatic hernia.

424 The yeast connection: is Candida linked to breastfeeding associated pain?
Janet I. Andrews; Diedre K. Fleener; Shawn A. Messer; Wendy F. Hansen; Michael A. Pfaller; Daniel J. Diekema
Candida albicans was found more often in breastfeeding mothers with self-reported breast pain, compared with asymptomatic breastfeeding mothers.

426 Does knowledge of cervical length and fetal fibronectin affect management of women with threatened preterm labor? A randomized trial
Amen Ness; John Visintine; Emily Ricci; Vincenzo Berghella
Knowledge of cervical length and fetal fibronectin shortens the time for evaluation for women with suspected preterm labor without increasing the rate of preterm birth.

428 Does length of labor vary by maternal age?
Mara B. Greenberg; Yvonne W. Cheng; Margaret Sullivan; Mary E. Norton; Linda M. Hopkins; Aaron B. Caughey
Even after controlling for potential confounders, we found that older women have significantly longer labors than younger women, particularly in the second stage.

431 The validity of cervical dilation as an indication of true labor between 32 weeks and 36 weeks 6 days of gestation
Sangeeta Jain; Angela Earhart; Nicole Ruddock; Tony Wen; Gary D. V. Hankins; George R. Saade
Cervical dilation is not a good indicator of true labor in preterm labor.

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JOURNAL CLUB

433 Value of HPV testing in follow-up of treated high-risk CIN1: a study by Alonso et al
Premal Thaker; George A. Macones
See related article, page 359, and full discussion, page e1

IMAGES IN GYNECOLOGY

435 A diagnostic challenge
Nadia Kabli; Jocelyne Arseneau; Togas Tulandi

LETTERS TO THE EDITORS

436 The new extended regimen for the management of premenstrual symptoms needs to be properly assessed
Jeevan P. Marasinghe; Chintana K. Hapuachchige Don; A.A.W. Amarasinghe

436 Reply
Andrea L. Coffee; Thomas J. Kuehl; Sherilyn Willis; Patricia J. Sulak

437 Repeated antenatal steroid trial methodology
Simon Gates

438 Reply
Ronald Wapner; Elizabeth Thom; Catherine Spong

438 Genetic epidemiologic studies of preterm birth: studies of disease or of “rescue by birth”?
J. Frederik Frøen; Halit Pinar; Errol R. Norwitz

439 Reply
Craig E. Pennell; Louis J. Muglia; Caroline Relton

440 Reliability of a preventability model in maternal death and morbidity needs further assessment
Jeevan P. Marasinghe; W. I. Amarasinghe; A. A. W. Amarasinghe

440 Reply
Stacie E. Geller; Marci G. Adams

441 Elevated uterine activity increases the risk of fetal acidosis at birth
Ernest M. Graham

441 Reply
P.C.A.M. Bakker; P.H.J. Kurver; D.J. Kuik; H.P. Van Geijn

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e1 Discussion: ‘Value of HPV testing in follow-up of treated high-risk CIN1’
by Alonso et al
Moderator: Premal Thaker; discussants: Nora T. Kizer; Trung Nguyen;
Israel Zighelboim; Jenifer Allsworth

Case Reports

e5 Neoadjuvant chemotherapy plus radical surgery in locally advanced cervical cancer during pregnancy: a case report
Innocenza Palaia; Milena Pernice; Marialida Graziano; Filippo Bellati;
Pierluigi Benedetti Panici
Neoadjuvant chemotherapy plus radical surgery could be a valid option in locally advanced cervical cancer diagnosed during pregnancy.

e7 Successful continuation of pregnancy after repair of a midgestational uterine rupture with the use of a fibrin-coated collagen fleece (TachoComb) in a primigravid woman with no known risk factors
Izumi Shirata; Ritsu Fujiwaki; Kenji Takubo; Toshihiko Shibukawa;
Kohji Sawada
Successful use of fibrin-coated collagen fleece to repair a spontaneous midgestational uterine rupture permitting continuation of pregnancy is reported.

Worth 1000 words

The editors invite submissions to new sections of the Journal that focus on visually arresting clinical materials.

Images in Obstetrics, Images in Gynecology
Have you encountered a remarkable clinical image—a clinical photograph or results of a diagnostic test such as an ultrasound scan, MRI film, slide, photomicrograph, DNA blot, or similar—that elucidates some aspect of obstetrics or gynecology in a way that words alone could never achieve?

Share it with AJOG readers in one of these new sections, published in alternating issues.

A short version of the text, with 1 image, appears in print; a longer version, typically with more images, online.

Surgeon’s Corner
This quarterly offering describes a new surgical technique or instrument or a new application of a standard one. Step-by-step photos are welcome. AJOG’s print edition includes a sample image and synopsis, referring readers to the full-length version online. Authors are encouraged to provide a video clip, with discussion, to be posted online with the article.

For manuscript length and other requirements, please see the Information for Authors at www.AJOG.org.
Nasal bone in screening for trisomy 21: defining hypoplasia

Jiri Sonek, MD, RDMS

For well over a century, it has been recognized that 1 of the common dysmorphic features of individuals with Down syndrome is a comparatively small nose. Anthropometric studies have shown that 50% of these individuals have an abnormally short nasal root, which provides objective support for this observation. Radiologic studies in terminated second-trimester fetuses with trisomy 21 have demonstrated a lack of ossification of the nasal bone (NB) in 33% of the cases and an abnormally short NB in 23% of the cases. Over the past 7 years, accumulated literature has shown examination of the NB with prenatal ultrasound scanning to be useful in screening for trisomy 21 in both the first and second trimesters. The difference in the examinations is that only the presence or absence of the NB is noted in the first trimester; in the second trimester, NB length (NBL) measurement has also been shown to be informative.

Combined data from studies that were performed between 11-13 weeks of gestation and where a standardized approach to NB evaluation was used to yield a total of 35,213 fetuses that were examined. The NB was absent in 66.8% of fetuses with trisomy 21 and in 0.9% of euploid fetuses. A review of second-trimester studies (3014 fetuses at 15-26 weeks of gestation) showed a prevalence of NB absence in 37% of fetuses with trisomy 21 and 0.9% of chromosomally normal fetuses. Data from studies that looked at the prevalence of NB hypoplasia found it to be present in 48.2% of trisomy 21 fetuses and in 2.4% of chromosomally normal fetuses (n = 5726). The prevalence of either NB absence or hypoplasia was found to be 60% in trisomy 21 fetuses and 1.4% in chromosomally normal fetuses (n = 2240). Evaluation of the NB improves the performance of the second-trimester genetic ultrasound scans in a statistically significant way.

The definition of NB absence is clear, and the diagnosis is relatively easy to make in the second trimester. However, a number of different definitions have been used in the literature to describe NB hypoplasia. They fall into 3 general categories: NBL adjusted for gestational age by the use of biparietal diameter (BPD) to NBL ratio (BPD/NBL), a single measurement cut-off, and defining hypoplasia on the basis of gestational age-adjusted NBL distributions in normal fetuses.

The use of BPD/NBL ratios was introduced by Bromley et al, who studied 239 euploid fetuses and 16 fetuses with Down syndrome between 16 and 23 weeks of gestation. They reported that 37% of the Down syndrome fetuses had an absent NB. For a BPD/NBL of 10, the detection rate was 81%, for a screen positive rate of 11%. The NBL in the euploid fetuses increased linearly, whereas NBL in the fetuses with Down syndrome remained relatively constant across the gestational ages that were studied.

Cicero et al defined NB hypoplasia as an NBL of $\leq$2.5 mm. The prevalence of absent NB was 32.4%. The prevalence of either NB hypoplasia or absence in the 34 fetuses with Down syndrome was 61.8%, with a screen positive rate of 1.2%. The prevalence of NB hypoplasia was highest in persons of Afro-Caribbean descent (8.8%).

Finally, a number of studies have defined NB hypoplasia as an NBL below a certain percentile (5th or 2.5th), which results in detection rates that vary from 48.4%-100%.

In the current issue of the Journal, Odibo et al provide additional evidence that prenatal ultrasound evaluation of the NB is useful to assess the risk of trisomy 21. The authors successfully examined the NB in 3197 of 3634 fetuses (88% of the cases) between 15 and 23 weeks of gestation. These data were used to generate regressed means for each gestational week. For fetuses of ethnicities other than white, correction factors were used. Of the 23 cases of trisomy 21, 6 cases (37%) had an absent NB; in the remaining 17 cases, NBL was measured.

The authors first compared the sensitivities and specificities at the point at which the screening performance of the multiples of the median (MoM) and BPD/NBL approaches was deemed optimal (MoM, <0.75; or BPD/NBL, >11). The sensitivities were 49% and 61%, respectively. The specificities were 92% and 84%, respectively. The sensitivity of the MoM approach was lower than the BPD/NBL approach, but the difference was not statistically significant. However, the MoM approach was found to have a statistically higher specificity.

The authors also compared the 2 approaches by fixing either the sensitivity or the screen positive rate. For a fixed sensitivity of 40%, the screen positive rates were 1% and 5.5% for the MoM and the BPD/NBL approaches, respectively. For a fixed 5% screen positive rate, the detection rates between the 2 approaches were similar at 39% and 43%, respectively.

Certain data that would be helpful to evaluate these results are not available in the article. Comparison of the distributions of values that were obtained in fetuses of various ethnicities to determine the need for the use of correction factors, the gestational age at which trisomy 21 fetuses were examined, the actual NBL measurements in the trisomy 21 fetuses, and a discussion regarding the fact that the NBL normal ranges in this study are generally lower than those that have been published previously are not presented.

Nonetheless, the data presented by Odibo et al indicate that the MoM approach is superior to the BPD/NBL ap-
proach. There are theoretic reasons to suggest that BPD/NBL ratios may not provide optimal results. First, the ratio method uses 2 fetal measurements and therefore 2 potential sources of error. Second, BPD/NBL ratios may not be constant as gestational ages change. Generally, when a ratio is created from a marker and a component of biometry, the resultant values tend to change with gestation because the relationship between biometry and gestation is often curvilinear rather than linear. Third, the use of the same ratio threshold across gestational age does not take into consideration that the discriminatory power of the marker may be gestational age–related.

An additional benefit to the MoM approach is that it not only serves as a way to define NB hypoplasia but also allows generation of likelihood ratios (LR) for various NBL measurements. Using LR’s to modify an a priori risk is the most efficient way to use continuous variable markers, especially if they are gestational age-specific, in screening for trisomy 21.

An important unresolved issue is whether efficiency of screening by NB evaluation changes during the first half of the second trimester. There is evidence that NBL in fetuses with trisomy 21 does not increase in the same fashion as in euploid fetuses. Therefore, it is possible that efficiency of screening with NBL during the first half of the second trimester improves with advancing gestational age. At present, we favor the MoM approach in our center.

REFERENCES
Cervical cancer accounts for more gynecology-related deaths worldwide than any other malady, thus making it the most important preventable disease in woman’s health today. Although likely an underestimate, Parkin et al\(^1\) reported that cervical cancer affected 493,243 women worldwide in 2002, which makes it the second most common female cancer and the third most common cause of female cancer death, with 273,505 deaths reported. Another way to analyze the importance of cervical cancer to society is to evaluate the years of life lost by young and middle-aged women (25-64 years old). On a global basis, cancer of the cervix is responsible for approximately 2% of the total (weighted) years of life lost.\(^2\) However, it is the most important cause of years of life lost in Latin America and the Caribbean. Cervical cancer also contributes the largest portion to years of life lost from cancer in the populous regions of Sub-Saharan Africa and South-Central Asia, where the actual risk of loss of life from this cause is even higher, although it is somewhat overshadowed by deaths from noncancerous causes, such as acquired immunodeficiency disease and tuberculosis.

In the developed world in general and the United States specifically, cervical cancer incidence and mortality rates have declined approximately 75% over the past 3 decades. Still, the disease remains a serious health threat, with an estimated incidence and mortality rate of 11,150 and 3670 in 2007, respectively.\(^3\) Incidence rates for Hispanic and Asian, especially Vietnamese, women are higher than those for non-Hispanic non-Asian American women. In addition, the African American mortality rate continues to be more than double that of white women, even though the mortality rate for African American women has declined more rapidly than the rate for white women.

Cervical cancer is preventable and generally curable if detected early. Important strategies to reduce the risk of cervical cancer include screening through the use of the Papanicolaou test, human papillomavirus (HPV) testing, and prophylactic HPV vaccination. Researchers have identified HPV, which is transmitted through sexual contact, as the main cause of cervical cancer. Although the exact financial burden of HPV is unknown, it is estimated that the annual direct medical costs that are associated with cervical cancer treatment in the United States range between 300 and 400 million US dollars and that the annual direct medical costs that are associated with cervical intraepithelial neoplasia (CIN) in the United States range between 700 million and 2.3 billion US dollars.\(^4\)

So, what is the most cost-effective and efficient method to reduce the incidence and death from cervical cancer? Clearly, widespread HPV vaccination is the most promising approach.\(^5\) Using noninfectious virus-like particles, HPV vaccination has been shown to be virtually 100% effective in preventing persistent type-specific HPV infections and their neoplastic sequelae.\(^6\) Fortunately, idiosyncratic toxicities have not been reported with HPV vaccination, although the durability of the vaccine induced immunity is unknown.

The 2006 Consensus Guidelines for the Management of Women with Abnormal Cervical Cancer Screening Tests\(^7\) and The 2006 Consensus Guidelines for the Management of Women with Cervical Intraepithelial Neoplasia or Adenocarcinoma in-situ,\(^8\) published in this issue of the Journal, provide clinicians with cost-effective recommendations for managing abnormal Papanicolaou test results and treatment of precancerous cervical lesions. The 146 experts, who included representatives from 29 professional organizations, federal agencies, and national and international health organizations, are to be commended for proposing the best clinical practices for cervical cancer control. Importantly, these guidelines were formulated within a framework of uncertainty and incomplete data and were derived from many sources, considering multifaceted epidemiologic, economic, social, political, and cultural factors. These revised treatment guidelines from the American Society for Colposcopy and Cervical Pathology join the screening recommendations from the American College of Obstetricians and Gynecologists, the American Cancer Society, as well as the US Preventive Services Task Force in an attempt to standardize cervical cancer prevention practices in the United States.\(^9\)

These undated consensus guidelines incorporate some of the significant advances in our understanding of the natural history of HPV infections and CIN. In addition, the guidelines emphasize the extremely small chance of a serious lesion in lieu of a biopsy specimen that shows CIN I with a preceding low-grade Papanico- laou test result, even if the colposcopic examination is unsatisfactory. Finally, more emphasis is placed on immediate “screen and treat” approaches when treating women with high-grade Papani-
Papanicolaou test results. HPV DNA testing is also incorporated into the treatment of women with Papanicolaou test results that show atypical glandular cells after their initial evaluation with colposcopy and endometrial sampling.

The guidelines are a product of a comprehensive and strenuous review process by cervical health experts; nonetheless, there are some notable limitations, largely because of the paucity of data from sufficiently powered studies, especially in the area of surveillance intervals and the negative predictive value of colposcopy. Concern exists therefore for the impact of false-negative colposcopy results in patients who will undergo lengthened screening intervals. In addition, the small risk of missing an occult cancer is obvious when CIN 2 or 3 are managed conservatively. Finally, the guidelines make no mention of HPV vaccination, nor do they consider future revisions in existing screening recommendations. Clearly, revisions in our approach to cervical cancer screening and prevention are needed as our understanding of the complex epidemiology of HPV and cervical cancer evolves and HPV vaccination becomes more widespread. Further study and careful modeling in this area are needed desperately.

To achieve cost-effective reductions in the cervical cancer burden, prevention initiatives must consider screening and immunization as integrated and organized entities (Figure). Alternative screening approaches that capitalize on advances in molecular genetics will likely be adopted in the future. Some experts currently would advocate for potentially leveraging HPV testing as a primary screening test, followed by triage with Papanicolaou cytologic findings.10 This alternative strategy would have the added benefit of providing immunosurveillance in vaccinated populations.

Further study of alternative screening models is needed to develop a system that can realize the cost savings in screening that is necessary to offset the added cost of universal HPV vaccination. The current guidelines are a short step toward this end; unfortunately, their net effect will likely not realize this necessary and worthy goal. Finally, although universal vaccination of teenagers and young women is a desirable policy, ethical and cultural barriers must be conquered before HPV vaccination is adopted widely across all sectors.11

REFERENCES
Oncology

2006 consensus guidelines for the management of women with cervical intraepithelial neoplasia or adenocarcinoma in situ

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The appropriate management of women with cervical intraepithelial neoplasia (CIN) is as critical a component of cervical cancer prevention programs as screening and managing abnormal screening test results. CIN is a relatively common problem, especially in women of reproductive age. Laboratory surveys from the mid-1990s from the College of American Pathologists suggest that more than 1 million women are diagnosed each year with low-grade cervical intraepithelial lesions, referred to as CIN 1, and that approximately 500,000 are diagnosed with high-grade cervical cancer precursor lesions, referred to as CIN 2,3. More recent data from the Kaiser Permanente Northwest health plan indicate a somewhat lower rate, with a projected annual incidence per 1000 women of 1.2 for CIN 1 and 1.5 for CIN 2,3. Improper management of CIN can increase the risk of cervical cancer on the one hand and complications from overtreatment on the other. Approximately 5 years ago the American Society for Colposcopy and Cervical Pathology (ASCCP) joined other professional societies and federal and international organizations to develop the 2001 Consensus Guidelines for Managing Women with Cervical Intraepithelial Neoplasia. The goal was to minimize risks by weighing the best available evidence. Since 2001, considerable new information has become available on the natural history of CIN, particularly in adolescents and young women, and the impact of treatment for CIN on future pregnancies. Our understanding of how to manage women with cervical adenocarcinoma in situ (AIS), a human papillomavirus (HPV)-associated precursor to invasive cervical adenocarcinoma, also has progressed. Therefore, in 2005 the ASCCP and its partner organizations (listed in Appendix A), began the process of revising the 2001 consensus guidelines. This culminated in a consensus conference held at the National Institutes of Health in September 2006. This report provides the recommendations developed with respect to managing women with CIN and AIS. Recommendations for managing women with abnormal cervical cancer screening tests appear in an accompanying article. A more comprehensive discussion of the recommendations and their supporting evidence, algorithms, and a glossary of terms are available on the ASCCP website (www.asccc.org).

Key words: adenocarcinomas in situ of the cervix, cervical intraepithelial neoplasia, cryotherapy, loop electrosurgical excision procedure, treatment

GUIDELINE DEVELOPMENT PROCESS

The process used to develop the 2006 guidelines was similar to that for the 2001 guidelines and is described in depth in other publications. Guidelines were developed through a multistep process. Working groups initially defined ques-
CIN lesions. Ablative methods include the affected tissue are utilized for treating and excisional modalities that remove destroy the affected cervical tissue in vivo. The terms “recommended,” “preferred,” “acceptable,” and “unacceptable” are used to describe various interventions. The letters A through E are used to indicate “strength of recommendation” for or against the use of a particular option. Roman numerals I-III are used to indicate the “quality of evidence” for a given recommendation. The “strength of recommendation” and “quality of evidence” are provided in parenthesis after each recommendation.

2006 Consensus Guidelines

General comments

The histological classification incorporated into these guidelines is a 2-tiered system that applies the terms CIN 1 to low-grade lesions and CIN 2,3 to high-grade precursors. Cytological low-grade squamous intraepithelial lesion (LSIL) is not equivalent to histological CIN 1 and cytological high-grade squamous intraepithelial lesion (HSIL) is not equivalent to histological CIN 2,3.

It is important to recognize that these guidelines should never substitute for clinical judgment. Clinical judgment should always be used when applying a guideline to an individual patient because it is impossible to develop guidelines that apply to all situations.

Treatment methods

Both ablative treatment methods that destroy the affected cervical tissue in vivo and excisional modalities that remove the affected tissue are utilized for treating CIN lesions. Ablative methods include cryotherapy, laser ablation, electrosurgical needle conization, and cold coagulation. Excisional methods that provide a tissue specimen for pathological examination include cold-knife conization, loop electrosurgical excision procedures (widely referred to as LEEP or LLETZ), laser conization, and electrosurgical needle conization.

Although there are only a limited number of randomized trials comparing these different treatment modalities, it appears that all of the ablative and excisional modalities listed above have a similar efficacy with respect to eliminating CIN and reducing a woman’s risk of future invasive cervical cancer.

It has been recognized for some time that cold-knife conization increases a woman’s risk of future preterm labor, a low birthweight infant, and cesarean section. Other treatment methods were thought to have no adverse effects on future pregnancies. This is no longer the case. Several large retrospective series have now reported that women who have undergone a loop excision procedure or a laser conization are also at increased risk for future preterm delivery, a low birthweight infant, and premature rupture of membranes. Although in most studies ablative methods have not been shown to be associated with a similar adverse effect on pregnancy outcome, it is difficult to measure small effects on pregnancy outcome, and therefore, it is possible that ablative methods have an adverse effect on future pregnancies.

There are no accepted nonsurgical therapies for CIN. Several topical agents have been either evaluated or are in clinical trials, but none has been proven as effective as excision or ablation. Similarly, although there is considerable interest in therapeutic HPV vaccines, none have been proven effective.

These considerations indicate that the decision as to which therapeutic option to use in an individual patient depends on considerations such as patient age; parity; desire for future child-bearing; preferences; prior cytology and treatment history; and history of default from follow-up, operator experience, and nonvisualization of the transformation zone.

Posttreatment follow-up

The treatment failure rate for CIN using either ablative or excisional methods has varied between 1% and 25%. Systematic reviews indicate overall pooled failure rates of 5-15% for the different modalities with no significant difference between the modalities. Most failures occur within 2 years after treatment. In addition to developing recurrent/persistent CIN, women who have been treated for CIN 2,3 remain at increased risk for developing invasive cervical cancer for a protracted period of time. A recent systematic review reported that the incidence of invasive cervical disease in treated women remains about 56 per 100,000 for at least 20 years after treatment, substantially greater than that in the general US population (5.6 per 100,000 women-years). Therefore, follow-up is essential.

A number of follow-up protocols have been recommended. These include cytology, colposcopy, combinations of cytology and colposcopy, and HPV deoxyribonucleic acid (DNA) testing at a variety of intervals. None of the follow-up protocols have been evaluated in randomized clinical trials, and because the various follow-up approaches are so different, it is difficult to compare them. Systematic reviews of the performance of HPV DNA testing for post-treatment follow-up have found that its performance is quite good and exceeds that of cytological follow-up. Overall, the pooled sensitivity of HPV testing for identifying recurrent/persistent CIN reaches 90% by 6 months after treatment and has been shown to remain at this level for at least 24 months. In contrast, the pooled sensitivity of cytology is approximately 70%. In some studies, but not others, use of a combination of HPV testing and cytology resulted in an increased sensitivity.

Special populations

Adolescents (aged 13-20 years) and young women are considered a special population. There is a very low risk for invasive cervical cancer in this group, but CIN lesions are common. CIN in adolescents also has a very high rate of spontaneous regression of CIN lesions. Pregnant women are another special population. The risk of progression of CIN 2,3 to invasive cervical cancer during pregnancy is minimal, and the rate of spontaneous regression postpartum is relatively high. Treatment of CIN
during pregnancy is associated with complications and a high rate of recurrence or persistence. Therefore, the only indication for therapy of cervical neoplasia in pregnant women is invasive cancer.

**CIN 1**

Literature cited at the time of the 2001 Consensus Conference recognized that CIN 1 represents a heterogeneous group of lesions. This heterogeneity is due in large part to the poor reproducibility of a histological diagnosis of CIN 1. Less than half of lesions diagnosed as CIN 1 by individual pathologists are classified as CIN 1 when reviewed by a panel of pathologists. Although most of CIN 1 lesions are associated with high-risk types of HPV, the distribution of high-risk types in CIN 1 lesions is different from that seen in CIN 2,3 lesions. In addition, CIN 1 lesions can be associated with non–high-risk types of HPV.

CIN 1 lesions are also heterogeneous with respect to ploidy status and other markers of neoplasia.

There is a very high rate of spontaneous regression of low-grade cervical lesions in the absence of treatment. For example, a prospective study of Brazilian women with a cytological result of LSIL found that more than 90% regressed within 24 months. Another study from The Netherlands found that over 4 years all women with LSIL, who were infected with non–high-risk types of HPV regressed to normal cytology as did 70% of those infected with high-risk types of HPV. Even higher rates of regression occur in adolescents and young women. Moscicki et al found that 91% of adolescents and young women with LSIL spontaneously cleared their lesions with 36 months, irrespective of associated HPV type.

Recent data suggest that CIN 1 uncommonly progresses to CIN 2,3, at least within the first 24 months. In the ASCUS/LSIL Triage Study, many of the CIN 2,3 lesions subsequently identified in women diagnosed with CIN 1 appeared to represent lesions that were missed during the initial colposcopic evaluation. Risk for having a CIN 2,3 lesion identified during the subsequent 2 years after initial colposcopy was nearly identical in women with a histological diagnosis of CIN 1 (13%) and in women whose initial colposcopy and biopsy were negative (12%).

It should be noted that the risk of having an undetected CIN 2,3 or adenocarcinoma in situ lesion is expected to be greater in women with CIN 1 preceded by a HSIL or atypical glandular cells (AGC) cytology result than for women with CIN 1 preceded by an ASC or LSIL cytology result. CIN 2,3 is identified in 84-97% of women with HSIL cytology evaluated using a loop electrosurgical excision procedure. Therefore, in the 2006 guidelines, separate recommendations are made for women with CIN 1 preceded by an HSIL or AGC cytology result.

**Recommended management of women with CIN 1**

**CIN 1 preceded by atypical squamous cells of undetermined significance (ASC-US); atypical squamous cells, cannot exclude HSIL, ASC-H, or LSIL cytology.** The recommended management of women with a histological diagnosis of CIN 1 preceded by an ASC-US, ASC-H, or LSIL cytology is follow-up with either HPV DNA testing every 12 months or repeat cervical cytology every 6 to 12 months. (BII) If the HPV DNA test is positive or if repeat cytology is reported as ASC-US or greater, colposcopy is recommended. If the HPV test is negative or 2 consecutive repeat cytology tests are “negative for intraepithelial lesion or malignancy,” return to routine cytological screening is recommended. (AII)

If CIN 1 persists for at least 2 years, either continued follow-up or treatment is acceptable. (CII) If treatment is selected and the colposcopic examination is satisfactory, either excision or ablation is acceptable. (A1) A diagnostic excisional procedure is recommended if the colposcopic examination is unsatisfactory, the endocervical sampling contains CIN, or the patient has been previously treated. (AIII)

**CIN 1 preceded by HSIL or AGC-NOS cytology**

Either a diagnostic excisional procedure or observation with colposcopy and cytology at 6 month intervals for 1 year is acceptable for women with a histological diagnosis of CIN 1 preceded by HSIL or atypical glandular cells—not otherwise specified (AGC-NOS) cytology, provided in the latter case that the colposcopic examination is satisfactory and endocervical sampling is negative. (BIII) In this circumstance it is also acceptable to review the cytological, histological, and colposcopic findings; if the review yields a revised interpretation, management should follow guidelines for the revised interpretation. (BII)

If observation with cytology and colposcopy is elected, a diagnostic excisional procedure is recommended for women with repeat HSIL or AGC-NOS cytological results at either the 6- or 12-month visit. (CIII) After 1 year of observation, women with 2 consecutive “negative for intraepithelial lesion or malignancy” results can return to routine cytological screening. A diagnostic excisional procedure is recommended for women with CIN 1 preceded by a HSIL or AGC-NOS cytology in whom the colposcopic examination is unsatisfactory, except in special populations (eg, pregnant women). (BII)

**CIN 1 in special populations**

**Adolescent women.** Follow-up with annual cytological assessment is recommended for adolescents with CIN 1. (AII) At the 12 month follow-up, only
adolescents with HSIL or greater on the repeat cytology should be referred to colposcopy. At the 24 month follow-up, those with an ASC-US or greater result should be referred to colposcopy. (AII) Follow-up with HPV DNA testing is unacceptable. (EII)

**Pregnant women.** The recommended management of pregnant women with a histological diagnosis of CIN 1 is follow-up without treatment. (BII) Treatment of pregnant women for CIN 1 is unacceptable. (EII)

### CIN 2,3
CIN 2,3 includes lesions previously referred to as moderate dysplasia (i.e., CIN 2) and severe dysplasia/carcinoma in situ (i.e., CIN 3).36 Although CIN 2 lesions are more heterogenous and more likely to regress during long-term follow-up than are CIN 3 lesions, histological distinction between CIN 2 and CIN 3 is poorly reproducible.43–45 Therefore, CIN 2 is utilized as the threshold for treatment in the United States to provide an added measure of safety, and recommendations for the management of women with histologically diagnosed CIN 2 and CIN 3 are combined in the 2006 Consensus Guidelines.36

**Recommended management of women with CIN 2,3**

**Initial management.** Both excision and ablation are acceptable treatment modalities for women with a histological diagnosis of CIN 2,3 and satisfactory colposcopy, except in special circumstances (see following text). (AII) A diagnostic excisional procedure is recommended for women with recurrent CIN 2,3. (AII) Ablation is unacceptable and a diagnostic excisional procedure is recommended for women with a histological diagnosis CIN 2,3 and unsatisfactory colposcopy (AII). Observation of CIN 2,3 with sequential cytology and colposcopy is unacceptable, except in special circumstances (see following text). (EII) Hysterectomy is unacceptable as primary therapy for CIN 2,3. (EII)

**Follow-up after treatment**
Acceptable posttreatment management options for women with CIN 2,3 include HPV DNA testing at 6–12 months. (BII) Follow-up using either cytology alone or a combination of cytology and colposcopy at 6 month intervals is also acceptable. (BII) Colposcopy with endocervical sampling is recommended for women who are HPV DNA positive or have a repeat cytology result of ASC-US or greater. (BII) If the HPV DNA test is negative or if 2 consecutive repeat cytology tests are “negative for intraepithelial lesion or malignancy,” routine screening for at least 20 years commencing at 12 months is recommended. (AII) Repeat treatment or hysterectomy is recommended only if the appearance of the lesion worsens or if cytology suggests invasive cancer. (BII) Surveillance with cytology and colposcopy is recommended no sooner than 6 weeks postpartum. (CIII)

**CIN 2,3 in Special Populations**

**Adolescent and young women**
For adolescents and young women with a histological diagnosis of CIN 2,3 not otherwise specified, either treatment or observation for up to 24 months using both colposcopy and cytology at 6 month intervals is acceptable, provided colposcopy is satisfactory. (BIII) When a histological diagnosis of CIN 2 is specified, observation is preferred but treatment is acceptable. When a histological diagnosis of CIN 3 is specified or when colposcopy is unsatisfactory, treatment is recommended. (BIII)

If the colposcopic appearance of the lesion worsens or if HSIL cytology or a high-grade colposcopic lesion persists for 1 year, repeat biopsy is recommended. (BIII) After 2 consecutive “negative for intraepithelial lesion or malignancy” results, adolescents and young women with normal colposcopy can return to routine cytological screening. (BII)

Treatment is recommended if CIN 3 is subsequently identified or if CIN 2,3 persists for 24 months. (BII)

**Pregnant women**
In the absence of invasive disease or advanced pregnancy, additional colposcopic and cytological examinations are acceptable in pregnant women with a histological diagnosis of CIN 2,3 at intervals no more frequent than every 12 weeks. (BII) Repeat biopsy is recommended only if the appearance of the lesion worsens or if cytology suggests invasive cancer. (BII) Referral for reevaluation until at least 6 weeks postpartum is acceptable. (BII) A diagnostic excisional procedure is recommended only if invasion is suspected. (BII) Unless invasive cancer is identified, treatment is unacceptable. (EII) Reevaluation with cytology and colposcopy is recommended no sooner than 6 weeks postpartum. (CIII)

**AIS**
AIS is much less commonly encountered than is CIN 2,3. In 1991-1995 the overall incidence of squamous carcinoma in situ of the cervix in white women in the United States was 41.4 per 100,000, whereas the incidence of AIS was only 1.25 per 100,000.25 Although the overall incidence of AIS remains rather low, the incidence increased by approximately 6-fold from the 1970s to 1990s.25 Management of women with AIS is both challenging and controversial. Many of the assumptions that are used to justify conservative management approaches in women with CIN 2,3 lesions do not apply to AIS. For example, the colposcopic changes associated with AIS can be minimal, so it can be difficult to determine the extent of a lesion. AIS frequently extends for a considerable distance into the endocervical canal making complete excision difficult. AIS is also frequently multifocal and frequently has
“skip lesions” (ie, lesions which are not contiguous). Thus negative margins on a diagnostic excisional specimen do not necessarily mean that the lesion has been completely excised.

Because of these considerations hysterectomy continues to be the treatment of choice for AIS in women who have completed child-bearing. However, AIS often occurs in women who wish to maintain their fertility. A number of studies have now clearly demonstrated that an excisional procedure is curative in the majority these patients. The failure rate after an excisional procedure (eg, recurrent/persistent AIS or invasive adenocarcinoma) ranges from 0% to 9%.46-50 A comprehensive review of the published literature conducted in 2001 identified 16 studies that included a total of 296 women with AIS who had been treated with a diagnostic excisional procedure.49 The overall failure rate was 8%.5 Margin status is one of the most clinically useful predictors of residual disease.51-54 Recent data suggest that endocervical sampling at the time of an excisional biopsy is also predictive of residual disease.51 Some, but not all, studies have suggested that there is an increased recurrence rate as well as an increase in positive margins when a loop excision procedure as opposed to cold-knife conization is used.48,49,55 Irrespective of conization method, clinicians should remember that margin status and interpretability of the margins are important for future treatment planning and management. Moreover, it should be emphasized that an excisional biopsy is required in all women with AIS prior to making any subsequent management decisions.

**Recommended management of women with AIS**

Hysterectomy is preferred for women who have completed child-bearing and have a histological diagnosis of AIS on a specimen from a diagnostic excisional procedure. (CIII) Conservative management is acceptable if future fertility is desired. (AII) If conservative management is planned and the margins of the specimen are involved or endocervical sampling obtained at the time of excision contains CIN or AIS, reexcision to increase the likelihood of complete excision is preferred. Reevaluation at 6 months using a combination of cervical cytology, HPV DNA testing, and colposcopy with endocervical sampling is acceptable in this circumstance. Long-term follow-up is recommended for women who do not undergo hysterectomy. (CIII)

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**REFERENCES**

Since the publication of the 2001 consensus guidelines, new information has become available, which includes the key follow-up results from the National Cancer Institute (NCI)–sponsored ASCUS (atypical squamous cells of undetermined significance)/LSIL (low-grade squamous intraepithelial lesions) Triage Study (ALTS).\(^1,2\) Moreover, molecular testing for high-risk types of human papillomavirus (HPV) is being used together with cervical cytology for screening in women 30 years of age and older. Although “interim guidance” for the use of HPV DNA testing in the screening setting was proposed in 2004, recommendations for how to manage the combination of test results have not formally been evaluated by a large, multidisciplinary group.\(^3\) Once the 2001 guidelines were implemented in a variety of clinical settings, it became apparent that there were a number of areas in which changes were needed. This pertains particularly to special populations such as adolescents and postmenopausal women. Therefore, in 2005, the American Society for Colposcopy and Cervical Pathology (ASCCP), together with its partner professional societies and federal and international organizations (listed in Appendix A), began the process of revising the guidelines. This culminated in the 2006 consensus conference that was held at the National Institutes of Health in September 2006. This report provides the recommendations developed with respect to managing women with cytological abnormalities. Recommendations for managing women with cervical intraepithelial neoplasia (CIN) or adenocarcinoma in situ (AIS) appear in the accompanying article. A more comprehensive discussion of the recommendations and their supporting evidence will be made available on the ASCCP website (www.asccp.org).

**Guideline Development Process**

The process used to develop the 2006 Consensus Guidelines was similar to that for the previous guidelines and is discussed in depth in other publications.\(^4,5\) Guidelines were developed through a multistep process. Working groups reviewed literature published after 2000 before developing guidelines that were subsequently revised based on input from the professional community at large, obtained using an Internet-based bulletin board. At the consensus conference, guidelines with supporting evidence were presented and underwent discussion, revision, and approval. The terminology utilized in the new guidelines is identical to that used previously, as is the 2-part rating system (Table).\(^4,5\) The terms “recommended,” “preferred,” “acceptable,” and “unacceptable” are used in the guidelines to describe various interventions. The letters A through E are used to indicate strength of recommendation for or against the use of a particular option. Roman numerals I-III are
used to indicate the “quality of evidence” for a given recommendation. The “strength of recommendation” and “quality of evidence” are provided in parentheses after each recommendation.

**2006 Consensus Guidelines**

**General comments**

Although the guidelines are based on evidence whenever possible, for certain clinical situations, there is limited high-quality evidence, and in these situations the guidelines have, by necessity, been based on consensus expert opinion. It is also important to recognize that these guidelines should never substitute for clinical judgment. Clinical judgment should always be used when applying a guideline to an individual patient because it is impossible to develop guidelines that apply to all situations.

The 2001 Bethesda System terminology is used for cytologic classification. This terminology utilizes the terms low-grade squamous intraepithelial lesion (LSIL) and high-grade squamous intraepithelial lesion (HSIL) to refer to low-grade lesions and high-grade cervical cancer precursors, respectively. The histologic classification used is a 2-tiered system that applies the terms CIN 1 to low-grade lesions and CIN 2,3 to high-grade precursors. It is important to note that cytologic LSIL is not equivalent to histologic CIN 1 and cytologic HSIL is not equivalent to histologic CIN 2,3. Algorithms detailing the different management recommendations are available at the ASCCP website ([www.asccp.org](http://www.asccp.org)). A glossary of terms used in the guidelines is in Appendix B.

The current guidelines expand clinical indications for HPV testing based on studies using validated HPV assays. One cannot assume that management decisions that are based on results of HPV tests that have not been similarly validated will result in the outcomes that are intended by these guidelines. Furthermore, the application of these guidelines using such tests may increase the potential for patient harm. The appropriate use of these guidelines requires that laboratories utilize only HPV tests that have been analytically and clinically validated with proven acceptable reproducibility, clinical sensitivity, specificity, and positive and negative predictive values for cervical cancer and verified precursor (CIN 2,3), as documented by Food and Drug Administration (FDA) approval and/or publication in peer-reviewed scientific literature. It is also important to stress that testing should be restricted to high-risk (oncogenic) HPV types. Testing for low-risk (nononcogenic) HPV types has no role in the evaluation of women with abnormal cervical cytological results. Therefore, whenever “HPV testing” is referred to in the guidelines, it applies only to testing for high-risk (oncogenic) HPV types.

**Special populations**

The exact same cytologic result has a different risk of CIN 2,3 or cancer (CIN 2+) in various groups of women. One such special population is adolescent women (aged 20 years and younger) who have a high prevalence of HPV infections, more minor-grade cytologic abnormalities (atypical squamous cells [ASC] and LSIL) but very low risk for invasive cervical cancer, compared with older women. This is because the vast majority of HPV infections spontaneously clear within 2 years after infection and are of little long-term clinical significance. Therefore, performing colposcopy for minor cytologic abnormali-
ties in adolescents should be discouraged because it can potentially result in harm through unnecessary treatment.

Pregnant women are also considered a special population. The only indication for therapy of cervical neoplasia in pregnant women is invasive cancer. Therefore, it is reasonable to defer colposcopy in pregnant women at low risk for having cancer. Finally, it should be cautioned that endocervical curettage is contraindicated in pregnant patients.

**Atypical squamous cells**

ASC is subcategorized into atypical squamous cells of undetermined significance (ASC-US) and atypical squamous cells, cannot exclude HSIL (ASC-H). There are several factors that need to be taken into consideration when managing women with ASC. One is that a cytological result of ASC is the least reproducible of all cytologic categories. Another is that the prevalence of invasive cancer is low in women with ASC (approximately 0.1-0.2%). Finally, it is important to note that the prevalence of CIN 2,3 is higher among women with ASC-H than women with ASC-US. Because of this, ASC-H should be considered to represent equivocal HSIL.

Clinical data from ALTS and other studies have demonstrated that 2 repeat cytologic examinations performed at 6-month intervals, testing for HPV, and a single colposcopic examination are all safe and effective approaches to managing women with ASC-US. Therefore, the 2001 Consensus Guidelines recognized that all 3 approaches were acceptable for managing women with ASC-US. The scientific basis for the 2001 recommendation has been strengthened over the last 5 years by additional clinical studies, additional analyses of the ALTS data, and metaanalyses of published studies. "Reflex" testing refers to testing either the original liquid-based cytology residual specimen or a separate sample collected at the time of the initial screening visit for HPV testing. This approach eliminates the need for women to return to the office or clinic for repeat testing, rapidly assures many women that they do not have a significant lesion, spares 40-60% of women from undergoing colposcopy, and has been shown to have a favorable cost-effectiveness ratio.

Because a single colposcopic examination can miss significant lesions, women who are referred for colposcopy and found not to have CIN 2,3 require additional follow-up. ALTS evaluated different postcolposcopy follow-up strategies and found that HPV testing performed 12 months after the initial colposcopy and 2 repeat cytology examinations performed at 6 month intervals performed similarly. Combining cytology with HPV testing did not increase sensitivity and reduced specificity.

**Special populations**

The prevalence of HPV DNA positivity changes with age among women with ASC-US. Rates of HPV DNA positivity are much higher in younger, compared with older, women with ASC-US. Thus, using HPV testing to manage adolescents with ASC-US would refer large numbers of women at low risk for having cancer to colposcopy. ASC-US is less common in postmenopausal than premenopausal women, and the risk of significant pathology in postmenopausal women with a history of cervical screening is relatively low. HPV testing is actually more efficient in older, compared with younger, women with ASC-US because it refers a lower proportion to colposcopy.

ASC-US is quite common in HIV-infected women. Previously, based on studies that had reported a high prevalence of both HPV DNA positivity and significant cervical pathology in this population, it was recommended that all immunosuppressed women with ASC-US undergo colposcopy. More recent studies have found a lower prevalence of CIN 2,3 and HPV DNA positivity; therefore, immunosuppressed women should be managed in the same manner as women in the general population. The risk of cancer is relatively low among pregnant women with ASC-US, and some studies have found that antepartum colposcopic evaluation does not add to management.

**Recommended Management of Women with ASC-US**

**General management approaches**

A program of DNA testing for high-risk (oncogenic) types of HPV, repeat cervical cytologic testing, or colposcopy are all acceptable methods for managing women over the age of 20 years with ASC-US. (A1) When liquid-based cytology is used or when cocollection for HPV DNA testing can be done, “reflex” HPV DNA testing is the preferred approach. (A1)

Women with ASC-US who are HPV DNA negative can be followed up with repeat cytologic testing at 12 months. (BII) Women who are HPV DNA positive should be managed in the same fashion as women with LSIL and be referred for colposcopic evaluation. (AII) Endocervical sampling is preferred for women in whom no lesions are identified (BII) and those with an unsatisfactory colposcopy (AII) but is acceptable for women with a satisfactory colposcopy and a lesion identified in the transformation zone. (CII) Acceptable postcolposcopy management options of women with ASC-US who are HPV positive, but in whom CIN is not identified, are HPV DNA testing at 12 months or repeat cytological testing at 6 and 12 months. (BII) It is recommended that HPV DNA testing not be performed at intervals less than 12 months. (EIII)

When a program of repeat cytologic testing is used for managing women with ASC-US, it is recommended that cytologic testing be performed at 6-month intervals until 2 consecutive “negative for intraepithelial lesion or malignancy” results are obtained. (AII) Colposcopy is recommended for women with ASC-US or greater cytologic abnormality on a repeat test. (AII) After 2 repeat “negative for intraepithelial lesion or malignancy” results are obtained, women can return to routine cytologic screening. (AII)

When colposcopy is used to manage women with ASC-US, repeat cytologic testing at 12 months is recommended for women in whom CIN is not identified.
(BIII) Women found to have CIN should be managed according to the 2006 Consensus Guidelines for the Management of Cervical Intraepithelial Neoplasia.

Because of the potential for overtreatment, the routine use of diagnostic excisional procedures such as the loop electrosurgical excision procedure is unacceptable for women with an initial ASC-US in the absence of histologically diagnosed CIN 2,3. (EII)

ASC-US IN SPECIAL POPULATIONS

Adolescent women

In adolescents with ASC-US, follow-up with annual cytologic testing is recommended. (BII) At the 12-month follow-up, only adolescents with HSIL or greater on the repeat cytology should be referred to colposcopy. At the 24-month follow-up, those with an ASC-US or greater result should be referred to colposcopy. (AI) HPV DNA testing and colposcopy are unacceptable for adolescents with ASC-US. (EII) If HPV testing is inadvertently performed, the results should not influence management.

Immunosuppressed and postmenopausal women

HIV-infected, other immunosuppressed women, and postmenopausal women with ASC-US should be managed in the same manner as women in the general population. (BII)

Pregnant women

Management options for pregnant women over the age of 20 years with ASC-US are identical to those described for nonpregnant women, with the exception that it is acceptable to defer colposcopy until at least 6 weeks postpartum. (CIII) Endocervical curettage is unacceptable in pregnant women. (EII)

RECOMMENDED MANAGEMENT OF WOMEN WITH ASC-H

The recommended management of women with ASC-H is referral for colposcopic evaluation. (AI) In women in whom CIN 2,3 is not identified, follow-up with HPV DNA testing at 12 months or cytological testing at 6 and 12 months is acceptable. (CIII) Referral to colposcopy is recommended for women who subsequently test positive for HPV DNA or who are found to have ASC-US or greater on their repeat cytologic tests. (BII) If the HPV DNA test is negative or if 2 consecutive repeat cytologic tests are negative for intraepithelial lesion or malignancy, return to routine cytologic screening is recommended. (AI)

LSIL

Over the last decade, the rate of LSIL has increased in the United States and in 2003 the mean LSIL reporting rate was 2.9% for liquid-based specimens. A result of LSIL is a good indicator of HPV infection. A recent metaanalysis reported that the pooled estimate of high-risk (oncogenic) HPV DNA positivity among women with LSIL was 76.6%. The prevalence of CIN 2 or greater identified at initial colposcopy among women with LSIL is 12-16%. Data from ALTS indicate that the risk of CIN 2,3 is the same in women with LSIL and those with ASC-US who are high risk (oncogenic) HPV DNA positive. This supports managing both groups of women in an identical manner except in special populations such as postmenopausal women.

SPECIAL POPULATIONS

Adolescents

Prospective follow-up studies of adolescents with LSIL have shown very high rates of regression to normal, although it is not unusual for regression to take years to occur. As with ASC-US, the high prevalence of HPV DNA positivity in adolescents with LSIL makes HPV testing of little value in this population. Some, but not all, studies have found that the prevalence of both HPV DNA positivity and CIN 2,3 decline with age in women with LSIL. This suggests that postmenopausal women with LSIL can be managed less aggressively than premenopausal women and that triage using HPV testing may be attractive.

Recommended Management of Women with LSIL

Colposcopy is recommended for managing women with LSIL, except in special populations (see following text). (AI) Endocervical sampling is preferred for nonpregnant women in whom no lesions are identified (BII) and those with an unsatisfactory colposcopy (AI), but is acceptable for those with a satisfactory colposcopy and a lesion identified in the transformation zone. (CII) Acceptable postcolposcopy management options for women with LSIL cytology in whom CIN 2,3 is not identified are testing for high-risk (oncogenic) types of HPV at 12 months or repeat cervical cytologic testing at 6 and 12 months. (BII) If the HPV DNA test is negative or if 2 consecutive repeat cytologic tests are negative for intraepithelial lesion or malignancy, return to routine cytologic screening is recommended. (AI) If either the HPV DNA test is positive or if repeat cytology is reported as ASC-US or greater, colposcopy is recommended. (AI) Women found to have CIN should be managed according to the appropriate 2006 Consensus Guidelines on the Management of Cervical Intraepithelial Neoplasia. In the absence of CIN identified histologically, diagnostic excisional or ablative procedures are unacceptable for the initial management of patients with LSIL. (EII)

LSIL IN SPECIAL POPULATIONS

Adolescents

In adolescents with LSIL, follow-up with annual cytologic testing is recommended. (AI) At the 12-month follow-up, only adolescents with HSIL or greater on the repeat cytology should be referred to colposcopy. At the 24-month follow-up, those with an ASC-US or greater result should be referred to colposcopy. (AI) HPV DNA testing and colposcopy are unacceptable for adolescents with ASC-US. (EII) If HPV testing is inadvertently performed, the results should not influence management.

Postmenopausal women

Acceptable options for the management of postmenopausal women with LSIL include “reflex” HPV DNA testing, repeat...
cytological testing at 6 and 12 months, and colposcopy. (CIII) If the HPV DNA test is negative or CIN is not identified at colposcopy, repeat cytology in 12 months is recommended. If either the HPV DNA test is positive or the repeat cytology is ASC-US or greater, colposcopy is recommended. (AI) If 2 consecutive repeat cytologic tests are negative for intraepithelial lesion or malignancy, return to routine cytologic screening is recommended.

Pregnant women

Colposcopy is preferred for pregnant, nonadolescent women with LSIL cytology. (BII) Endocervical curettage is unacceptable in pregnant women. (EIII) Deferring the initial colposcopy until at least 6 weeks postpartum is acceptable. (BIII) In pregnant women who have no cytologic, histologic, or colposcopically suspected CIN 2,3 or cancer at the initial colposcopy, postpartum follow-up is recommended. (BIII) Additional colposcopic and cytologic examinations during pregnancy are unacceptable for these women. (DIII)

HSIL

The mean reporting rate of HSIL in US laboratories is 0.7%. The rate of HSIL varies with age. In 1 US center, the rate of HSIL in women 20-29 years of age is 0.6%, compared with 0.2% and 0.1% in women 40-49 years and 50-59 years of age, respectively. The finding of a HSIL in women 20-29 years of age is 0.6%, compared with 0.2% and 0.1% in women 40-49 years of age and 50-59 years of age, respectively. The finding of a HSIL in women 40-49 years and 50-59 years of age is 0.6%, compared with 0.2% and 0.1% in women 20-29 years of age.

Recommended Management of Women with HSIL

An immediate loop electrosurgical excision or colposcopy with endocervical assessment is an acceptable method for managing women with HSIL, except in special populations (see following text). (BII) When CIN 2,3 is not identified histologically, either a diagnostic excisional procedure or observation with colposcopy and cytology at 6 month intervals for 1 year is acceptable, provided in the latter case that the colposcopic examination is satisfactory and endocervical sampling is negative. (BII) In exceptional circumstances, a diagnostic excisional procedure is acceptable. (BIII) If during follow-up a high-grade colposcopic lesion is identified or HSIL cytology persists for 1 year, biopsy is recommended. (BIII) If CIN 2,3 is identified histologically, management should follow the 2006 Consensus Guideline for the Management of Women with Cervical Intraepithelial Neoplasia. (BII) If HSIL persists for 24 months without identification of CIN 2,3, a diagnostic excisional procedure is recommended. (BIII) After 2 consecutive “negative for intraepithelial lesion or malignancy” results, adolescents and young women without a high-grade colposcopic abnormality can return to routine cytological screening. (BIII) A diagnostic excisional procedure is recommended for adolescents and young women with HSIL when colposcopy is unsatisfactory or CIN of any grade is identified on endocervical assessment (BII).

HSIL in Special Populations

Adolescent women

In adolescents with HSIL, colposcopy is recommended. Immediate loop electrosurgical excision (ie, “see-and-treat”) is unacceptable in adolescent women. (AI) If CIN 2,3 is not identified histologically, observation for up to 24 months using both colposcopy and cytology at 6-month intervals is preferred, provided the colposcopic examination is satisfactory and endocervical sampling is negative. (BIII) In exceptional circumstances, a diagnostic excisional procedure is acceptable. (BIII) If during follow-up a high-grade colposcopic lesion is identified or HSIL cytology persists for 1 year, biopsy is recommended. (BIII) If CIN 2,3 is identified histologically, management should follow the 2006 Consensus Guideline for the Management of Women with Cervical Intraepithelial Neoplasia. (BII) If HSIL persists for 24 months without identification of CIN 2,3, a diagnostic excisional procedure is recommended. (BIII) After 2 consecutive “negative for intraepithelial lesion or malignancy” results, adolescents and young women without a high-grade colposcopic abnormality can return to routine cytological screening. (BIII) A diagnostic excisional procedure is recommended for adolescents and young women with HSIL when colposcopy is unsatisfactory or CIN of any grade is identified on endocervical assessment (BII).

Pregnant women

Colposcopy is recommended for pregnant women with HSIL. (AI) It is preferred that the colposcopic evaluation of pregnant women with HSIL be conducted by clinicians who are experienced in the evaluation of colposcopic changes induced by pregnancy. (BIII) Biopsy of lesions suspicious for CIN 2,3 or cancer
is preferred; biopsy of other lesions is acceptable (BIII). Endocervical curettage is unacceptable in pregnant women. (EIII) Diagnostic excision is unacceptable unless invasive cancer is suspected based on the referral cytology, colposcopic appearance, or cervical biopsy. (EII) Re-evaluation with cytology and colposcopy is recommended no sooner than 6 weeks postpartum for pregnant women with HSIL in whom CIN 2,3 is not diagnosed. (CII)

**Atypical glandular cells (AGC)**

AGC results are relatively uncommon, with a mean reporting rate of only 0.4% in the United States in 2003. Although AGC is frequently caused by benign conditions, such as reactive changes and polyps, clinicians should be aware that it is not uncommon for AGC to be associated with a significant underlying neoplastic condition including adenocarcinomas of the cervix, endometrium, ovary, and fallopian tube. Recent series have reported that 9-38% of women with AGC have significant neoplasia (CIN 2,3, AIS, or cancer), and 3-17% have invasive cancer.55,56,57

The rate and type of significant findings in women with AGC varies with age. Although a variety of glandular lesions, including malignancies, are associated with AGC, CIN is the most common significant finding identified in women with AGC.58 Gynecologic malignancy is less common in women under the age of 35 years than in older women.54 Pregnancy does not appear to change the underlying associations between AGC and gynecologic neoplasia.

Neither HPV testing nor repeat cervical cytology has the requisite sensitivity to be utilized alone as an initial triage test for women with AGC.55,56 Because of the spectrum of neoplasia linked to AGC, initial evaluation must include multiple testing modalities.55,56 These include colposcopy, endocervical evaluation and sampling, HPV testing, and endometrial evaluation. Because of the high incidence of neoplasia and the poor sensitivity of all test modalities, diagnostic excisional procedures may be necessary, despite initial negative testing, for women with AGC-favor neoplasia,” AIS, or repeat AGC cytology.4

**Other Glandular Abnormalities**

Benign-appearing endometrial cells in a woman 40 years of age and older and endometrial stromal cells or histiocytes are occasionally encountered cytologically. Approximately 0.5-1.8% of cervical cytology specimens from women 40 years of age and older will have endometrial cells.61 Benign-appearing exfoliated endometrial cells in premenopausal women are rarely associated with significant pathology.61 Similarly, the presence of endometrial stromal cells/histiocytes rarely has clinical significance. In contrast, benign-appearing endometrial cells in postmenopausal women are not infrequently associated with significant endometrial pathology.62 Although hormone replacement therapy can increase the rate of shedding of benign-appearing endometrial cells, the prevalence of significant pathology remains elevated in this setting.51,62 Benign-appearing glandular cells derived from small accessory ducts, foci of benign adenosis, or prolapse of the fallopian tube into the vagina are sometimes seen in cytology specimens after total hysterectomy and have no clinical significance.

**Recommended Management of Women with AGC**

**Initial workup**

Colposcopy with endocervical sampling is recommended for women with all subcategories of AGC and AIS. (AII) Endometrial sampling is recommended in conjunction with colposcopy and endocervical sampling in women 35 years and older with all subcategories of AGCs and AIS. (BII) Endometrial sampling is also recommended for women under the age of 35 years with clinical indications suggesting they may be at risk for neoplastic endometrial lesions. These include unexplained vaginal bleeding or conditions suggesting chronic anovulation. It is recommended that women with atypical endometrial cells be initially evaluated with endometrial and endocervical sampling. Colposcopy can be either performed at the initial evaluation or deferred until the results are known. If no endometrial pathology is identified, colposcopy is recommended. (AII) If not already obtained, HPV DNA testing at the time of colposcopy is preferred in women with atypical endocervical, endometrial, or glandular cells not otherwise specified (NOS). (CII) The use of HPV DNA testing alone or a program of repeat cervical cytology is unacceptable for the initial triage of all subcategories of AGC and AIS. (EII)

**Subsequent evaluation or follow-up**

The recommended postcolposcopy management of women of unknown HPV status with atypical endocervical, endometrial, or glandular cells NOS who do not have CIN or glandular neoplasia identified histologically is to repeat cytologic testing combined with HPV DNA testing at 6 months if they are HPV DNA positive and at 12 months if they are HPV DNA negative. (CII) Referral to colposcopy is recommended for women who subsequently test positive for high-risk (oncogenic) HPV DNA or who are found to have ASC-US or greater on their repeat cytologic tests. If both tests are negative, women can return to routine cytologic testing. (BII) The recommended postcolposcopy management of women of unknown HPV status with atypical endocervical, endometrial, or glandular cells NOS who do not have CIN or glandular neoplasia identified histologically is to repeat cytologic testing at 6-month intervals. After 4 consecutive “negative for intraepithelial lesion or malignancy” results are obtained, women can return to routine cytologic testing. (CIII)

If CIN, but no glandular neoplasia, is identified histologically during the initial workup of a woman with atypical endocervical, endometrial, or glandular cells NOS, management should be according to the 2006 Consensus Guidelines for the Management of Women with Cervical Intraepithelial Neoplasia. If invasive disease is not identified during the initial colposcopic workup, it is recommended that women with atypical endocervical...
or glandular cells “favor neoplasia” or endocervical AIS undergo a diagnostic excisional procedure. (AII) It is recommended that the type of diagnostic excisional procedure used in this setting provide an intact specimen with interpretable margins. (BII) Concomitant endocervical sampling is preferred. (BII)

**AGC in Special Populations**

**Pregnant women**

In pregnant women, the initial evaluation of AGC should be identical to that of nonpregnant women, except that endocervical curettage and endometrial biopsy are unacceptable. (BII)

**Other Forms of Glandular Abnormalities**

**Benign-appearing endometrial cells**

For asymptomatic premenopausal women with benign endometrial cells, endometrial stromal cells, or histiocytes, no further evaluation is recommended. (BII) For postmenopausal women with benign endometrial cells, endometrial assessment is recommended regardless of symptoms. (BII)

**Benign-appearing glandular cells after hysterectomy**

For posthysterectomy patients with a cytolologic report of benign glandular cells, no further evaluation is recommended. (BII)

**HPV DNA Testing When Used for Screening**

Despite the successes of cytology as a cervical cancer screening method, cytology has a number of significant limitations.63 These limitations have led to considerable interest in using a combination of HPV testing and cytology as screening.64 Most newly acquired HPV infections clear spontaneously and the prevalence of HPV DNA positivity drops with age from a peak in adolescents and women in their 20s.1,65 Therefore, HPV testing should be used only for routine screening in women 30 years of age and older.1,66 A number of large studies have evaluated screening using a combination of HPV testing and cervical cytology (either liquid-based or conventional cytology).67,68 In screening studies from North America and Europe, the pooled sensitivity and specificity of HPV testing for the detection of CIN 2 or greater in women 35 years and older is 95% and 93%, respectively.69 For comparison, pooled sensitivity and specificity of cytology at a threshold of ASC-US are 60% and 97%, respectively. Sensitivity using a combination of HPV testing and cytology is significantly higher than that of either test alone with negative predictive values of 99-100%.69,70 Molecular testing for high-risk (oncopgenic) types of HPV is now approved by the FDA for use as an adjunct to cervical cytology for screening in women 30 years of age and older.71 Interim guidance on how to manage women with different combinations of screening results was developed by a NCI, ASCCP, and American Cancer Society joint workshop in 2003.3 The 2006 Consensus Conference formally reviewed and modified the previous interim guidance. The two controversial areas are when women negative by both cytology and HPV testing should be rescreened and how to manage cytology-negative, HPV-positive women. Women who are negative by both cytology and HPV testing have a less than 1 in 1000 risk of having CIN 2 or greater, and prospective follow-up studies have shown that the risk of developing CIN 3 over a 10-year period is quite low.3,72,73 Less than 2% of cytology- and HPV-negative Danish women 40-50 years of age developed CIN 3 or greater during 10 years of follow-up.72 Identical results have been reported for women 30 years of age and older in Portland, OR.73 Health policy modeling studies demonstrate that 3 year screening using a combination of cytology and HPV testing in women 30 years and older provides equivalent or greater benefits than those provided by annual conventional cytology.74 Therefore, women who are negative by both cytology and HPV testing should not be rescreened before 3 years.

Many women in screened populations who test positive for HPV will have a negative cervical cytology. In a series of more than 213,000 women 30 years and older enrolled in Kaiser Northern California, the overall prevalence of HPV positivity was 6.5%, and 58% of the HPV-positive women had a concurrent negative cytology.60 HPV-positive women require counseling with respect to their risk for CIN 2 or greater, source of their infection, and their infectivity. The risk of having an undetected CIN 2 or greater is quite low in cytology-negative, HPV-positive women in screened populations, ranging from 2.4-5.1%.75-78 For comparison, CIN 2 or greater was detected at enrollment colposcopy in 10.2% of women of unknown HPV status with ASC-US in ALTS.1 It is also important to note that even in women 30 years and older, the majority of HPV-positive women become HPV negative during follow-up. After a median follow-up of 6 months, 60% of HPV-positive women in a prospective study from France became HPV-negative.78 Based on these considerations, conservative follow-up with repeat cytology and HPV testing at 12 months appears to be the best management approach for cytology-negative, HPV-positive women. Women who on repeat testing are persistently HPV positive should undergo colposcopy, whereas women who are negative on both tests can be rescreened in 3 years.

**HPV Genotyping**

Emerging data suggest that the specific type of high-risk (oncogenic) HPV that a woman has may be an important indicator of her risk for CIN 2 or greater. Among cytology-negative women 30 years of age and older in the Portland study, CIN 3 was identified during 10 years of follow-up in 21% and 18% of those with HPV 16 or 18, respectively, at enrollment.73 In contrast, the risk of CIN 3 among women with other high-risk HPV types was only 1.5%. Schlecht et al79 also found a higher incidence of cytological HSIL during follow-up in Brazilian women who were positive for HPV 16 or 18, compared with women with other high-risk HPV types, although the difference in incidence was not as marked as observed in Portland.

Genotyping assays to determine specific high-risk HPV type(s) have not
yet been approved by the FDA. However, should the FDA approve HPV genotyping assays, it would be reasonable to utilize genotyping in cytology-negative, HPV-positive women in the same manner as high-risk HPV testing is utilized in women with ASC-US. Samples from cytology-negative, HPV-positive women would be genotyped for specific high-risk types of HPV, and women with specific high-risk types, such as HPV 16 or 18, would be referred for colposcopy.73 Women with other high-risk types would be told to return in 12 months for retesting for both cytology and HPV. This would allow women at increased risk for having a false-negative cytology result to be referred to colposcopy.

**Recommended Management Different Combinations of Results**

**General recommendations**

It is recommended that HPV DNA testing target only high-risk (oncogenic) HPV types. There is no clinical utility in testing for other (nononcogenic) types. (A1) Testing for other (nononcogenic) HPV types when screening for cervical neoplasia, or during the management and follow-up of women with abnormal cervical cytology or cervical neoplasia, is unacceptable. (E1)

**Recommendations for women with different combinations of results**

For women 30 years of age and older who have a cytology result of “negative for an intraepithelial lesion or malignancy” but test positive for HPV, repeat cytology and HPV testing at 12 months is preferred. (BII) If on repeat testing HPV is test positive for HPV, repeat cytology is recommended. (AII) Women found to have an abnormal result on repeat cytology should be managed according to the appropriate 2006 Consensus Guidelines outlined earlier.

**Recommendations for HPV genotyping**

Until an FDA-approved assay becomes available, a recommendation for use of type-specific HPV genotyping cannot be made. Once such assays are FDA approved, emerging data support the triage of women 30 years of age and older with a cytology result of “negative for an intraepithelial lesion or malignancy” but who are HPV positive with HPV genotyping assays to identify those with HPV 16 and 18. (AII)

**ACKNOWLEDGMENTS**

We would like to thank all of the participants and formal observers to the 2006 Consensus Conference who worked so hard to develop the guidelines. Their names and organizations are available at www.asccpp.org. We would like to also thank Ms Kathy Poole for administrative support during the development of the guidelines and Dr Anna Barbara Moscicki, who chaired the adolescent working group. These guidelines were developed with funding from the American Society for Colposcopy and Cervical Pathology and the National Cancer Institute. Its contents are solely the responsibility of the authors and the American Society for Colposcopy and Cervical Pathology and do not necessarily represent the official views of the National Cancer Institute.

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APPENDIX A

Participants and participating organizations

Organizer: American Society for Colposcopy and Cervical Pathology (ASCCP)

Participants: A complete listing of all of the participants and formal observers of the 2006 Consensus Conference and their institutions is available at www.asccp.org.

Participating organizations: American Academy of Family Physicians; American Cancer Society; American College Health Association; American College of Obstetricians and Gynecologists; American Social Health Association; American Society for Clinical Pathology; American Society for Colposcopy and Cervical Pathology; American Society of Cytopathology; Association of Reproductive Health Professionals; Centers for Disease Control and Prevention, Division of Viral and Rickettsial Diseases; Centers for Disease Control and Prevention, Division of Cancer Prevention and Control; Centers for Disease Control and Prevention, Division of Laboratory Systems; Centers for Medicaid and Medicare Services; College of American Pathologists; Food and Drug Administration; International Academy of Cytology; International Federation for Cervical Pathology and Colposcopy; International Federation of Gynecology and Obstetrics; International Gynecologic Cancer Society; International Society of Gynecological Pathologists; National Cancer Institute; National Association of Nurse Practitioners in Women’s Health; Papanicolau Society of Cytopathology; Pan American Health Organization; Planned Parenthood Federation of America; Society of Canadian Colposcopists; Society of Gynecologic Oncologists; Society of Gynecologic Oncologists of Canada; Society of Obstetricians and Gynaecologists of Canada.

APPENDIX B

Definitions of terms (copyright 2001, 2006 ASCCP)

Colposcopy is the examination of the cervix, the vagina, and, in some instances the vulva with the colposcope after the application of a 3-5% acetic acid solution coupled with obtaining colposcopically directed biopsies of all lesions suspected of representing neoplasia.

Endocervical sampling includes obtaining a specimen for either histological evaluation using an endocervical curette or cytobrush or for cytological evaluation using a cytobrush.

Endocervical assessment is the process of evaluating the endocervical canal for the presence of neoplasia using either a colposcope or endocervical sampling.

Endometrial sampling including obtaining a specimen for histological evaluation using an endometrial biopsy or a “dilatation and curettage” or hysteroscopy.

Diagnostic excisional procedure is the process of obtaining a specimen from the transformation zone and endocervical canal for histological evaluation and includes laser conization, cold-knife conization, loop electrosurgical excision procedure (LEEP), and loop electrosurgical conization.

Satisfactory colposcopy indicates that the entire squamocolumnar junction and the margin of any visible lesion can be visualized with the colposcope.

Adolescent women are females 20 years of age and younger (ie, from 13th to 21st birthdays).
With the introduction of multiple new, promising screening and diagnostic tests, we are more capable of preventing cervical cancer and more precisely characterizing a woman’s risk for cervical cancer. However, we must devise new rational management strategies to avoid confusion among clinicians facing unfamiliar combinations of test results and provide optimal consistent care for women. For example, consider the following management scenarios. Patient A has low-grade squamous intraepithelial lesion (LSIL) cytology followed by a colposcopically directed biopsy showing cervical intraepithelial neoplasia (CIN) grade 1. Patient B has atypical squamous cells of undetermined significance (ASC-US) cytology, a positive triage carcinogenic human papillomavirus (HPV) test that sends her to colposcopy, at which time no abnormality is identified. How should each of these women be managed? Some clinicians might consider treatment for the “histologically confirmed CIN” of patient A but watchful waiting for patient B. In fact, both women are at the same risk of detection of CIN3/cancer (10-15%) within the subsequent 2 years. Clinicians must understand the implications of various test combinations in terms of a woman’s risk for CIN3/cancer, and given the same risk, it is logical and important to consider the same management strategy.

As another illustration that affects millions in the United States yearly, when should women who have ASC-US cytology but are carcinogenic HPV deoxyribonucleic acid (DNA) negative on triage be reexamined? As recently recognized by the American Society for Colposcopy and Cervical Pathology (ASCCP) Consensus Conference experts, their risk of subsequent CIN3/cancer is less than 2% over the following 2 years, no more than the risk among women with negative Papnicolaou tests (whose carcinogenic HPV status is unknown). Testing carcinogenic HPV negative identifies the half of ASC-US that represents benign look-alikes. Thus, routine screening interval is appropriate.

Advances in screening and diagnosis make it increasingly possible to prevent cervical cancer. However, if misused or poorly understood, these new tools will only increase costs and potentially harm patients without benefit. As a framework for standardized care that maximizes patient safety and well-being, we propose that a risk model be adopted to guide clinical management now and in the future. The model would use thresholds of increasing risk for cervical precancer and treatable cancer to guide clinical decision making for screening intensity, diagnostic evaluation, or treatment. Experts would decide on these risk thresholds and stratum based on the patient risk to benefit, independent of current (eg, cytology, carcinogenic HPV testing, and colposcopy) and future methods of measuring risk. A risk management model for cervical cancer prevention, based on appropriate clinical actions that correspond to risk stratum, can result in better allocation of resources to and increased safety for women at the greatest risk and increased well-being for women at the lowest risk.

Key words: biopsy, carcinogenic human papillomavirus, cervical cancer, cervical cytology, cervical precancer, colposcopy, human papillomavirus vaccination, loop electrosurgical excision procedure, Papanicolaou smears, risk

The general point is this: as new technologies are introduced, confusion will result unless there is an underlying logic to what we do with increasing knowledge about our patients. We propose that as an organizing principle it is reasonable to use risk for detecting CIN3/(treatable) cancer until the next scheduled screening visit as the metric to determine how often and how intensively to follow-up or treat patients, such that comparable risk leads to comparable interventions.

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Each step in this process—cytology, colposcopy, and histology—has limitations in terms of sensitivity and specificity for our target for treatment: CIN3/cancer (we will refer to CIN3 as the best surrogate of invasive cancer risk). To compensate for such test limitations, we have relied on repeated screening on an annual or biennial basis. In addition, we have used CIN2 as a threshold for treatment to provide an additional margin of safety, even though many CIN2 lesions are destined to regress. The addition of more accurate methods of screening and diagnosis could increase both the sensitivity and efficiency of the process by decreasing the number of repetitions needed to achieve programmatic efficacy. However, the addition of new tests will raise questions about the appropriate management of a positive result.

The ASCCP guidelines highlight an expanded role for the detection of carcinogenic HPV DNA in the clinical management of women with cervical abnormalities. Specifically, these guidelines recommend the use of HPV assays for the following: (1) reflex testing or “triage” for equivocal cytology (ie, ASC-US); (2) postcolposcopic follow-up of women with abnormal cytology found not to have CIN2 or greater; and (3) posttreatment follow-up to assess the risk of recurrence. In addition, carcinogenic HPV testing as an adjunct to cytology in primary cervical cancer screening of women 30 years old and older is now accepted, and there may be a future clinical role for HPV genotyping to identify HPV16 and HPV18, the 2 riskiest types of HPV. The introduction of carcinogenic HPV testing into clinical practice is based on the necessary role of persistent carcinogenic HPV infections in the development of cervical precancer and cancer. The clinical rationale is the already proven increased sensitivity of carcinogenic HPV testing for detection of cervical precancer and cancer and its greater reliability than cytology. Carcinogenic HPV testing has been introduced to increase the efficiency of cervical cancer screening by better risk stratification than cytology alone.

**Risk Stratification**

The concept of risk stratification (distinguishing the few women at risk from the many who are not at risk) is the principle underlying any screening test. With regard to carcinogenic HPV DNA testing, finding carcinogenic types in cervical specimens does not provide a diagnosis of CIN3 or cancer; rather, it identifies a group of women among whom CIN3/cancer is more likely. Complementary tests may provide even more powerful risk stratification. For example, cytologically normal women who test negative for carcinogenic HPV have a very low risk of cervical precancer for the next 5-10 years and are at virtually no risk of developing frank cancer. Conversely, women who have high-grade squamous intraepithelial lesion (HSIL) cytology, a positive test for carcinogenic HPV, and a colposcopic impression of high-grade cervical neoplasia have an 80% likelihood of having CIN3 or worse within 2 years; even if these women are biopsied and not immediately found to have CIN2 or greater, they are still at greater than 60% risk of CIN3 or greater within the next 2 years.

Algorithms based on specific interventions or tests, including cytologic interpretations, biopsy findings, or HPV testing may become outdated as technology evolves. HPV testing could be supplanted in the future by even more sophisticated molecular tests that measure the interaction of virus and human host. Also, HPV vaccination will affect all parts of cervical cancer prevention, although not eliminating the need for some kind of screening. Thus, it is rational to make clinical decisions based on the knowledge of the risk of precursor (ie, CIN3), which can be estimated for various test results and combinations of test results in prospective studies and clinical trials. Clinical algorithms, with agreed-upon priori thresholds, would then apply for any new clinically validated test or diagnostic assay with well-documented risk associated with a positive or negative result.

Careful emphasis on risk, rather than procedure, would clarify many points of confusion. For example, the emphasis on histologic grading, which is unreliable, would decline in favor of more predictive endpoints. Most CIN1 diagnoses follow ASC-US or LSIL cytology. More rarely, CIN1 diagnoses follow HSIL cytology and may represent a higher risk for CIN3 or greater. However, the risk associated with all CIN1, without regard for the preceding cytologic results, will be less than for LSIL cytology. Many practitioners do not realize that a biopsy-derived diagnosis of CIN1 carries a lower risk than LSIL cytology or even carcinogenic HPV-positive ASC-US cytology (nota bene, LSIL, and HPV-positive ASC-US carry the same risk of CIN3 or greater) because CIN1 implies that colposcopic biopsy has ruled out the most evident precancerous lesions. Whereas cytologic LSIL is a reliable sign of HPV infection, a histologic diagnosis of CIN1 is especially nonreproducible and lacks the predictive utility to decide management options. In addition, CIN2 should be seen as an equivocal finding predicting an elevated “risk” of cancer arising somewhere in the cervical transformation zone, not necessarily within that lesion.

We treat (excise with loop electrosurgical excision procedure [LEEP]) the entire transformation zone, not focal lesions, because biopsies showing CIN2 or even CIN3 indicate that the field of epithelium is at risk of cancer. CIN2 is also poorly reproducible and often nothing more than a sign of acute HPV infection upon review. So treatment, especially for CIN2, might be more accurately viewed as a cancer risk-reducing intervention.

**Risk Thresholds**

Professional clinical societies and cancer prevention experts together will make the decisions as to the proper clinical responses to which risk levels, considering benefits as well as total cost, which includes financial and negative health consequences. It is worth discussing to what extent protocols versus individual clinician judgment should dictate which tests are used, when, and how they should be interpreted. In general, we believe, because screening is inherently a popula-
### TABLE
A table of cervical precancer risk based on current screening and management tools with proposed clinical follow-up

<table>
<thead>
<tr>
<th>Stage of screening or clinical management</th>
<th>Cytology results</th>
<th>Carcinogenic HPV test results (HPV positive or HPV negative)</th>
<th>References</th>
<th>Proposed clinical follow-up</th>
<th>Absolute risk of precancer (CIN3)*</th>
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</thead>
<tbody>
<tr>
<td>Screening</td>
<td>Negative</td>
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<td>Routine</td>
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<td>HPV negative</td>
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<td></td>
</tr>
<tr>
<td>Screening</td>
<td>Negative</td>
<td>HPV positive</td>
<td>12, 13</td>
<td>12 months</td>
<td>2% to less than 10%</td>
</tr>
<tr>
<td>Screening</td>
<td>LSIL</td>
<td>HPV negative</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td>ASC-US</td>
<td>HPV positive</td>
<td>27</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After colposcopy†</td>
<td>Negative</td>
<td>HPV positive</td>
<td>15</td>
<td></td>
<td></td>
</tr>
<tr>
<td>LEEP</td>
<td></td>
<td>HPV positive</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening/atriage</td>
<td>ASC-US</td>
<td>HPV positive</td>
<td>29</td>
<td>Colposcopy</td>
<td>10% or greater</td>
</tr>
<tr>
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<td>LSIL</td>
<td>HPV positive</td>
<td>17</td>
<td></td>
<td></td>
</tr>
<tr>
<td>After colposcopy†</td>
<td>HPV positive</td>
<td>15</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Post-LEEP</td>
<td>ASC-US or greater (or)</td>
<td>HPV positive</td>
<td>28</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Screening</td>
<td>HSIL</td>
<td>HPV negative</td>
<td>14</td>
<td>Colposcopy/treatment?</td>
<td>40% or greater</td>
</tr>
<tr>
<td>After colposcopy†</td>
<td>HSIL</td>
<td>HPV negative</td>
<td>15</td>
<td>Treatment?</td>
<td>40% or greater</td>
</tr>
<tr>
<td>CIN2 biopsy</td>
<td></td>
<td>HPV positive</td>
<td>18</td>
<td>Treatment</td>
<td></td>
</tr>
<tr>
<td>Colposcopy (high grade)</td>
<td>HSIL</td>
<td>HPV+</td>
<td>14</td>
<td>Treatment?</td>
<td>60% or greater</td>
</tr>
</tbody>
</table>

* Risk until the next screening.

† Cytology and HPV results are those from the first follow-up visit 6 months after colposcopy.

Cytology and HPV results are those from the first follow-up visit 6 months after colposcopy. Women with a less than 2% risk of precancer within the subsequent 2-3 years (eg, women who test carcinoma HPV negative in routine screening) might be at an acceptable low risk to remain in regular interval screening. Regular screening may include 1- to 2-year screening with cytology alone or every 3 years with cytology and carcinogenic HPV cotesting for women 30 years old and older. Women with a 2% to less than 10% risk (eg, women with negative cytology who test carcinogenic HPV-positive in screening or women with ASC-US cytology unqualified by carcinogenic HPV testing) might warrant rescreening in a year. Women with a 10% or greater risk (eg, women with LSIL cytology or carcinogenic HPV-positive ASC-US) may warrant immediate colposcopic evaluation including multiple biopsies to maximize the sensitivity of colposcopy in high-risk women.\(^\text{19}\)

More controversial, women identified with a risk of precancer above a certain threshold may warrant treatment, even if an initial colposcopic evaluation and directed biopsy does not identify a precancerous lesion on that day. Treatment of risk may be justified at some thresholds because we now better appreciate the limitations in the sensitivity of colposcopy for finding precancerous lesions.\(^\text{5}\)

There are legitimate concerns regarding a strategy of treatment based on risk that would lead to overtreatment of some...
women. In principle, we should recognize that the current treatment threshold of CIN2 already results in significant overtreatment of many women whose lesions would regress spontaneously; we accept this overtreatment to provide a margin of safety against misclassification of diagnosis and risk. Importantly, some women may be more likely to be lost to follow-up and, given high risk, may benefit from treatment before the confirmation of disease. For example, women with HSIL cytology are still at a 40% risk of having a precancerous lesion, even if the initial colposcopy is negative. Thus, in those populations in which loss to follow-up for colposcopy and treatment is a real concern, immediate treatment of women with HSIL cytology may be warranted to provide safety. Older women at high risk of precursor, for whom the potential adverse effects of treatment on fertility are not a consideration, may benefit from treatment without colposcopic confirmation because precancerous lesions tend to be higher in the endocervical canal and therefore more easily missed by colposcopy.

As described above, in the extreme, a combination of testing positive for carcinogenic HPV or HSIL cytology and having a colposcopic impression of high grade carries an approximately 80% risk of CIN3 or greater. The question becomes whether colposcopically directed biopsy, a flawed diagnostic procedure, provides any added information. With informed decision making between patient and clinician that takes into account patient well-being, it may be more cost effective and less invasive to immediately treat women with very high-risk profiles than to require 2 or more clinical visits including an intensive follow-up with multiple biopsies to find the precancerous lesion and then treat.

Accepted thresholds for deciding who needs closer surveillance, colposcopy, and treatment will aid in clinical decisions regarding any new validated test or diagnostic procedure. A new assay that yields a positive result with defined clinical risk will trigger action consistent with that risk. Thus, detection of new biomarker X by a laboratory- and clinically validated assay Y, which carries a 15% risk of CIN3/cancer in 2 years should be managed identically as LSIL cytology for which colposcopic evaluation is currently recommended.

**The Importance of Context in Clinical Decisions**

Context is another element that figures into the risk predicted by a particular set of clinical test results. A risk associated with a positive test, like a carcinogenic HPV DNA test or CIN2 biopsy, is situational. An illustrative example is as follows: young women who have recently become sexually active have a high prevalence of carcinogenic HPV but an extremely low prevalence of precancerous lesions. In fact, the positive predictive value of a carcinogenic HPV test is so low in these women that its use in screening is not recommended. By comparison, HPV prevalence drops and the prevalence of precancerous lesions rises in women who are 10-15 years after sexual debut, and its use is now recommended as a cotest with cytology in women 30 years old and older in the United States (median age of sexual debut is approximately 17 years). Another example is the risk of precancer following an ASC-US cytology in screening (2% to less than 10%) vs post-LEEP (10% or greater), which should trigger different clinical responses for...
each. Thus, the circumstances for the test result must be considered (eg, prior colposcopy, prior treatment, or HPV vaccination) to accurately project the risk and the appropriate clinical response. Figure 2 illustrates the principles of risk stratification and contextual use of a test.

**FIGURE 2**
Cumulative incidence of precancer

The figure shows the cumulative incidence of precancer (CIN3) and cancer (CIN3 or greater) by HPV status and illustrates two aforementioned outlined principles: risk stratification and contextual use of a test. A positive test for carcinogenic HPV in women 30 years old and older even with negative cytology can identify those women at risk of precancer and cancer. More importantly, those who tested negative are at an extremely low risk over many years and should not be screened again for several years; the current screening recommendation is that women who are cytology and carcinogenic HPV negative should not be rescreened before 3 years.

If the positive test for carcinogenic HPV is then triaged using HPV16 and HPV18 detection, there is further risk stratification, with HPV16-positive or HPV18-positive women being at an extremely high risk of developing precancer or cancer. In fact, this risk was greater than the risk observed for women with LSIL, for whom colposcopy is recommended. Thus, when a validated HPV genotyping assay becomes available, colposcopy for HPV16-positive or HPV18-positive, cytology-negative women may be warranted. However, the appropriate management for HPV16-negative/HPV18-negative, carcinogenic HPV-positive, cytology-negative women becomes less clear because the absolute risk is low, yet women remain at risk for the 30% of the cancer not caused by HPV16 and HPV18. This clinical conundrum will become more common to the screening of women 10-15 years after they have received vaccination against HPV16 and HPV18 infections. Thus, the risk of cervical precancer and cancer among women who test positive for carcinogenic HPV will differ between those who have and have not been previously vaccinated. (n.b., the X-axis does not represent continuous time and the time points represent midpoints within a follow-up time period. Follow-up time was crudely divided into an initial period of 0-9 months (Papanicolaou smears that were rapidly repeated, presumably prompted by a previous cytologic abnormality or suspicious symptoms), followed by yearly intervals for a total time of 122 months. These intervals roughly paralleled the intervals at which women returned for annual smears).

In summary, we have unprecedented capability to identify women with the greatest and lowest risk of cervical precancer and cancer using current and soon-to-be-available prevention tools. Our capacity to accomplish this is much more robust than the extensively cited and used model for breast cancer risk by Gail et al because we can now or will soon be able to ascribe risks of cervical precancer ranging from less than 1% to over 90% risk.

Given the moderate sensitivity of colposcopy/colposcopically directed biopsy to identify women with precancer and the availability of more objective tools to measure the risk, we suggest that a change of paradigm is in order: identify and manage based on aggregate risk for CIN3/precancer. Importantly, as we shift from a model with false certainty based on a colposcopic impression and diagnosis to a model of relative and absolute risk with acknowledged uncertainties, we will protect clinicians from medicolegal consequences for a highly successful but imperfect intervention. A risk management model for cervical cancer prevention, based on clinical actions corresponding to risk stratum, can result in better allocation of resources and increased safety for women at the greatest risk and increased well-being for women at the lowest risk.

**REFERENCES**

2. Safaeian M, Solomon D, Wacholder S, Schiffman M, Castle PE. Risk of pre-cancer and follow-up management strategies for women...
Cervical intraepithelial neoplasia grade 1 (CIN1) and its cytologic equivalent low-grade squamous intraepithelial lesion (L-SIL) are considered to be the morphologic appearance of cells with productive infections by human papillomavirus (HPV). Nearly 85% of CIN1/LSIL are caused by high-risk (HR) HPV types, as are almost 100% of CIN2-3 and cervical carcinomas, and only 15% are caused by nonprogressive, low-risk HPV types. Nevertheless, a vast majority of CIN1/L-SIL lesions, even those caused by HR-HPV, are transient and will spontaneously regress. As a result, no treatment for these lesions is proposed in current protocols.

A review of the literature from 1950-1992 noted that, although the likelihood of regression for CIN1 lesions was 60%, 10-15% progressed to CIN2-3, and 0.3% progressed to invasive carcinoma. Unfortunately, current diagnostic tools do not allow us to identify which cases of CIN1/L-SIL are at risk of developing CIN2-3 and which lesions will regress. Nevertheless, a few risk factors have been identified. Persistence of HR-HPV infection has been shown to be a prerequisite for the development of CIN2-3 and cervical cancer. Old age is considered an indirect factor of HPV persistence. Finally, it has been shown that a significant percentage of women with an initial diagnosis of CIN1/L-SIL actually harbor a CIN2-3; women with unsatisfactory colposcopy or endocervical lesions are particularly prone to hide a CIN2-3 lesion.

The American Society for Colposcopy and Cervical Pathology (ASCCP) sponsored a consensus conference in 2001 to develop evidence-based guidelines for women with CIN. Repeated cytology at 6 and 12 months or DNA testing for HR-HPV types at 12 months was the pre-
ferred management option\textsuperscript{5} for women with CIN1/L-SIL and satisfactory colposcopy according to these protocols. However, in a few particular situations a diagnostic excisional procedure was considered more appropriate than observation, mainly because of the possibility of missing a high-grade lesion or an occulted invasive cancer. These situations included unsatisfactory colposcopy or positive endocervical curettage, persistence of CIN1/L-SIL or HR-HPV infection for longer than 2 years and older than 40 years.\textsuperscript{13,14}

Nevertheless, although the criteria to treat CIN1/L-SIL lesions have been well-defined, most reported series evaluating the follow-up of patients treated for CIN are focused only on high-grade lesions. Thus, the aim of our study was to evaluate the results of posttreatment follow-up in a cohort of women with CIN1/L-SIL that meet the ASCCP risk criteria relative to a series of pretreatment and posttreatment variables.

**Materials and Methods**

**Patient selection**

We included in the study 77 women with CIN1/L-SIL who were treated by loop electrosurgical excision procedure (LEEP) in the Department of Obstetrics and Gynecology of the Hospital Clinic of Barcelona between March 1998-February 2005. All women had at least 2 repeated cytologies of L-SIL. Criteria for treatment were as follows: unsatisfactory colposcopy, large lesions or positive endocervical curettage (29 women), long-time persistent lesion (at least 2 yrs; 20 cases) or women older than 40 years (28 cases). Women with HIV infection or with other causes of immunosuppression were excluded from the study. A colposcopically directed biopsy with a CIN1 result was available in 62 cases.

**Diagnostic techniques**

Conventional cytology was performed and evaluated according to the criteria of Bethesda 2001. Colposcopy was performed as described elsewhere.\textsuperscript{15} Colposcopic findings were described according to the criteria of the International Federation for Cervical Pathology and Colposcopy (Barcelona 2002). All women with abnormal cytology (ASC-US or higher) or positive result in HPV testing and an abnormal transformation zone underwent a colposcopically directed biopsy. When the transformation zone was not visible or only partially visible or no colposcopic abnormality was identified, an endocervical study (with endocervical brush or a Kervokian curette) was also performed.

Specimens for HPV testing were collected using the Digene cervical sampler kit (Digene, Gaithersburg, MD). The samples were stored at -20°C until further processing was available. HPV detection was performed by using the commercially available Hybrid Capture II (HC2) system (Digene). All the samples were analyzed only for the presence of HR-HPV types (1, 16, 18, 32, 34, 35, 39, 40, 45, 51, 52, 56, 58, 59, and 68). The cutoff of 1 relative light unit (RLU; 1.0 pg/mL) was used to classify a specimen as positive or negative.\textsuperscript{16} The RLU value of each individual sample was recorded. RLU value provides an indication of the viral load.\textsuperscript{18}

All diagnostic biopsy specimens, cone specimens, and all cytologies establishing a diagnosis of L-SIL were independently and blindly evaluated by 2 pathologists (J.O., E.C.). When differences between the 2 independent evaluations were detected, a new evaluation by the 2 observers was conducted and a consensus diagnosis was reached.

**Treatment procedure and specimen diagnosis**

LEEP was performed according to previously described protocols.\textsuperscript{15} All patients signed the informed consent previously to the LEEP procedure. The loop was selected according to the size of the area to be excised. When an endocervical extension was suspected, a second selective endocervical sweep was performed. LEEP specimens were anatomically orientated and pinned to a cork support and fixed in 10% formalin. The excision specimens were thoroughly examined, after processing the whole specimen in 3-12 paraffin blocks (median, 6). If there was more than 1 sweep, each one of them was independently included. Surgical margins were identified with ink and were carefully examined. All slides of the LEEP specimen were carefully reviewed by a gynecologic pathologist (J.O.) and a final diagnosis obtained after applying the standard criteria for the grading of CIN lesions.\textsuperscript{17}

**Follow-up routine**

Posttreatment control was scheduled at 6, 12, 18, and 24 months during the first 2 years and yearly after this period. When surgical margins were affected, the first control was conducted at 3 months. The mean follow-up period was 12.6 ± 6.8 months (range, 3-38). In every visit after LEEP, a Papanicolaou (Pap) test smear and colposcopy were performed. A sample for HPV DNA was collected at 6 or 12 months after treatment.

Residual/recurrent CIN1/L-SIL was diagnosed on the basis of 2 repeated cytologies and/or colposcopically directed biopsy or endocervical study. For the diagnosis of progression to CIN2-3, a positive colposcopy-directed biopsy or endocervical sample was required. Women with 2 consecutive negative Pap test smears and normal colposcopy were considered negative for residual/recurrent lesion whatever the HC2 result. CIN1 was considered as residual/recurrent disease; the evidence of CIN of grades 2 or 3 was considered a progression of the disease.

**Statistical methods**

Data were analyzed with the program STATA (v 8.0; StataCorp 2003, College Station, TX). The efficacy of post-LEEP detection tools for the diagnosis of residual/recurrent disease were evaluated as sensitivity, specificity, and positive and negative predictive values for qualitative variables (cytology, cone margins, and HR-HPV positivity) and by using receiver operating characteristic (ROC) analysis for quantitative variables (HR-HPV load). Relationship between cytology, surgical margins, and residual or recurrent disease was analyzed with the $\chi^2$ statistical analysis or the Fisher’s exact test. The relationship between HR-HPV load and residual/recurrent disease was...
analyzed with the Student t test after logarithmic transformation, with results expressed as a geometric mean. The odds ratio (OR) and 95% confidence interval (CI) were estimated by logistic regression analysis. Multivariate models were estimated by using the step-wise method.

**Results**

**Results of the LEEP analysis**

The histologic diagnoses obtained in the LEEP specimen are shown in Table 1. Fifty-two women (67.6%) had a CIN1 confirmed in the LEEP specimen and 17 patients (22%) had a CIN2-3. In 8 women (10.4%), no lesion was identified after a thorough examination of the LEEP specimen. Positive pretreatment HPV testing was significantly more frequent in women harboring a CIN2-3 (12/12, 100%) or CIN1 (29/33, 93.5%) than in women with a negative histology in the LEEP specimen (1/7, 14.3%; P < .001). No differences in viral load were observed between women showing a CIN1 or patients with CIN2-3 in the LEEP specimen (1387.3 ± 2599.2 vs 915.0 ± 880.5; P = NS).

**Post-LEEP follow-up**

Residual/recurrent disease was identified in 22 women (28.6%). The diagnosis of residual/recurrent disease was established by colposcopically directed biopsy in 16 cases and by 2 or more cytolgies of L-SIL in 6 women. Eighty-two percent of all recurrences (18/22) were detected during the first year after treatment (100% of patients progressing to CIN2-3/H-SIL; 79% of women recurring as CIN1/L-SIL).

In the group of women with CIN1 in the LEEP specimen, residual/recurrent disease was identified in 17 of 52 (32.7%) women during the follow-up (12 cases diagnosed by biopsy specimen and 5 by 2 or more cytologies). Fifteen women recurred as CIN1/L-SIL and 2 women recurred as CIN2-3. Mean lag time between LEEP and diagnosis of recurrent disease was 8.8 ± 8.9 months (range, 3-36). There were no differences in the time to develop recurrence between cases developing CIN2-3/H-SIL and CIN1/L-SIL (6.5 ± 2.1 vs 10.2 ± 9.6 months; P = NS).

Residual/recurrent disease was identified in 4 of 17 (23.5%) patients with CIN2/3, 1 of them recurred as a CIN2-3/H-SIL and 3 as CIN1/L-SIL. One of 7 women (14.3%) with negative LEEP histology had residual/recurrent disease develop. The patient, who had a positive pretreatment HPV detection, recurred as CIN1/L-SIL.

Pretreatment HR-HPV testing was positive in all cases eventually developing residual/recurrent disease (15 tested cases of the overall 22 women with persistent/recurrent disease).

The 2 groups of women with no lesion and with CIN2-3 in the LEEP specimen were excluded from further analysis, which was limited to the 52 women with confirmed CIN1 in the LEEP specimen.

**Prediction of residual/recurrent disease**

The mean age of nonrecurrent cases was 35.1 ± 8.8 years, whereas in cases that developed disease, it was 37.5 ± 9.7 years.

---

**Table 1**

<table>
<thead>
<tr>
<th>Diagnosis in the LEEP specimen</th>
<th>n (%)</th>
<th>CIN 1</th>
<th>CIN 2-3</th>
<th>P</th>
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</thead>
<tbody>
<tr>
<td>n (%)</td>
<td>8 (10.4%)</td>
<td>52 (67.6%)</td>
<td>17 (22.0%)</td>
<td>NA</td>
</tr>
<tr>
<td>Age (y)</td>
<td>38.4 ± 10.3</td>
<td>36.7 ± 9.4</td>
<td>37.7 ± 7.5</td>
<td>NS</td>
</tr>
<tr>
<td>% of positive pretreatment HPV</td>
<td>14.3%</td>
<td>92.5%</td>
<td>100%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Residual/recurrent disease [n, (%)]</td>
<td>1 (14.3%)</td>
<td>17 (32.7%)</td>
<td>4 (23.5%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

**Table 2**

<table>
<thead>
<tr>
<th>Residual/recurrent disease</th>
<th>N</th>
<th>n</th>
<th>(%)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;35</td>
<td>23</td>
<td>8</td>
<td>34.8%</td>
<td>NS</td>
</tr>
<tr>
<td>&gt;35</td>
<td>29</td>
<td>9</td>
<td>31.0%</td>
<td>NS</td>
</tr>
<tr>
<td>Pretreatment</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HPV load (RLU)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1-100</td>
<td>13</td>
<td>2</td>
<td>15.4%</td>
<td>.005</td>
</tr>
<tr>
<td>&gt;100</td>
<td>18</td>
<td>9</td>
<td>50.0%</td>
<td>.005</td>
</tr>
<tr>
<td>Cone margins</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Negative</td>
<td>36</td>
<td>9</td>
<td>25.0%</td>
<td>NS</td>
</tr>
<tr>
<td>Positive</td>
<td>16</td>
<td>8</td>
<td>50.0%</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>
(P = NS). Geometric mean HR-HPV load in RLU before treatment was 61.5 ± 199.8 in nonrecurrent cases, and 682.8 ± 1400.8 in women developing residual/recurrent disease (P = .035).

Surgical margins were positive in 16 cases (30.7%). Of the overall group of patients with positive margins, the exocervical margin was involved in 10 (62.5%), the endocervical margin in 5 (31.2 %), and both margins in 1 case (6.2%). In 1 case, the deep glandular margin was involved. The cone margins were negative in the 2 cases recurring as CIN2-3/H-SIL and in 8 of 15 (53.3%) cases recurring as CIN1/L-SIL.

As shown in Table 2, HR-HPV load above 100 RLU before treatment and positive cone margins were associated with significantly higher risk of residual/recurrent disease.

Identification of residual/recurrent disease

An abnormal cytology (ASC-US or higher) at the first follow-up control was observed in 18 women (34.6%). Posttreatment HC2 was positive in 24 women (46.1%). No patients with residual disease were negative for HR-HPV during the follow-up.

Table 3 shows the sensitivity, specificity, positive predictive value (PPV), and negative predictive value (NPV) for residual/recurrent disease after LEEP of cone margin result, single cytology and HR-HPV detection with HC2.

Logistic regression analysis

Table 4 shows the results of the univariate logistic regression analysis of factors predicting or identifying residual/recurrent disease. No relationship was observed between age and residual/recurrent disease. On the multivariate analysis only posttreatment HR-HPV detection and load showed statistical significance (OR, 2.3; 95% CI, 1.22-4.31; P = .01).

Comment

The main goal of our study is that it is specifically focused on the treatment of patients with CIN1/L-SIL. Although the criteria to select women with CIN1/L-SIL lesions that should receive treatment have been clearly defined,14 all previous reports have been focused only on high-grade lesions, or present the results of CIN1 and CIN2-3 together.

More than one-fifth of the women of our study treated because of L-SIL/CIN1, actually harbored a high-grade lesion in the LEEP specimen. These data are in keeping with previous reports showing that 23-55% of patients undergoing LEEP for CIN1/L-SIL actually have CIN 2–3,14 and stresses the need of excision for patients with L-SIL/CIN1 with unsatisfactory colposcopy, positive endocervical curettage, large lesions, long-time persistent lesion, or who are older than 40 years, as proposed by the ASCCP.7 No lesion was identified in the LEEP specimen in 8 women of our series. Interestingly, all but 1 of them had a negative pretreatment HR-HPV test, suggesting that these lesions were caused by LR-HPV.2 Thus, our work suggests that excision treatments should probably be avoided in women with negative HR-HPV test, even when the ASCCP criteria of risk for CIN1/L-SIL are accomplished.

The association between HR-HPV load and the severity of CIN or the progression of low-grade lesions remains controversial. Several studies suggest that viral load could be a possible biomarker in the natural history of infection. With the use of semiquantitative or quantitative HPV DNA tests, several observational studies have found high correlation between high viral load and high-grade lesions.18–20 In contrast, no differences were found in our study between pretreatment HR-HPV load and CIN grade in the LEEP specimen, as observed in other studies.21,22

<table>
<thead>
<tr>
<th>Parameter</th>
<th>OR</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive cone margin</td>
<td>3.00</td>
<td>0.871-10.333</td>
<td>.082</td>
</tr>
<tr>
<td>Pretreatment HR-HPV load</td>
<td>Doubling value</td>
<td>1.267</td>
<td>0.999-1.606</td>
</tr>
<tr>
<td>Posttreatment HR-HPV load</td>
<td>Doubling value</td>
<td>1.715</td>
<td>1.287-2.285</td>
</tr>
</tbody>
</table>

Pre- and posttreatment HR-HPV were evaluated as quantitative variables. Increases in risk for doubling the values of viral load are expressed.
Residual/recurrent disease was identified in 22/77 women of the whole group (28.6%). Four of 17 (23.5%) women with CIN2-3 in the LEEP specimen had residual/recurrent disease develop. This percentage is consistent with the data previously reported by other authors and similar to the percentage of residual/recurrent disease recently reported by our group. Surprisingly, residual/recurrent disease was more frequent in women with CIN1 (17/52, 32.7%), although the differences were not significant. Moreover, 2 cases with CIN1 in the LEEP specimen recurred as a high-grade lesion. This stresses the need for strict follow-up after LEEP excision, even for women with CIN1.

Nearly 93% of cases of CIN1 were positive for HR-HPV testing before LEEP, a similar percentage to the 85% obtained with CIN2-3. Moreover, women with loads above 100 RLU had 3 times the risk of having residual/recurrent disease develop and may warrant special attention with a more rigorous follow-up protocol. HR-HPV testing showed, nevertheless, significantly less specificity than cytology (80.0% vs 91.4%). This is in accordance with previously reported data on specificity and PPV of HC2 and cytology, and confirms that post-treatment follow-up of patients should include both cytology and HPV test.

In conclusion, patients with L-SIL/CIN1 with unsatisfactory colposcopy, positive endocervical curettage, extensive or long-time persistent lesion or who are older than 40 years of age should be treated because of a significant risk of harboring a CIN2-3 lesion. However, a conservative approach should be considered in women with basal negative HR-HPV test. Patients with high pretreatment HR-HPV loads should be considered at risk of having residual/recurrent disease develop and may warrant special attention with a more rigorous follow-up protocol. HC2 during the follow-up has an extremely high sensitivity for detecting women with residual/recurrent disease.

No relationship was observed in our series between age and risk of residual/recurrent disease as previously reported in series of CIN2-3. Also in keeping with previous reports of CIN2-3, the status of resection margins was a predictor of residual disease, but 50% of women with positive cone margins did not recur and, on the contrary, 25% of women with negative cone margins had persistent/recurrent disease develop. Thus, our data support previous evidence indicating that status of resection margins has only a limited usefulness in predicting residual disease and that further treatments, such as conization or hysterectomy, should not be based only on cone resection margins.

Our study shows that HR-HPV detection with the use of HC2 during the follow-up has an extremely high sensitivity for detecting women with residual/recurrent disease. In our group this test alone reached a sensitivity and NPV of 100%, retaining a good specificity (80.0%). These figures, which are similar to those previously reported for HC2 in women treated for CIN2-3, are higher than the sensitivity obtained by a single cytology (88.2% and 94.1%, respectively). HR-HPV testing showed, nevertheless, significantly less specificity than cytology (80.0% vs 91.4%). This is in accordance with previously reported data on specificity and PPV of HC2 and cytology, and confirms that post-treatment follow-up of patients should include both cytology and HPV test.

In conclusion, patients with L-SIL/CIN1 with unsatisfactory colposcopy, positive endocervical curettage, extensive or long-time persistent lesion or who are older than 40 years of age should be treated because of a significant risk of harboring a CIN2-3 lesion. However, a conservative approach should be considered in women with basal negative HR-HPV test. Patients with high pretreatment HR-HPV loads should be considered at risk of having residual/recurrent disease develop and may warrant special attention with a more rigorous follow-up protocol. HC2 during the follow-up has an extremely high sensitivity for detecting women with residual/recurrent disease.

ACKNOWLEDGMENTS

We are grateful to Drs Manel Solé, Lúcia Alós, and Lluís Colomo and Ms Fuencisla Maderuelo and Teresa Cuberes for their help in the cytologic evaluation of cervical smears. We also thank Ms Noemí Ferrer for her help in the review of the clinical charts and Peter Szost for the English revision.

REFERENCES


Defining nasal bone hypoplasia in second-trimester Down syndrome screening: does the use of multiples of the median improve screening efficacy?

Anthony O. Odibo, MD, MSCE; Harish M. Sehdev, MD; David M. Stamilio, MD, MSCE; Alison Cahill, MD; Linda Dunn, MD; George A. Macones, MD, MSCE

OBJECTIVE: The purpose of this study was to compare the efficacy of the use of nasal bone (NB) multiples of the median (MoM) with the biparietal diameter (BPD)/NB ratio as definitions of NB hypoplasia that is associated with Down syndrome in the second trimester.

STUDY DESIGN: We conducted a prospective cohort study of women who underwent an anatomic survey between 16 and 22 weeks of gestation. The fetal NB and other markers of fetal aneuploidy were evaluated. MoMs for the NB length at each gestational age category were calculated and adjusted for maternal race. NB hypoplasia was defined either as an absent NB or by a ratio of the BPD/NB or by NB lengths <0.75, 0.5, and 0.25 MoM for the gestational age, respectively. Fetuses or infants with Down syndrome were compared with those without for the presence of NB hypoplasia.

RESULTS: Among 3634 women whose condition was evaluated, NB assessment was obtained in 3197 women (88%). There were 23 cases of Down syndrome that were detected. Receiver operating characteristic curve comparison revealed NB MoM =0.75 to be the best definition of NB hypoplasia (area under receiver operating characteristic curve, 0.75). NB <0.75 MoM had a sensitivity and specificity of 49% (95% CI, 26%-69) and 92% (95% CI, 91%-93%), respectively, compared with 61% (95% CI, 38%-80%) and 84% (95% CI, 82%-85%), respectively for BPD/NB >11. The difference in the sensitivity of 12% (95% CI, 5%-31) with the BPD/NB ratio >11 vs with an NB MoM <0.75 was not significant (P=.25). The difference in the specificity of 8% (95% CI, 7.5-9.5) with the BPD/NB ratio >11 vs an NB MoM <0.75 was significant (P=.0001).

CONCLUSION: In the second trimester of pregnancy, the use of nasal bone length <0.75 MoM for the gestational age was the best definition for Down syndrome detection and resulted in improved specificity.

Key words: Down syndrome, nasal bone hypoplasia, screening efficiency


A n absent fetal nasal bone (NB) or NB hypoplasia is now an established marker for aneuploidy in the second trimester of pregnancy.1-7 The optimal definition of NB hypoplasia, however, is still uncertain. Although previous studies have suggested a biparietal diameter (BPD)/NB ratio as the best definition of NB hypoplasia,4,6 other investigators have suggested that using multiples of the median (MoM) of NB for the gestational age (GA) was a better definition.8,9 One of these reports did not provide screening efficiency of the use of NB MoM8; the other report derived one-half of their Down syndrome cases from a retrospective review of archived images.9

In a study that combined NB with other markers for Down syndrome, the specificity of NB hypoplasia, which was defined as BPD/NB >11, was only 82%.7 Therefore, in an attempt to explore definitions of NB hypoplasia with higher specificity, we performed the present study that compares the screening efficiency for Down syndrome with the BPD/NB ratio or NB MoMs.

MATERIALS AND METHODS
This is a secondary analysis of data from a prospective cohort study of women who underwent an anatomic survey between 16 and 22 weeks of gestation from January 2002 to December 2005. The fetal NB and other markers of fetal aneuploidy, which included nuchal fold, femur and humeral lengths, choroid plexus cysts, major fetal anomalies, echogenic bowel, pylorostenosis, and hypoplastic fifth digits,
were evaluated. Approval from the institutional review boards of all study centers was obtained.

We assessed fetal NB, as previously described by Sonek and Nicolaides. Briefly, the facial profile of the fetus was obtained in the mid-sagittal plane and by rocking the transducer sideways (Figure 1). The angle of insonation was maintained at 45 or 135 degrees. The NB is seen as a triangular echogenic structure in this view. All sonographic evaluations were performed by experienced, American Registry of Diagnostic Medical Sonographers–certified sonographers, with training in first-trimester screening ultrasound scanning. Median levels of NB were calculated with the use of polynomial regression and best fit analysis compared with GA in weeks. For the generation of the medians, we used NB measurements from the fetuses of white women without any chromosomal abnormality. For race other than white, normal medians were calculated with the use of a correction factor of 1.06 for African American women, 0.96 for Asian women, and 1.01 for Hispanic women. The correction factors were derived by comparing the median values of white race to other races for our population. MoMs for the NB length at each GA category were then calculated. NB hypoplasia was defined either as an absent NB or by a ratio of the BPD/NB >11 or by NB lengths <0.75, 0.5, and 0.25 MoM for the GA, respectively. Fetuses or infants with Down syndrome were compared with those without for the presence of NB hypoplasia.

RESULTS

Among 3634 women who were evaluated over the 4-year period, NB assessment was obtained in 3197 women (88%). There were 23 cases of Down syndrome that were detected. A description of the demographics for the study population is shown in Table 1. Most of our patients were white, and the most common indication for evaluation was routine anatomical survey.

Fetal karyotypes were confirmed either by chromosomal analysis when available or by newborn examination. Statistical analysis was performed with chi-square test for categoric variables and Student t test for continuous variables. The primary outcome was the association between NB and fetal aneuploidy. All analyses were performed with the use of STATA software (version 9.0; STATA Corp, College Station, TX).

TABLE 1
Demographics of study population (n = 3634)

<table>
<thead>
<tr>
<th>Demographic</th>
<th>N</th>
<th>%</th>
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</thead>
<tbody>
<tr>
<td>Mean age (y)*</td>
<td>30 ± 6.8</td>
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</tr>
<tr>
<td>Mean GA (wk)*</td>
<td>19.1 ± 1.7</td>
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</tr>
<tr>
<td>Race</td>
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<td>Advanced maternal age</td>
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</tr>
<tr>
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<td>62</td>
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<tr>
<td>Chestnut Hill Hospital</td>
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</table>

* Data are given as mean ± SD.
Figure 2 is the scatter plot and the regressed median line of NB vs GA (in weeks). Figure 3 is the regression diagnostic of the residuals vs fitted values, which shows that, despite a few outliers (especially below the zero line), the model was overall well fit. From the regressed medians, NB MoMs were calculated for the GA between 15 and 23 weeks of gestation (Table 2).

The screening efficiency for Down syndrome detection with NB MoMs and BPD/NB ratio >11 were then compared (Table 3). Receiver operating characteristic (ROC) plots that compare the screening efficiency of BPD/NB ratios and NB MoMs are shown in Figure 4. The optimal threshold from the curves correspond to NB MoM <0.75 and BPD/NB >11. NB <0.75 MoM had a sensitivity and specificity of 49% and 92%, respectively, compared with 61% and 84%, respectively, for BPD/NB >11. The 95% CIs for the screening performance of NB with each definition are shown in Table 3. The area under the ROC curves for NB MoM and BPD/NB ratios were 71% and 72%, respectively. When limited to the area of the ROC with a false-positive rate of <5%, the area under the ROC curves for the BPD/NB ratio was 22% and for the NB MoM was 21%. The difference in the sensitivity of 12% (95% CI, -5.31) with the BPD/NB ratio >11 vs an NB MoM <0.75, was not significant (P < .25). The difference in the specificity of 8% (95% CI, 7.5-9.5) with the BPD/NB ratio >11 vs an NB MoM <0.75 was significant (P < .0001).

When the sensitivity for detecting Down syndrome is fixed at 40%, the false positive rate with a BPD/NB ratio was 5.5% and with an NB MoM was 1%. The BPD/NB and NB MoM threshold levels that resulted in the 40% sensitivity were a ratio >12.5 and NB MoM <0.625, respectively. Alternatively, with a fixed false-positive rate of 5% for Down syndrome detection, the sensitivity for Down syndrome detection with BPD/NB ratio vs NB MoM as definitions of NB hypoplasia were 39% and 43%, respectively. The BPD/NB ratio and NB MoM threshold levels that resulted in the 5% FPR were a ratio >12.6 and MoM <0.72, respectively.

**Comment**

Our study demonstrates that the use of NB MoM in the second trimester was a more efficient definition of NB hypoplasia.

---

**Appendix A:**

<table>
<thead>
<tr>
<th>GA (wk)</th>
<th>Subjects (n)</th>
<th>Regressed median (cm)</th>
<th>0.75 MoM</th>
<th>0.5 MoM</th>
<th>0.25 MoM</th>
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<td>0.15</td>
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<td>0.29</td>
<td>0.14</td>
</tr>
<tr>
<td>23</td>
<td>30</td>
<td>0.64</td>
<td>0.48</td>
<td>0.32</td>
<td>0.16</td>
</tr>
</tbody>
</table>

* Adjusted for black, Asian, and Hispanic women, with the use of a correction factor of 1.06, 0.98, and 1.01, respectively.
sia, in that it was associated with an improved specificity in Down syndrome detection with no significant difference in sensitivity. This information is of clinical importance in the ability to reduce maternal anxiety from reduced false-positive rates when NB hypoplasia is used for Down syndrome screening. Previous reports by our group and others had proposed BPD/NB ratio ≥11 to be the optimal definition of NB hypoplasia.³,⁶,⁷ The sensitivity of NB hypoplasia for Down syndrome detection in the second trimester with the use of the previous definitions varied from 37%-81%, and the specificity varied from 80%-100%.²-⁷,¹⁰ The lower values in the sensitivity ranges and the higher values in the specificity ranges were obtained when absent NB was used as the definition of NB hypoplasia. The current study shows that to optimize the usefulness of NB as a second-trimester marker for trisomy 21, NB MoMs should be adopted as the definition of NB hypoplasia. The sensitivity that is obtained with NB MoM, however, is lower than those reported by Gianferrari et al.⁹ This calls for more studies to validate this novel definition of NB hypoplasia.

The current study has focused only on the issue of optimizing the definition of NB hypoplasia. We previously demonstrated that, when NB is combined with other second trimester markers of aneuploidy, there was an improvement in the detection of Down syndrome, compared with the use of NB as a single marker.⁷ Although, the present study does not address the issue of the efficiency of NB MoM when combined with other markers, there is no reason to believe that a similar improvement in trisomy 21 detection will not be obtained.

Our study has some limitations. NB evaluations were all performed with 2-dimensional sonograms only. Recent evidence from 3-dimensional rendering suggests that NB can be unilaterally or bilaterally absent.¹¹-¹³ Until the technique of 3-dimensional rendering becomes well-standardized to avoid high false-positive rates, we believe that the information that is obtained by 2-dimensional NB evaluation is reliable. We agree with Sonek et al.¹⁰ that, because the phenomenon of unilateral absence of NB is rare in euploid fetuses, it may not affect the efficiency of screening significantly with the use of 2-dimensional sonography.

In conclusion, our study demonstrates that, in second-trimester screening for Down syndrome, the use of NB MoM was the best definition of NB hypoplasia because it was associated with improvement in the specificity of trisomy 21 detection without significantly affecting the sensitivity.

REFERENCES

Placental angiogenesis markers sFlt-1 and PlGF: response to cigarette smoke

Ramkrishna Mehendale, MD, PhD; Judith Hibbard, MD; Asgerally Fazleabas, PhD, HCLD; Richard Leach, MD

OBJECTIVE: Excess soluble vascular endothelial growth factor receptor, fms-like tyrosine kinase-1 (sFlt-1), and reduced placental growth factor (PIGF) mediate the genesis of preeclampsia. Cigarette smoking reduces the risk of preeclampsia. We hypothesized that placental secretion of sFlt-1 and PIGF was affected by exposure to cigarette smoke extract.

STUDY DESIGN: Term placental villous explants were cultured with cigarette smoke extract. Media were analyzed for sFlt-1 and PlGF. Apoptosis was measured by TUNEL staining. Results are reported as sFlt-1 or PlGF picogram/milliliter/milligram wet weight of explant.

RESULTS: Exposure to cigarette smoke extract reduced secretion of sFlt-1 in a dose-dependent manner. There was no difference in apoptosis. In contrast with sFlt-1, PIGF did not decline when incubated with cigarette smoke extract.

CONCLUSION: Exposure of placental villous explants to cigarette smoke extract results in a proangiogenic state with reduced sFlt-1 and relative abundance of PIGF. This is the reverse of changes that are seen in preeclampsia and may explain the reduction of preeclampsia in smokers.

Key words: cigarette smoke, placenta, preeclampsia, sFlt-1, smoking

Preeclampsia, a disease that resists elucidation of cause, affects approximately 8% of pregnancies, and results in both neonatal and maternal morbidity and mortality rates; despite extensive studies, there is still no therapy to prevent or cure this disease. Notably, 15% of all preterm births result from preeclampsia; major risk factors for this disease are primiparity, previous preeclampsia, and chronic hypertension. A number of therapies (eg, salt restriction, zinc, magnesium, fish oil, calcium, and vitamins) have all failed to show significant benefit in the prevention of preeclampsia. Reduction in maternal morbidity can be achieved by delivery of the fetus, but this may compete directly with fetal interests in earlier gestations.

Numerous mechanisms have been implicated in the pathogenesis of the preeclampsia syndrome, which include endothelial activation, oxidative stress, immune activation, and blunted endothelial relaxation of the vasculature. The cardinal pathologic feature noted in the preeclamptic placenta is perturbation of trophoblast invasion into the myometrium and a reduction in the remodeling of the endomyometrial vasculature from high to low resistance vessels, which suggests a role for angiogenic modulators in the development of preeclampsia. Seminal work by Maynard et al demonstrated the involvement of soluble fms-like tyrosine kinase-1 (sFlt-1), which is the soluble form of the vascular endothelial growth factor receptor, in the pathogenesis of preeclampsia.

Paradoxically, it has been noted that the risk of preeclampsia is lower in smokers than in nonsmokers. This effect was first described in 1959 by Lowe and has been verified by a number of investigators. It is notable that, in men and nonpregnant women, the levels of sFlt-1 in smokers were one-half that of nonsmokers; similarly, in pregnancy, serum levels of sFlt-1 were lower in smokers, compared with nonsmokers.

These data led us to hypothesize that cigarette smoke may reduce the secretion of sFlt-1 from placental tissue. This study was undertaken to measure sFlt-1 secretion from placental villous explants that were exposed to cigarette smoke extract in vitro.

Materials and Methods

Cigarette smoke extract

Cigarette smoke extract was prepared with a modification of the method described by Ambalavanar et al. Briefly, air was drawn through 2 lit cigarettes (Marlboro 100s; Philip Morris USA, Richmond, VA) with a vacuum apparatus that bubbled the mainstream smoke through 30 mL of medium. The medium was then cold filtered with a 22-μm filter. Aliquots of this extract were stored at -20°C until used for culture experiments. High-performance liquid chromatography analysis of the extract was performed with a nicotine standard (#36733; Sigma-Aldrich, St. Louis, MO).
Placental villous explants
Placenta was obtained from term healthy patients who underwent vaginal delivery or cesarean section and were collected from patients who had no hypertensive or systemic vascular disease and no suspicion of chorioamnionitis or intrauterine growth restriction. Patients with a self-reported smoking history were also excluded. All tissue was obtained under an institutional review board–approved protocol. A 2 × 2–cm piece of the placental cotyledon was collected in cold culture medium, and dissection was completed within 2 hours of delivery.

The placental tissue was microdissected, and 2–5 mg villous explants from the interior of the cotyledon were generated. These were placed in 24-well flat-bottomed culture plates and covered with 0.5 mL of DMEM/F12 that contained 10% fetal calf serum and penicillin/streptomycin. The explants were incubated overnight at 37°C at the appropriate oxygen tension required by the experiment with 5% CO2 and nitrogen. Media were changed every 24 hours, and care was taken to minimize the time outside the hypoxia chambers.

Explant cultures
Placental villous explants were cultured in 24-well plates with 4 explants per treatment group in 500 μL of media (DMEM/F12 1:1 with 10% nonstripped fetal calf serum and penicillin/streptomycin). This was changed after the initial 12 hours (overnight) incubation. For dose-response experiments, smoke extract was added at a dose of 0, 12.5, 25, or 50 μL per well. Media were changed subsequently every 24 hours, and treatments continued with every change of medium for a total of 72 hours. Spent media were collected, centrifuged to remove debris, aliquoted, and stored at -80°C until assayed. At the end of the culture, the villous explants were blotted gently, and the wet weights were recorded. For some experiments, the explants were fixed and paraffin embedded for TUNEL staining.

Term placental tissue is normoxic at 10% oxygen; ambient air is 20%, and preimplantation tissue oxygen is approximately 2%.19,20 Because this potentially may alter the effect of cigarette smoke on sFlt-1, we also conducted explant cultures at these oxygen tensions. After initial establishment of the model, further experiments with placental growth factor (PIGF) were carried out only under the optimized conditions of normal oxygen tension in the term placenta, approximately 8-10%. For hypoxia experiments, villous explants were cultured similarly in quadruplicate in separate 24-well plates for each oxygen tension. The media were changed every 24 hours, and care was taken to minimize the time outside the hypoxia chambers.

Hypoxia chambers
Plexiglas chambers that had been adapted to provide a humidified environment of 5% CO2 and varying levels of oxygen and to balance nitrogen with the use of a servo control mechanism were used to provide 2% and 10% oxygen tension (BioSpherix, Redfield, NY). These chambers were placed inside standard incubators to maintain an ambient temperature of 37°C. The servo control used O2 and CO2 sensors, and levels were electronically monitored throughout the period of incubation. There were no fluctuations in oxygen or CO2 levels, except for 1 hour immediately after change of culture media. Experiments at 20% O2 tension (ambient oxygen) were carried out in standard cell culture incubators.

Assays
Measurement of sFlt-1 and PIGF was performed with commercially available enzyme-linked immunosorbent assay kits (DVR100 for sFlt-1 and DPG00 for PIGF; R&D Systems, Minneapolis, MN). The volume of media used was determined by pilot assays and ranged from 10-25 μL. Media were assayed in duplicate for all experiments.

Apoptosis was assessed with a commercially available fluorescent terminal deoxynucleotidyl transferase biotin-dUTP nick end labeling (TUNEL) kit (Dead End Fluorometric G3250; Promega, Madison, WI). After being stained according to the manufacturer’s instructions, the sections were mounted in Vectashield with DAPI (Vector Laboratories, Burlingame, CA). Three sections per explant were examined, and 3 images from each section that maximized the tissue represented in the field were used to determine the percentage of TUNEL-positive nuclei.

Statistics
The data represent mean and SE of quadruplicate explants per treatment group. The data were analyzed by analysis of variance with repeated measures; interaction effects included when appropriate. Significance was set at P of <.05.

RESULTS
Smoke extract
High-performance liquid chromatography analysis of smoke extract showed a concentration of 4.5 ng/μL of nicotine. Smoke extract used at the dose of 12.5–50 μL (56.25–225 ng) in 500 μL of culture medium is similar to blood levels of nicotine in heavy smokers (approximately 200 ng/mL).

Effect of smoke extract on sFlt-1 secretion
The secretion of sFlt-1 decreased with time in culture; however, this was not statistically significant, and there was no interaction with treatment with smoke extract. In contrast, there was a statistically significant reduction in the secretion of sFlt-1 with increasing dose of smoke extract. Specifically, the highest dose of cigarette smoke extract resulted in a significantly lower secretion of sFlt-1, compared with control (Figure 1).

Secretion of sFlt-1 increased in controls under conditions of 10% oxygen, compared with 20% and 2% oxygen across time in culture. However, this was not statistically significant. After treatment with smoke extract, a statistically significant decrease in the secretion of sFlt-1 at 10% and 20% oxygen was noted, compared with controls (Figure 2).

Effect of smoke extract on PIGF
PIGF experiments were carried out only under 10% oxygen. The secretion of PIGF decreased with time in controls. Treatment with smoke extract appeared to increase the secretion of PIGF during

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the second (48 hours) and third (72 hours) day of culture (Figure 3), compared with controls. However, these differences were not statistically significant ($P = .06$).

**TUNEL staining**

The percentage of TUNEL-positive nuclei was 11.76% ± 2.0% in the control group and varied between 8.56% and 10.47% in explants that were treated with smoke extract (Figure 4). The percentage of TUNEL-positive nuclei did not change after treatment with cigarette smoke extract ($P = .63$), which indicates the absence of apoptosis because of exposure to smoke extract.

**COMMENT**

Our results indicate that exposure to cigarette smoke significantly reduces the secretion of sFlt-1 from term placental villous explants. This effect is dose dependent and appears to be persistent under ambient (20%) and 10% oxygen, which is the normoxic level within the term placenta. Furthermore, the exposure to smoke extract maintains the secretion of PlGF. We demonstrated no change in the degree of apoptosis. These findings suggest that exposure to smoke extract may reverse the increase in sFlt-1 and the reduction in PlGF serum levels that have been reported in patients with preeclampsia.9,21

The comprehensive metaanalysis by Conde-Agudelo et al,22 which included 34 studies, demonstrated that there is a significant reduction in the risk of preeclampsia by maternal cigarette smoking, with an overall odds ratio of 0.68 (95% CI, 0.67-0.69). Also, reduction in risk was significantly greater with an increase in the number of cigarettes smoked. The relationship between smoking and preeclampsia was consistent across study size and geographic location. Hammoud et al,15 who analyzed a German perinatal database of 157,857 singleton pregnancies, noted that the prevalence of smoking was 23% and that there was an inverse correlation between the rate of preeclampsia and the number of cigarettes smoked per day. A protective effect on the development of preeclampsia in smokers was verified, with an odds ratio of 0.64 (95% CI, 0.59-0.70); there was a significant dose response relationship. Lain et al14 compared 50 cases of preeclampsia with 50 gestational age and body mass index–matched control subjects. Tobacco exposure was quantified by the measurement of urinary cotinine concentration and categorized as nonexposed (<50 ng/mL), light exposure (50-300 ng/mL), and heavy exposure (>300 ng/mL). Overall

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**FIGURE 1**

Effect of increasing doses of cigarette smoke extract on sFlt-1 secretion at 20% oxygen conditions ($n = 4$ for each dose)

![Graph showing effect of different doses of smoke extract on sFlt-1 secretion](image)

**FIGURE 2**

Effect of hypoxia on sFlt-1 secretion at 48 hours of culture

![Graph showing effect of oxygen tension on sFlt-1 secretion](image)
odds ratio for exposed vs nonexposed subjects for the development of preeclampsia was 0.31 (95% CI, 0.12-0.79). A similar effect has also been observed in twin pregnancies.23 Some studies have suggested that there may be a protective effect of smoke exposure, in ever smokers or in women who have recently ceased smoking, from the development of gestational hypertension but not preeclampsia.24 These data suggest that exposure to tobacco smoke significantly reduces the risk of preeclampsia. This effect is independent of geography or reporting bias and is dose-dependent. Our work supports the aforementioned clinical findings and provides a biologic reason for this association that links it to the underlying pathogenesis of preeclampsia.

There is speculation about the components of cigarette smoke that may produce the effects on sFlt-1 and PlGF that we have noted. Genbacev et al,25 who investigated the effect of cigarette smoking on trophoblast phenotype, studied first trimester placental villus explants from patients who were exposed to passive or active smoke and compared the expression of von Hippel Landau protein, a protein that suppresses angiogenesis, to those treated with nicotine in vitro. They noted that in vivo smoke exposure downregulates this protein, enhances angiogenesis, and perhaps improves placental invasion. However, explants that were exposed to nicotine demonstrated the opposite effect with an upregulation of von Hippel Landau protein, which would lead to reduced angiogenesis and hence poor placental invasion. However, explants that were exposed to nicotine demonstrated the opposite effect with an upregulation of von Hippel Landau protein, which would lead to reduced angiogenesis and hence poor placental invasion as seen in preeclampsia. Thus, nicotine is unlikely to be the compound responsible for risk reduction for preeclampsia with maternal smoking.

Carbon monoxide or antioxidant systems have been hypothesized by Bainbridge et al26 to explain these observations. Apoptosis by TUNEL staining, after a hypoxia-reoxygenation injury model of term human placental villous explants, was reduced with exposure to carbon monoxide.27 However, apoptosis is increased in placentae from preeclamptic patients; it is possible that the protective effect of smoking in preeclampsia may be caused by the exposure to carbon monoxide in cigarette smoke. However, the protective effect of smoking has also been seen in ever smokers and smokers who stopped smoking in early gestation and are no longer exposed to continuous carbon monoxide,22 which implicates other factors in this protective effect. Our experiments with an aqueous extract that was stored at -20°C and used in very small quantities (maximum of 50 μL) are likely to have negligible amounts of carbon monoxide.
levels in our cultures, which suggests that there may be other components of cigarette smoke that provide the protective effect against preeclampsia.

In summary, this report offers an explanation for the enigma of cigarette smoking and the reduction in the risk of preeclampsia. Only term placental tissue was used, but further experiments currently are underway to corroborate these results in earlier gestation. Further investigation into the mechanism of this effect in terms of changes in the messenger RNA for the angiogenic factors and their upstream regulators are pending. We agree with those investigators who have suggested that the enigma of smoking, with its deleterious effects of intrauterine growth restriction and abruption, but the beneficial effect in regard to preeclampsia may help elucidate the pathophysiologic factors of this disease.

REFERENCES
Self-reported cognitive functioning in formerly eclamptic women

Annet M. Aukes, MS; Ineke Wessel, PhD; Albertien M. Dubois, MS; Jan G. Aarnoudse, MD, PhD; Gerda G. Zeeman, MD, PhD

OBJECTIVE: Recently, persistent brain white matter lesions were demonstrated in eclamptic women when imaged 6 weeks after delivery. Moreover, many of these women complain about cognitive limitations years after the eclamptic pregnancy. Therefore, in a cohort of such women, we assessed cognitive failures in daily life.

STUDY DESIGN: Thirty formerly eclamptic women completed the Cognitive Failures Questionnaire. Scores were compared with scores of formerly preeclamptic (n = 31) and healthy parous control participants (n = 30) with the use of a priori Student t test. Groups were matched in terms of current age and years elapsed since index pregnancy.

RESULTS: Women who have had eclampsia scored significantly higher on the Cognitive Failures Questionnaire, compared with healthy parous control subjects (43.5 ± 14.6 vs 36.1 ± 13.9, respectively; P < .05).

CONCLUSION: Women who have had eclampsia reported significantly more cognitive failures years after the index pregnancy. It is hypothesized that this might be due to some degree of cerebral white matter damage. This subjective assessment of cognitive function must be confirmed with objective neurocognitive testing and related to neuroimaging findings.

Key words: cognitive failure, eclampsia, Posterior Reversible Leuco-Encephalopathy Syndrome (PRES), preeclampsia

From the School of Behavioral and Cognitive Neurosciences (Ms Aukes) and the Division of Clinical and Developmental Psychology, Faculty of Behavioural and Social Sciences (Dr Wessel), University of Groningen, and the Department of Obstetrics and Gynecology, University Medical Center Groningen (Drs Aarnoudse, and Zeeman, Ms Dubois and Ms Aukes), Groningen, The Netherlands.


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MATERIALS AND METHODS

Participants

The University Medical Center Groningen (UMCG) Obstetrics Department is part of the 8 university teaching hospitals in The Netherlands and serves as a tertiary referral center. The annual delivery rate at the UMCG varied from 1600-1900 in the last 5 years. The Department has used an electronic delivery database since 1988, which was used to identify participants. Seventy-three women who were diagnosed with eclampsia were admitted to the UMCG between 1988 and 2005. Three women died in the interim, 2 of whom died because of the complications of eclampsia (hepatic rupture with bleeding for several days followed by multiple organ failure and severe cerebral edema that resulted in infratentorial herniation). The third woman died of cervical carcinoma several years after pregnancy. We were unable to contact 28 of these women, which resulted in 42 women who were eligible to participate. Each formerly eclamptic woman was matched for age and year of index pregnancy with a formerly preeclamptic woman and a healthy parous control subject. Control subjects with epilepsy or other neurologic or psychiatric disorders that are known to influence cognitive functioning were excluded, as were those women with a history of alcohol or substance abuse. Preeclampsia/eclampsia was defined according to the definition by the International Society for the Study of Hypertension in Pregnancy. Thus, a total of 126 women were invited to participate in the study and received a questionnaire package by mail. Women in all 3 groups received a Dutch translation of the CFQ together with a questionnaire concerning demographic characteristics that included employment, education, and marital status and former and current psychosocial functioning. Participants were also asked about traumatic brain injury and brain surgery in the past and current use of medication. A total of 92 women returned the questionnaires, which resulted in a response rate of 73%. Of the group of formerly eclamptic women, 31 women completed the questionnaire, as did 31 women of the formerly preeclamptic group and 30 women of the healthy control group. A few of the formerly eclamptic women declined to participate because they considered the questions too confrontational. All medical records were reviewed to confirm the diagnosis of preeclampsia/eclampsia and to extract clinical information. Through review of the medical records, documentation of 1 woman was insufficient to confirm the diagnosis of eclampsia; therefore, she was excluded from the study. This resulted in a total number of 30 formerly eclamptic women participating in this study. The study was approved by the local investigational review board, and informed consent for this study was signed by all participants.

Cognitive failures questionnaire

The CFQ is a questionnaire that assesses the likelihood of committing errors in the completion of daily tasks which the participant should be capable of doing (ie, the routines of every day life). Participants were instructed to complete the items with specific reference to the past 6 months. The CFQ consisted of 25 items that were scored on a 5-point scale (range, 0 [never]-4 [very often]). Thus, the total score ranged from 0-100, with higher scores indicating more frequently occurring cognitive failures. A recent factor analytic study confirmed the usefulness of the total score as an index of general cognitive failures and 4 subscales that pertained to more specific areas of cognitive failures. These subscales were memory (7 items; range, 0-28) to assess participant forgetfulness (eg, “Do you find you forget appointments?”), distractibility (9 items; range, 0-36) to assess disturbance of internally focused attention (eg, “Do you daydream when you ought to be listening to something?”), blunders (7 items; range, 0-28) to reflect social blunders and motor control (eg, “Do you say something and realize afterwards that it might be taken as insulting?”; “Do you bump into people?”), and names (2 items; range, 0-8; eg, “Do you find you forget people’s names?”).

Statistical analysis

Because of our expectation that cognitive failures would occur specifically in the eclamptic group, planned comparisons (t tests) were carried out to analyze total CFQ scores and scores on the subscales. Demographic characteristics and parameters relevant to psychosocial functioning were analyzed with t tests or χ² analyses where appropriate. All tests were 2-tailed with alpha set at .05.

RESULTS

Relevant characteristics at the time of the index pregnancy are shown in Table 1. In the formerly eclamptic groups, no differences were found between participating and nonparticipating women (Table 1). Formerly eclamptic women scored significantly higher on the CFQ, compared with healthy parous control subjects (Table 2). The difference in total CFQ scores between formerly eclamptic and preeclamptic women showed a nonsignificant trend (P = .08). Eclamptic women who experienced 3 seizures had significantly higher CFQ total scores, compared with women who only had 1 seizure (Table 2), which suggests that each seizure had cumulative harmful effects, that resulted in increased CFQ scores. As for the CFQ subscales, formerly eclamptic women had higher scores compared with both formerly preeclamptic women and healthy parous control subjects; however, no significant differences were found. Table 3 shows relevant demographic and psychosocial characteristics for all 3 groups. Current age of the participants in all groups was similar. There was no difference in elapsed time since the index pregnancy between any of the groups.

There was no difference in the number of women who were currently not working outside the home. However, 4 of the 8 formerly eclamptic women (50%) reported to be unable to work because of their current health status. Moreover, these women received sickness or disability benefits in contrast to none of the formerly preeclamptic women. In this latter group, 5 women who reported not to be working outside the home stated that this was their personal choice. Sig-
significantly more women of the pre- eclamptic group reported a history of migraine, compared with the control subjects (P = .02). No participant reported a history of epilepsy or any other relevant intercurrent medical condition. Two women in the control group reported a cerebral contusion without any permanent sequelae in the past. There was no difference in level of education among the groups. Use of tobacco and alcohol was similar in all 3 groups. The current use of antihypertensive medication was significantly higher in the formerly preeclamptic group, compared with the formerly eclamptic and control groups (P = .040 and .003, respectively). A similar number of women in each group reported having experienced past episodes of lack of interest and/or phases of feeling down. Compared with the control subjects, significantly more women in the formerly eclamptic and preeclamptic groups reported that these episodes were related specifically to the index pregnancy or delivery (P = .010 and .004, respectively). Eight of the formerly eclamptic women had received psychologic treatment because of these problems, which was significantly greater compared with the control women (P = .002). There was no difference among any of the groups in number of women who were receiving current psychologic therapy.

**COMMENT**

The main finding of this study is that, several years after a pregnancy that was complicated by eclampsia, women reported impaired cognitive functioning compared with healthy parous women. In addition, women who experienced multiple eclamptic seizures reported greater cognitive impairment, compared with those who experienced 1 seizure. This is a remarkable finding because the predominant view holds that eclampsia concerns a 1-time event without any known long-term consequences, provided that intracranial hemorrhage does not precede or follow the acute moment.

The difference in CFQ outcomes may indicate that eclamptic seizures are more harmful than has been thought previously. The concept that the occurrence of eclamptic seizures does not affect ma-

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**TABLE 1**

Gestational characteristics of index pregnancy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Eclampsia (n = 30)</th>
<th>Preeclampsia (n = 31)</th>
<th>Control subjects (n = 30)</th>
<th>Nonparticipating formerly eclampsia (n = 39)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage of primiparous women (%)</td>
<td>80</td>
<td>57</td>
<td>52</td>
<td>64</td>
</tr>
<tr>
<td>Gestational age (wk)*</td>
<td>33.2 ± 4.5</td>
<td>34.6 ± 5.0</td>
<td>39.8 ± 1.3</td>
<td>32.3 ± 4.1</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>1849 ± 961</td>
<td>2132 ± 1226</td>
<td>3551 ± 477</td>
<td>1628 ± 818</td>
</tr>
<tr>
<td>Percentage cesarean section delivery (%)</td>
<td>71</td>
<td>61</td>
<td>8</td>
<td>79</td>
</tr>
</tbody>
</table>

No. of seizures (n)

<table>
<thead>
<tr>
<th>No. of seizures</th>
<th>Eclampsia</th>
<th>Preeclampsia</th>
<th>Control subjects</th>
<th>Nonparticipating former eclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16 (53)</td>
<td>10 (33)</td>
<td>4 (13)</td>
<td>3 (8)</td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Unknown</td>
<td></td>
<td></td>
<td></td>
<td>1 (3)</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.

---

**TABLE 2**

CFQ scores

<table>
<thead>
<tr>
<th>Score</th>
<th>Group</th>
<th>Eclampsia (n = 30)</th>
<th>Preeclampsia (n = 31)</th>
<th>Control subjects (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CFQ total score (0-100)</td>
<td>43.5 ± 14.6*</td>
<td>36.9 ± 13.9</td>
<td>36.1 ± 13.9</td>
<td></td>
</tr>
<tr>
<td>Memory (0-28)</td>
<td>10.8 ± 5.1</td>
<td>9.0 ± 3.9</td>
<td>9.2 ± 4.7</td>
<td></td>
</tr>
<tr>
<td>Distractibility (0-36)</td>
<td>16.6 ± 5.6</td>
<td>14.4 ± 5.3</td>
<td>13.5 ± 5.6</td>
<td></td>
</tr>
<tr>
<td>Blunders (0-28)</td>
<td>11.2 ± 4.3</td>
<td>9.4 ± 4.5</td>
<td>8.9 ± 3.6</td>
<td></td>
</tr>
<tr>
<td>Names (0-8)</td>
<td>4.8 ± 2.2</td>
<td>4.1 ± 2.3</td>
<td>4.6 ± 1.8</td>
<td></td>
</tr>
</tbody>
</table>

CFQ total score (0-100)

| 1 seizure (n = 16) | 39.1 ± 10.9 |
| 2 seizures (n = 10)| 43.8 ± 16.3 |
| 3 seizures (n = 4)| 60.0 ± 14.5*|

Results are given as means ± SD.

* P = .040; t(58) = 2.0 vs control.
† P = .005; t(18) = −3.2 vs eclampsia with 1 seizure.
ternal outcome as long as the maternal and fetal condition is being monitored closely during a seizure is no longer acceptable. The significant difference in outcome between the formerly eclamptic and pre-eclamptic women resembled those of the healthy parous control subjects. This lack of significant difference is likely due to a type II error. Future studies with larger groups should better determine differences in functioning between formerly eclamptic and pre-eclamptic women.

Virtually nothing is known about long-term consequences of PRES in either obstetric or nonobstetric patients, regarding their neurocognitive and social functioning. However, there is some evidence that preeclampsia may increase the risk of psychiatric conditions such as depression or posttraumatic stress disorder, relative to women with uncomplicated pregnancies. The present study used questions about episodes in the past that were characterized by a loss of interest and/or depressed mood as a crude index of past depression. The finding that formerly eclamptic and preeclamptic women indicated that they had experienced more of such episodes that were tied specifically to the index pregnancy or delivery is consistent with this previous literature on psychiatric sequelae of preeclampsia/eclampsia.

Interestingly, the prevalence of migraine was found to be higher in both formerly preeclamptic and eclamptic women in our study. The association between preeclampsia/eclampsia and migraine has been reported previously. Both conditions are thought to be characterized by hyperperfusion of the brain, are related to changes in female sex steroid hormones, and have similar symptoms (headache, visual disturbances, and nausea). However, the exact pathophysiologic condition of and the association between, migraine and preeclampsia/eclampsia remains unknown. In addition, white matter lesions in the cerebral posterior circulation territory are demonstrated more frequently

### Table 3

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Group</th>
<th>Eclampsia (n = 30)</th>
<th>Preeclampsia (n = 31)</th>
<th>Control subjects (n = 30)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current age (y)*</td>
<td></td>
<td>38.7 ± 6.6</td>
<td>40.3 ± 5.2</td>
<td>38.7 ± 7.0</td>
</tr>
<tr>
<td>Elapsed time since index pregnancy (y)*</td>
<td></td>
<td>7.6 ± 5.0</td>
<td>6.8 ± 4.5</td>
<td>5.8 ± 4.1</td>
</tr>
<tr>
<td>Education y†</td>
<td></td>
<td>8.7 ± 1.5</td>
<td>7.9 ± 1.9</td>
<td>8.6 ± 2.1</td>
</tr>
<tr>
<td>Married or cohabited (n)</td>
<td></td>
<td>25 (83%)</td>
<td>26 (84%)</td>
<td>25 (83%)</td>
</tr>
<tr>
<td>No work outside of home (n)</td>
<td></td>
<td>8 (27%)</td>
<td>5 (16%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Of whom receive disability/sickness benefits</td>
<td></td>
<td>4 (50%)‡</td>
<td>0</td>
<td>2 (33%)</td>
</tr>
<tr>
<td>Tobacco use (n)</td>
<td></td>
<td>5 (17%)</td>
<td>9 (29%)</td>
<td>6 (20%)</td>
</tr>
<tr>
<td>Alcohol use (n)</td>
<td></td>
<td>15 (50%)</td>
<td>19 (61%)</td>
<td>18 (60%)</td>
</tr>
<tr>
<td>Antihypertensive medication (n)</td>
<td></td>
<td>2 (7%)</td>
<td>8 (26%)§</td>
<td>0</td>
</tr>
<tr>
<td>Migraine (n)</td>
<td></td>
<td>9 (30%)</td>
<td>14 (45%)†</td>
<td>5 (17%)</td>
</tr>
<tr>
<td>Episodes of feeling down and/or lack of interest (n)</td>
<td></td>
<td>17 (57%)</td>
<td>15 (48%)</td>
<td>17 (57%)</td>
</tr>
<tr>
<td>Of which related to pregnancy or postpartum period</td>
<td></td>
<td>9 (53%)§</td>
<td>9 (60%)§</td>
<td>2 (12%)</td>
</tr>
<tr>
<td>Psychologic therapy because of pregnancy-related problems (n)</td>
<td></td>
<td>8 (27%)§</td>
<td>3 (10%)</td>
<td>0</td>
</tr>
</tbody>
</table>

* Data are given as mean ± SD.  
* Education was measured by an increasing scale (1-11), depending on the highest level of completed education.  
* $P = .06$ vs women with preeclampsia.  
* $P = .05$ vs women with eclampsia.  
* § $P ≤ .01$ vs control subjects.  
* † $P ≤ .05$ vs women with eclampsia.  
* ‡ $P ≤ .01$ vs control subjects.
in the general population in people who experience migraine, especially in women. This may also be of particular relevance to women with eclampsia.

It should be noted that CFQ scores reflect self-reported difficulties that pertain to everyday slips of attention and action. Although the CFQ has demonstrated satisfactory internal consistency, retest reliability, and cross-cultural validity, additional measures are needed to illuminate the origins of elevated scores. For example, a previous study found elevated CFQ scores in organic (predominantly dementia) and functional (ie, depression or anxiety) conditions. Yet, the authors were unable to identify specific CFQ profiles that discriminated between those organic and functional groups. Thus, although the present findings are potentially consistent with an interpretation in terms of brain white matter lesions, alternative explanations cannot be ruled out. For example, it remains possible that cognitive failures in the eclampsia group were due to nonorganic variables, such as increased levels of anxiety or depression. Furthermore, it is also possible that higher cognitive failures scores reflect the specific conviction of eclamptic women that they experience memory and concentration difficulties. That is, it may be that objectively eclamptic women are comparable to healthy control subjects, yet they interpret the occurrence of cognitive failures as evidence for the negative sequelae of their eclamptic seizures. It is possible that some women attribute a more negative meaning to cognitive lapses than others, which results in higher estimates of their occurrence over the index period of the previous 6 months as identified in the CFQ instructions. However, the increase of cognitive dysfunction with increased number of seizures and the finding that more women in the eclamptic group were unable to work and received disability benefits may be taken as support for the notion that differences in reported cognitive failures reflected genuine dysfunctions in daily life, rather than a self-report bias.

Several additional methods limitations to this study deserve attention. First, the retrospective nature of the study may have resulted in selection bias. For example, if formerly eclamptic women with more cognitive dysfunction were eager to participate, seeking recognition of self-perceived cognitive limitations, the cognitive impairment might be overestimated. In addition, the CFQ is a retrospective instrument that is subject to the limitations of human memory. Second, because the CFQ is a subjective measure of cognitive function, neuropsychological examination will be needed to demonstrate cognitive abilities and limitations objectively. A third limitation to this study is that the investigated groups are small. However, in the context of the rare incidence of eclampsia, this represents a sizeable study, the results of which may be clinically important.

In conclusion, formerly eclamptic women report some degree of cognitive impairment many years after the index pregnancy. Whether the cognitive impairment that was found in this study cohort of formerly eclamptic subjects might be due to some degree of permanent cerebral white matter disturbance remains to be seen. Further research is now ongoing to confirm our findings, with the use of objective neurocognitive testing such as functional magnetic resonance imaging and neuroimaging to assess the relationship with brain white matter lesions in formerly eclamptic women. The potential for the identification (and eventually the prevention and treatment) of chronic cognitive and psychosocial impairment in this particular group of young mothers is of important societal relevance and will also draw attention to possible long-term sequelae after PRES in other, nonpregnancy-related patient categories. The paradigm that eclampsia is a condition of which the women can expect full clinical recovery may need to be revised, and the importance of the prevention of eclamptic seizures should be emphasized.

ACKNOWLEDGEMENTS
We thank Marjon Wiegman for her assistance in this study.

REFERENCES


Clinical trial of interconceptional antibiotics to prevent preterm birth: subgroup analyses and possible adverse antibiotic–microbial interaction

Alan T. N. Tita, MD, MPH, PhD; Suzanne P. Cliver, BA; Alice R. Goepfert, MD; Michael Conner, MD; Robert L. Goldenberg, MD; John C. Hauth, MD; William W. Andrews, PhD, MD

OBJECTIVE: The purpose of this study was to explore whether endometrial microbial colonization and plasma cell endometritis are risk factors for adverse pregnancy outcomes, and whether these outcomes are influenced by interactions between interconceptional antibiotics and the micro-flora.

STUDY DESIGN: Subgroup analyses of data from a double-blind, randomized, placebo-controlled trial of a course of metronidazole plus azithromycin given every 4 months to women with a prior preterm delivery to prevent recurrent preterm delivery. Endometrial cultures and histology were obtained at randomization and repeated 2 weeks after the first treatment. Fifty-nine on antibiotics versus 65 on placebo had pregnancy outcomes. Prevalence of adverse pregnancy outcomes (pregnancy loss or preterm birth < 37 weeks) was stratified by treatment group and endometrial characteristics. Subgroups were assessed and screened for potential interaction (P values for significance set a priori at ≤ .01), prior to formal statistical testing for interaction (P values ≤ .05).

RESULTS: The prevalence of adverse pregnancy outcome was 62.7% in the presence of endometrial microbial colonization at baseline (any microbe) and 50% in the absence of colonization (RR = 1.25; 99% CI 0.42-3.7). Prevalence of adverse pregnancy outcomes was 61.9% with plasma cell endometritis, and 70.8% without; RR = 0.87 (0.50-1.5). There was a nonsignificant reduction in adverse pregnancy outcome in the absence of Gardnerella vaginalis or Gram-negative rods with RR (95% CI) = 0.60 (0.3-1.2) and 0.66 (0.4-1.2), respectively. In the presence of these microbes, antibiotics appeared to increase adverse outcomes: RR = 1.5 (1.1-2.0) and 1.5 (1.1-2.1), respectively. This reversal of impact represents a crossover interaction.

CONCLUSION: Neither baseline endometrial microbial colonization nor plasma cell endometritis were risk factors for adverse pregnancy outcome. However, colonization with specific microbes interacted with antibiotics to increase adverse outcomes.

Key words: antibiotics, endometrial micro-flora, interactions or effect modification, preterm birth


Preterm birth, which occurs in 12.7% of births in the United States, is a major cause of neonatal and infant mortality and long term morbidity. Among efforts to reduce preterm delivery (PTD), we recently reported findings from a double-blind, randomized clinical trial of interconceptional prophylactic antibiotics. A 1-week course of metronidazole plus azithromycin versus placebo was administered every 4 months to 241 women at high risk for PTD. These women also had endometrial cultures and histology performed at randomization and 2 weeks after initial treatment. Antibiotics did not reduce the prevalence of subsequent PTD or pregnancy loss among 124 women who conceived and had pregnancy outcomes. Instead, there was a trend towards lower mean gestational age (32.0 vs 34.4 weeks, P = .08) and birthweight (2046 vs 2464 g, P = .06) associated with antibiotics. This occurred even though the antibiotic regimen induced desirable changes in the endometrial micro-flora. Antibiotics prevented the acquisition and/or promoted the resolution of endometrial microbial colonization with highest impact on Gram-negative rods, especially Gardnerella vaginalis. Taken together with other studies of antibiotics in pregnancy that reported reductions in susceptible genital tract microbes in association with increased adverse pregnancy outcomes.
this paradoxical finding raised concern about a possible adverse interaction between the endometrial micro-flora and antibiotics.

Furthermore, an editorial review accompanying the original trial report highlighted clinical questions that could be addressed using data collected as part of the clinical trial. Key among these concerned whether positive endometrial cultures or chronic endometritis were associated with increased risk of adverse pregnancy outcomes such as PTD.

Therefore, we conducted subgroup analyses to explore whether baseline endometrial microbial colonization and plasma cell endometritis were risk factors for adverse pregnancy outcomes (preterm delivery < 37 weeks or pregnancy loss), and to ascertain any interaction between the endometrial micro-flora and antibiotic therapy.

**Materials and Methods**

We performed subgroup analyses of data obtained as part of a previously reported double-blind, placebo-controlled randomized clinical trial. The methodology has already been described in detail. Briefly, between January 1998 and August 2001 research personnel recruited women with singleton pregnancies ending in a spontaneous preterm birth or pregnancy loss between 16 and 34 weeks. Women who were not at risk of becoming pregnant (had a cesarean hysterectomy or sterilization, or had Norplant or IUD placement) were excluded from the study, as were those with a multiple gestation or fetal anomaly in the index pregnancy. Our Institutional Review Board approved the study and all women provided written informed consent.

At approximately 4 months’ postpartum, study candidates were evaluated, and baseline endometrial specimens were obtained and specific cultures performed for aerobic and anaerobic bacteria, as well as *Ureaplasma urealyticum*, *Mycoplasma species*, *Neisseria gonorrhoeae*, group B *Streptococcus*, *Trichomonas vaginalis*, and a ligase chain reaction test for *Chlamydia trachomatis*. Histopathology was also performed on the endometrial specimens. Plasma cell endometritis was defined as the presence of any plasma cell on 40X magnification. These nonpregnant women were randomized to active-drug versus placebo groups using a computerized random number sequence. The active-drug group received 2 doses of azithromycin 1.0 g given 4 days apart plus sustained-release metronidazole 750 mg daily for 7 days. The control group received identical-appearing placebos. Treatment was repeated every 4 months until conception or until the study was terminated. Women were reevaluated 2 weeks after randomization at which time specimens were obtained for repeat endometrial cultures and histopathology.

Follow-up continued for 5 years when the study was discontinued after achievement of 68% of the recruitment goal because the funding agency ceased support. A total of 241 women had been randomized and 134 conceived. Seven women electrolytically terminated their pregnancies while 3 had no outcome data. This report focuses on the 124 remaining women.

Adverse pregnancy outcome was defined as a composite of PTD < 37 weeks or spontaneous pregnancy loss. In the primary report, these outcomes were reported separately (PTD ≥ 15 weeks and spontaneous abortion < 15 weeks). We combined them here (additional analyses were also performed to confirm consistent results on alternative outcomes: 1. all PTD < 37 weeks [ie, excluding miscarriages, 9 on placebo vs 7 on antibiotics]; 2. spontaneous PTD < 37 weeks [ie, further excluding 10 indicated PTD; 5 in each arm]; and 3. composite of spontaneous PTD and pregnancy loss). Prevalence of adverse pregnancy outcome was stratified by treatment group and by characteristic of the endometrial micro-flora. The endometrial micro-flora was characterized by the presence or absence of individual microbes, selected groups of microbes, or plasma cell endometritis.

Baseline demographic comparisons were performed using Chi-square test or Student t test for categorical and continuous variables, respectively. Chi-square or Fisher exact tests were used as appropriate to compare the prevalence of adverse pregnancy outcomes for women with and without specific endometrial microbial characteristics within treatment subgroups. Because of planned multiple comparisons, P values < .01 were designated a priori to represent significant findings. Therefore, when computed, corresponding relative risks (RR) were bound by 99% (not 95%) confidence intervals (CI). Examination of results within the placebo group only was utilized to determine whether characteristics of the endometrial micro-flora were risk factors for adverse pregnancy outcome. Examination of findings for endometrial subgroups across treatment groups was also utilized to screen for possible interactions using the same stringent criteria. Chi-square test for heterogeneity with P value for significance set a priori at .05 (not the frequently used .1) as well as the stratification method for independent and joint effects (RR and 95% CI) were utilized to examine relationships suggesting an interaction.

Data management and statistical analyses were performed using SAS version 9.1 (Cary, NC) and Stata/SE version 9.2 (College Station, TX).

**Results**

Out of the 124 women, 59 were randomized to antibiotics and 65 to placebo. Women in both arms had similar baseline sociodemographic characteristics including age, race, smoking, prior miscarriage, gestational age at prior delivery, and time from randomization to index delivery as originally reported (Table 1). The prevalence of spontaneous PTD was 52% (27/52) in the antibiotic group versus 46% (26/56) in the placebo group while the prevalence of any PTD was 62% (32/52) versus 55% (31/56). Prevalence of miscarriage < 15 weeks was 12% (7/59) versus 14% (9/65), respectively. Therefore, the prevalence of adverse pregnancy outcome was 66.1% (39/59) versus 61.5% (40/65), P value = .60.

In Table 2 we present prevalence of adverse pregnancy outcomes stratified by the presence or absence of individual microbes, selected groups of microbes, and plasma cell endometritis and by treatment subgroup. Focusing on the placebo arm only, prevalence of adverse preg-
nancy outcomes was not significantly different in the presence (62.7%) versus absence (50%) of endometrial colonization with any microbe, corresponding to a RR (99% CI) of 1.25 (0.42-3.7). The corresponding RR after excluding indicated preterm births from the outcome was 1.08 (0.36-3.3). Similarly, plasma cell endometritis was not associated with adverse pregnancy outcomes: 61.9% when present versus 70.8% when absent, RR = 0.87 (0.50-1.5) and excluding indicated preterm births, 1.05 (0.53-2.1). Lactobacillus and Gram-positive rods in general were associated with a nonsignificant increase in adverse pregnancy outcome with RR = 1.52 (0.91-2.5), which became significant after indicated preterm births were excluded: 1.86 (1.01-3.44). The presence of Gemella morbillorum was associated with 100% adverse pregnancy outcome (regardless of treatment) but this relationship did not attain statistical significance. The other endometrial microbial species or groups, including presence of Gardnerella vaginalis, were not associated with increased prevalence of adverse pregnancy outcomes. For the antibiotic arm, Gardnerella vaginalis and Gram-negative rods were strongly associated with increased prevalence of adverse pregnancy outcomes (Table 2).

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Antibiotics (n = 59)</th>
<th>Placebo (n = 65)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>23.3 ± 5.7</td>
<td>23.7 ± 5.3</td>
<td>.689</td>
</tr>
<tr>
<td>African American (vs white)</td>
<td>80</td>
<td>78</td>
<td>.870</td>
</tr>
<tr>
<td>Unmarried</td>
<td>73</td>
<td>63</td>
<td>.244</td>
</tr>
<tr>
<td>Smoker</td>
<td>15</td>
<td>20</td>
<td>.490</td>
</tr>
<tr>
<td>Education ≤ 12 years</td>
<td>29</td>
<td>38</td>
<td>.257</td>
</tr>
<tr>
<td>History of spontaneous abortion</td>
<td>39</td>
<td>31</td>
<td>.337</td>
</tr>
<tr>
<td>Gestational age of index pregnancy (wk)*</td>
<td>25.0 ± 4.7</td>
<td>26.0 ± 4.8</td>
<td>.261</td>
</tr>
<tr>
<td>Previous delivery to randomization (d)*</td>
<td>177 ± 235</td>
<td>164 ± 209</td>
<td>.740</td>
</tr>
<tr>
<td>Last treatment to conception time (d)*</td>
<td>169 ± 292</td>
<td>123 ± 169</td>
<td>.292</td>
</tr>
<tr>
<td>Interpregnancy interval (d)*</td>
<td>522 ± 414</td>
<td>554 ± 381</td>
<td>.664</td>
</tr>
</tbody>
</table>

Table reproduced from Am J Obstet Gynecol 2006;194:617-23 (reference 3).

* Results reported in mean ± standard deviation, all others are percentages.

### Table 2

<table>
<thead>
<tr>
<th>Microbial species or category</th>
<th>% Adverse outcome* (Placebo group, n = 65)</th>
<th>% Adverse outcome* (Antibiotic group, n = 59)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Present†</td>
<td>Absent†</td>
</tr>
<tr>
<td>Plasma cell endometritis</td>
<td>61.9 (13/21)</td>
<td>70.8 (17/24)</td>
</tr>
<tr>
<td>Any endometrial microbe</td>
<td>62.7 (37/59)</td>
<td>50.0 (3/6)</td>
</tr>
<tr>
<td>Gardnerella vaginalis</td>
<td>62.5 (20/32)</td>
<td>60.6 (20/33)</td>
</tr>
<tr>
<td>Lactobacillus species</td>
<td>75.9 (22/29)</td>
<td>50.0 (18/36)</td>
</tr>
<tr>
<td>Ureaplasma urealyticum or</td>
<td>62.5 (5/8)</td>
<td>61.4 (35/57)</td>
</tr>
<tr>
<td>Mycoplasma hominis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gemella morbillorum</td>
<td>100 (4/4)</td>
<td>59.0 (36/61)</td>
</tr>
<tr>
<td>Gram negative rods</td>
<td>64.7 (22/34)</td>
<td>58.1 (18/31)</td>
</tr>
<tr>
<td>Gram positive rods</td>
<td>75.9 (22/29)</td>
<td>50.0 (18/36)</td>
</tr>
<tr>
<td>Gram positive cocci</td>
<td>62.5 (20/32)</td>
<td>60.6 (20/33)</td>
</tr>
<tr>
<td>Aerobes</td>
<td>63.2 (36/57)</td>
<td>50.0 (4/8)</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>61.1 (11/18)</td>
<td>61.7 (29/47)</td>
</tr>
</tbody>
</table>

* Adverse pregnancy outcomes — preterm delivery < 37 weeks + miscarriages.

† Numbers in columns represent percentage of adverse pregnancy outcome (numerator/denominator) in presence or absence of microbial species or category.
Further examination of results across treatment groups to screen for potential interaction between antibiotic groups and micro-flora revealed Gardnerella vaginalis and Gram-negative rods in general as candidates because of their strong association with adverse pregnancy outcome in the antibiotic (P values < .0001), but not in the placebo arm. We also preselected colonization with any microbe because of its trend towards an association with adverse pregnancy outcome in the antibiotic arm alone (P value = .014). The relative risks of adverse pregnancy outcome associated with interconceptional antibiotics (vs placebo) stratified by presence or absence of each of these 3 preselected endometrial microbial species or groups are presented in Table 3.

The results in Table 3 indicate that the impact of antibiotics on pregnancy outcomes was highly significantly modified by endometrial colonization with Gardnerella vaginalis or Gram-negative rods in general (P values of .0014 and .0084, respectively). When Gardnerella vaginalis and Gram-negative rods were present prior to treatment, antibiotic treatment was, respectively, associated with a 45% and a 36% statistically significant increase in adverse pregnancy outcome. In the absence of either one, there was a nonsignificant 35-40% risk reduction in adverse pregnancy outcomes among women treated with antibiotics. Excluding indicated preterm births, the RR (95% CI) for the impact of antibiotics was 0.58 (0.3-1.1) in the absence of G. vaginalis and 1.56 (1.1-2.3) in its presence, yielding a P value for heterogeneity of .003. The impact of antibiotics however did not vary significantly according to the presence or absence colonization of the endometrium with any microbe.

To better describe the impact of this interaction between antibiotic treatment and endometrial microbes (Gardnerella or Gram-negative rods in general), we present their independent and joint effects in Table 4. Compared to the reference group of women on placebo and not colonized at baseline with Gardnerella, women on placebo and colonized by Gardnerella were not at increased risk for adverse pregnancy outcomes, RR (95% CI) = 1.0 (0.7-1.5). Antibiotics appeared to reduce the risk in the absence of colonization with Gardnerella: RR = 0.6 (0.3-1.1) and P value = .07. Importantly, the combination of antibiotics and colonization with Gardnerella was associated with a 50% increase in adverse outcomes; RR = 1.5 (1.1-2.0), P < .05. Assuming no interaction between Gardnerella and antibiotics on either of 2 commonly used scales (additive or multiplicative), using standard methods we computed that the expected relative risks for the joint impact of Gardnerella vaginalis and antibiotics would be both 0.6. Therefore, the ob-
served relative risk of 1.5 represents a strong negative interaction crossing to the other side of null on both scales. In other words, not only is the protective relative risk associated with antibiotics erased, the combination of antibiotics and baseline presence of Gardnerella is in fact harmful. This is known as a crossover or qualitative interaction or effect modification.\(^9,10\) The results for the observed independent and joint effects involving Gram-negative rods in general were practically identical to those for Gardnerella (Table 4). Compared with the expected joint relative risks on both scales of no interaction, the observed relative risk of 1.5 also indicated significant crossover interaction.

**Comment**

Overall, subgroup analyses of data from the innovative clinical trial of interconceptional antibiotics among high-risk women failed to identify endometrial colonization or plasma cell endometritis as risk factors for pregnancy loss or preterm delivery < 37 weeks. Of interest, endometrial colonization with *Gardnerella vaginalis* or Gram-negative rods in general did not appear to independently affect pregnancy outcomes. However, the antibiotic regimen comprising azithromycin and metronidazole was consistently associated with a nonsignificant 40% reduction in adverse outcomes in the absence of these microbes. Importantly, this apparent protective impact of antibiotics appeared to be negatively modified by colonization with Gardnerella or Gram-negative rods in general such that the joint impact of antibiotics and colonization with either of these microbes was instead associated with a 50% increase in adverse pregnancy outcomes, a finding consistent with an impressive crossover effect modification.

These findings must be considered and interpreted within the framework of the study limitations, especially those inherent in subgroup analyses.\(^9,10\) First, multiple comparisons increased the likelihood that a significant relationship may occur purely by chance (type 1 error). We addressed this issue by a priori using a stringent criterion for determining risk factor impact and preselecting significant relationships (*P* value < .01 as opposed to the commonly used .05). Regarding assessment for interactions, a *P* value < .1 is often used in exploratory studies as a cut-off for significance because of concerns that a true interaction might be missed. We however applied a more stringent threshold of *P* < .05 to reduce the likelihood of chance findings.

A second study limitation is the possibility that real relationships may be missed because of small sample size (type 2 errors). This might be the case with Lactobacillus (or Gram positive rods) as a risk factor for adverse outcome as well as *Gemella morbillorum*.

Our use of a composite adverse pregnancy outcome combining pregnancy loss and all PTD may be perceived as a third study limitation, especially as the all PTD outcome combines spontaneous and indicated PTD. However, a prior spontaneous PTD is a risk factor for an indicated PTD and vice versa,\(^1,1\) suggesting that shared causal mechanisms are involved. An example is chorioamnionitis, potentially modifiable by antibiotics. Nevertheless, we also conducted additional analyses using alternative outcomes including the exclusion of indicated preterm births and the results were the same.

Finally, endometrial specimens were obtained at a time remote from the future pregnancy of interest, compounded by the long mean time from last treatment to conception (4 to 5 months). It is biologically plausible that antibiotics may induce a sequence of changes in the endometrium that ultimately influences pregnancy outcomes. Also, the impact of potential endometrial microbes such as fungi, viruses, or other microbes that can only be identified using molecular techniques is unknown.

A crossover (or qualitative) interaction is a relationship in which impact (of antibiotics in this study) is harmful in 1 subgroup (endometrial colonization with specific microbes) but protective in the other (absence of specific microbial colonization). An analogous conceptualization of crossover interaction (see Table 4) is the scenario where the observed joint impact of 2 factors (antibiotics and endometrial microbes) is on the opposite side of null to the expected joint impact (computed from individual RRs) assuming no interaction existed between the factors. Compared to quantitative interactions where impact is in the same direction in both subgroups, but of different magnitude, qualitative interactions are less likely to be chance findings and less frequently encountered.\(^8,9\) An application of Bradford Hill’s classic considerations for causality\(^12\) including experimental evidence, a cause-effect temporal relationship suggests that the antimicrobial/endometrial microbial interaction might be real or at least a true marker for events occurring within the endometrium in association with antibiotics. Biologic plausibility and epidemiologic coherence derive from reports of increased adverse pregnancy outcomes (mainly PTD) in defined groups of women receiving antibiotics prior to or during pregnancy,\(^3,5,13-16\) although other studies have not reported this increase.\(^17,18\) To our knowledge, none have reported the likely presence of a similar crossover antibiotic-microbial interaction. Furthermore, the same endometrial microbes most affected by antibiotics\(^5\) are implicated in the reported interaction. If reproducible, the biologic mechanism underlying this interaction remains unclear. Metronidazole is bactericidal while azithromycin is mainly bacteriostatic.\(^19\) Perhaps by causing the release of bacterial products and/or inactivating bacteria and exposing them to inflammatory cells, antibiotics may engender an endometrial reaction that ultimately increases the risk of adverse pregnancy outcomes. A clinical analogy is observed deterioration associated with antibiotic treatment of Gram-negative sepsis\(^20\) or the Jarisch-Herxheimer reaction to syphilis treatment, although these reactions occur in a very short time frame. A less plausible mechanism is the selection of pathogenic endometrial microbes due to antibiotics: we did not observe such microbial overgrowth among over 50 bacterial species studied.\(^3\)

In prior reports we demonstrated that interconceptional antibiotic treatment did not reduce adverse pregnancy out-
comes to even though it reduced endometrial microbial colonization by specific microbes. New data in the current report do not support the simple hypothetical model that endometrial microbial colonization leads to adverse pregnancy outcomes. Furthermore, these data support a more complex hypothetical model that should account additionally for endometrial micro-flora/antimicrobial interaction. This modification of a protective impact of antibiotics by endometrial colonization with *Gardnerella vaginalis* (or Gram-negative rods) to jointly increase adverse pregnancy outcome remains exploratory. It should be assessed in future studies, ideally, designed and powered to detect such an interaction prior to any change in clinical practice. Even then, it might be useful to examine less invasive and cost effective ways by which the endometrial microenvironment might be accurately predicted by sampling the more accessible environments of the vagina and cervix.

**REFERENCES**


The association of crown-rump length discordance in twin gestations with adverse perinatal outcomes

Judy Tai, MD; William A. Grobman, MD, MBA

OBJECTIVE: The purpose of this study was to evaluate the association between the crown-rump length (CRL) difference in twin gestations and adverse pregnancy outcome.

STUDY DESIGN: Women with a first trimester ultrasound scan of a twin pregnancy who delivered between June 2000 and March 2006 at Northwestern Memorial Hospital were identified. The association between the difference in CRLs and pregnancy outcomes was explored.

RESULTS: For the 178 twins who were eligible for the study, the median difference in CRLs was 4.2% and the median difference in birthweights was 9.3%. Twins with a CRL difference of >85th percentile (11.1%) were more likely to have discordant birthweights and to have at least 1 of the pair be small-for-gestational age, be admitted to the special care nursery, and have perinatal morbidity.

CONCLUSION: CRL differences of >85th percentile are associated with several measures of adverse perinatal outcome.

Key words: crown-rump length, discordance, twins


The twin birth rate in the United States has increased approximately 50% over the past 20 years and now accounts for almost 3% of all births. Although twins represent a minority of births, they account for a disproportionate share of perinatal morbidity and deaths. One cause that frequently is associated with perinatal morbidity and death in twins is intrauterine growth restriction and third-trimester twin discordance.

Although growth discordance in the third trimester has been associated with adverse perinatal outcome, the implications of twin discordance that are detected in the first trimester of pregnancy remain less well understood. There are relatively few articles that have assessed this phenomenon; those articles that exist have not reached uniform conclusions. In some cases, the degree of crown-rump length (CRL) difference has been correlated with the amount of difference of the subsequent birthweights, although in other studies this correlation has not been found. Also, although very large differences in CRL have been associated with aneuploidy and anatomic abnormalities, the clinical outcomes of euploid fetuses without major congenital anomalies, but with differences in CRL, are not well described.

The objective of this study was to determine how the difference in the first-trimester CRL of twins is related to subsequent growth abnormalities and adverse perinatal outcome.

MATERIALS AND METHODS

Women who received a first trimester ultrasound scan of a twin pregnancy between 7 and 14 weeks of gestation and subsequently delivered between June 2000 and March 2006 at Northwestern Memorial Hospital were identified. Only pregnancies with 2 fetal heartbeats present at time of the first-trimester ultrasound scan were included. If women had >1 ultrasound scan during this time period, the CRL measurements were derived from the first ultrasound examination that was performed. Fetuses with known chromosomal or major congenital anomalies were excluded from further analysis, as were women who underwent first- or second-trimester pregnancy termination. Also excluded were those pregnancies that were monoamniotic.

Maternal charts were reviewed to ascertain antenatal and intrapartum characteristics. Chorionicity was assigned according to the ultrasonographic appearance of the twin pregnancy at the first-trimester ultrasound examination and confirmed by pathologic report. Birthweights, Apgar score at 1 and 5 minutes, and arterial and venous blood gases, when available, were recorded. Neonatal charts were further abstracted to ascertain the length of the neonatal hospital admission, admission to the special care nursery, and the presence of major morbidities, which included respiratory distress syndrome, intraventricular hemorrhage, patent ductus arteriosus, necrotizing enterocolitis, culture-proven sepsis, and neonatal death.

The difference in CRL (CRLΔ) was calculated as the difference in the twin CRLs divided by the CRL of the larger twin and expressed as a percentage. Similarly, the difference in birthweight (BWΔ) was calculated as the difference in birthweights divided by the birthweight of the larger twin and expressed...
Results

One hundred seventy-eight women met inclusion criteria. In 6 pregnancies, there was previable death of both twins, and birthweights were not obtained. For these cases and for the 3 pregnancies with an intrauterine fetal death of 1 twin long before delivery of the cotwin, birthweight discordance was not calculated. These pregnancies were included in evaluation of CRL of other pregnancy outcomes, as applicable. Thus, the 6 previable deaths were not considered in the analysis of a outcome such as “neonatal intensive care unit” admission but were analyzed with regard to the composite adverse outcome, which included death. No neonates were found to have any major congenital anomaly or were diagnosed with a chromosomal abnormality.

The median CRL of the 178 twins in the study was 4.2% (interquartile range, 1.9%-8.3%); the median BW of the 168 twins with birthweight available was 9.3% (interquartile range, 3.9%-16.4%). The median CRLs of monochorionic and dichorionic twin gestations were similar (3.6% [interquartile range, 0.0%-8.3%] vs 5.1% [interquartile range, 2.0%-8.3%], respectively; \( P = .18 \)). The CRL was significantly correlated with BW (\( r = 0.43; P < .001 \)). The CRL was further evaluated in relation to birthweight discordance through the construction of a receiver operating characteristic (ROC) curve. This ROC curve (Figure) had an area under the curve of 0.72 and revealed the 85th percentile of CRL (11.1%) to be an optimal point of demarcation to predict subsequent birthweight discordance. Thus, a CRL of >11% was further used to assess the relation between this extreme of CRL and other patient characteristics and adverse pregnancy outcomes.

There did not appear to be any relation between a CRL of at least 11% and any demographic characteristic of the patients. As Table 1 shows, there were no differences in maternal age, gestational age at first trimester ultrasonographic examination, or chorionicity, after stratification of the study group by this CRL.

Conversely, a CRL >11% was associated with a significantly increased risk of continued discordant twin growth, because these twins were significantly more likely to be delivered with discordant birthweights (Table 2). The association of a CRL of at least 11% with discordant birthweight was evident regardless of chorionicity, because this association was present in both monochorionic and dichorionic twin subgroups after stratiﬁcation. Sixty-seven percent of monochorionic twins with a CRL of at least 11% had discordant birthweights; the frequency of birthweight discordance was only 9.1% among those with a CRL <11% (\( P < .01 \)). With respect to dichorionic twins, those twins with a CRL of at least 11% had a 41% probability of birthweight discordance, although only 7.6% of those twins with a CRL <11% had this birthweight discordance (\( P < .01 \)).

Multiple other adverse outcomes were also increased in twins with a CRL of at least 11%. Those twins with a CRL in this range were at increased risk of at least 1 twin being growth restricted at birth. Moreover, there was evidence that these growth abnormalities had ramifications for perinatal health. Those twins with a CRL of >11% were significantly more likely to require admission to the neonatal intensive care unit and to experience at least 1 of the adverse outcomes in the composite perinatal morbidity measure. The test characteristics of a CRL of at least 11% as a predictor of selected adverse neonatal outcomes are presented in Table 3.
TABLE 2
Pregnancy outcomes stratified by CRLΔ >11%

<table>
<thead>
<tr>
<th>Outcome</th>
<th>CRL Δ &gt;11% (n = 29)</th>
<th>CRL Δ ≤11% (n = 149)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (wk)</td>
<td>35.6 (32.2-37.5)</td>
<td>36.4 (34.0-38.0)</td>
<td>.31</td>
</tr>
<tr>
<td>Delivery at &lt;37 wk (n)</td>
<td>19 (65.5%)</td>
<td>84 (56.4%)</td>
<td>.36</td>
</tr>
<tr>
<td>Twin-twin transfusion syndrome (n/N)*</td>
<td>3/7 (43.9%)</td>
<td>3/36 (8.3%)</td>
<td>.04</td>
</tr>
<tr>
<td>Birthweight discordance (n/N)</td>
<td>13/28 (46.4%)</td>
<td>11/141 (7.8%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Small-for-gestational age (n)*</td>
<td>19 (65.5%)</td>
<td>55/143 (38.5%)</td>
<td>&lt;.01</td>
</tr>
<tr>
<td>Birthweight discordance and at least 1 fetus small-for-gestational age (n/N)</td>
<td>9/28 (31.0%)</td>
<td>9/141 (6.4%)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Perinatal death (n)†</td>
<td>2 (6.9%)</td>
<td>8 (5.4%)</td>
<td>.67</td>
</tr>
<tr>
<td>Apgar &lt;7 at 5 min (n)†</td>
<td>5 (17.2%)</td>
<td>9/138 (6.5%)</td>
<td>.06</td>
</tr>
<tr>
<td>Arterial pH &lt;7.0 (n/N)†</td>
<td>2/19 (10.5%)</td>
<td>4/111 (3.6%)</td>
<td>.21</td>
</tr>
<tr>
<td>Neonatal intensive care unit admission (n)†</td>
<td>17 (58.6%)</td>
<td>50/143 (35.0%)</td>
<td>.02</td>
</tr>
<tr>
<td>Composite perinatal morbidity (n)†</td>
<td>14 (48.3%)</td>
<td>36 (24.2%)</td>
<td>&lt;.01</td>
</tr>
</tbody>
</table>

* Frequency for the monochorionic population only.
† Based on the occurrence in at least 1 fetus within the twin pregnancy.

Comment
There has been relatively little information regarding the pregnancy outcomes of women with twins who were examined in the first trimester with differences in their CRL. The information that does exist has focused on 2 outcomes: birthweight discordance or the presence of a fetus with a chromosomal or anatomic abnormality. Several authors, for example, have found that twins with large CRLΔs were more likely to have birthweight discordance. Kalish et al also demonstrated, in a population of dichorionic twins, that only those with a CRLΔ of >90th percentile (in their study population a difference in CRL of >10%) were more likely to include a fetus with a chromosomal or anatomic anomaly. This association was also found in the study by Salomon et al.

We chose to assess the relationship between the CRL difference in euploid and anatomically normal twin gestations and subsequent pregnancy outcome. This relationship has not been well explored and is potentially important for patient counseling, because parents of a twin pregnancy with discordant CRLs, but without evident aneuploidy or anatomic abnormality, will desire other prognostic information. We have confirmed that the difference in first-trimester CRL is correlated with differences in birthweight and that large differences in the CRLs are associated with eventual growth discordance and growth restriction in the third trimester and with twin-transfusion syndrome in monochorionic gestations. We have also showed that larger CRL differences are associated with adverse perinatal outcomes, which are represented by the increased frequency of perinatal morbidity and neonatal intensive care unit admission.

We have used a CRLΔ of >85th percentile to identify the group that is at risk of adverse outcome. There is nothing, however, that is inherently pathologic about the 85th percentile; it was used because, on the basis of the ROC analysis, it is a cutoff that adequately balances sensitivity and specificity. Indeed, other investigators have used other cutoffs. For example, Kalish et al′ uses ROC analysis to determine that a CRLΔ of >90th percentile was best for the prediction of dis-

TABLE 3
The test characteristics of a CRLΔ >11% as a predictor of selected adverse neonatal outcomes

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>PPV</th>
<th>NPV</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birthweight discordance</td>
<td>54%</td>
<td>90%</td>
<td>46%</td>
<td>92%</td>
</tr>
<tr>
<td>Small-for-gestation age*</td>
<td>26%</td>
<td>90%</td>
<td>66%</td>
<td>62%</td>
</tr>
<tr>
<td>Neonatal intensive care</td>
<td>25%</td>
<td>89%</td>
<td>59%</td>
<td>65%</td>
</tr>
<tr>
<td>Unit admission*</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Composite perinatal morbidity*</td>
<td>28%</td>
<td>88%</td>
<td>48%</td>
<td>76%</td>
</tr>
</tbody>
</table>

* Based on the occurrence in at least one fetus within the twin pregnancy.
NPV, negative predictive value; PPV, positive predictive value.
cordant third-trimester growth. Yet, in their study population, the 90th percentile was a CRLΔ of 10%, which is a difference quite similar to our finding of 11% as the optimal discriminator. Also, we have used a percentage difference as opposed to the absolute difference in CRL. It should be noted that we did analyze the absolute difference for its association with adverse outcomes (data not shown), but the absolute difference was not as good as the percentage difference at the prediction of these neonatal outcomes. This is not surprising, given that the absolute size of the CRL changes over such a wide range during the gestational ages under study.

The finding in the present study that the median CRLΔ of monochorionic twins is similar to that of dichorionic twins was also noted by Sebire et al.4 In contrast to our data, however, that study demonstrated an association between the CRLΔ and subsequent birthweight discordance only in the dichorionic subgroup. One potential reason for the difference in findings is that Sebire et al analyzed the association using the median CRLΔ and we have used a categoric CRLΔ variable. Also, the present study incorporated ultrasound information that was ascertained as early as 7 weeks of gestation; the study of Sebire et al4 (and of Kalish et al7) did not use ultrasound data from pregnancies that were before 10 weeks of gestation.

Northwestern Memorial Hospital is a tertiary care center, and this characteristic raises the possibility that the results that were derived from this study population are not generalizable to other twin populations. However, the median CRLΔ and the frequency of birthweight discordance are similar to findings that have been described by other investigators. Moreover, characteristics of the study population (such as the frequency of preterm birth, monochorionicity, and discordant twin birthweights) are similar to that expected in the general population.9-11 Although the frequency of small-for-gestational-age birthweight appears higher than expected, this finding is explained by the fact that we ascertained this outcome on the basis of singleton nomograms, because these nomograms have been shown to be more predictive of adverse perinatal outcomes than twin-specific nomograms.12,13

This study provides information that can be used to counsel women who are confronted with CRL discordance in the first trimester of pregnancy. In some sense, the information may be viewed as reassuring, because CRL discordance is not associated inevitably with adverse pregnancy outcome. Those twins with discordant CRLs still had a >50% chance of not having significant perinatal morbidity. Nevertheless, the risk of adverse outcomes is increased, and care providers should be cognizant of this association.

REFERENCES

Fetal cord blood mononuclear cells that are collected at term from HIV-1 infected women harbor transcriptionally active integrated proviral DNA

Jane E. Ellis, MD, PhD; Gregory A. Hair, PhD; Michael K. Lindsay, MD, MPH; Aftab A. Ansari, PhD; J. Bruce Sundstrom, PhD

OBJECTIVE: The purpose of this study was to determine levels of intrauterine infection and transcriptional activity in cord blood mononuclear cells that were collected at term from fetuses who were born to women who were infected with human immunodeficiency virus (HIV) and who received highly active antiretroviral therapy (HAART). 

STUDY DESIGN: RNA and DNA were isolated from maternal placental tissues and fetal cord blood specimens that were obtained at term from pregnant women who were infected with HIV and who received HAART. Levels of integrated HIV provirus and messenger RNA transcripts were determined by real-time polymerase chain reaction.

RESULTS: Detectable levels of transcriptionally active integrated provirus were present in approximately 27% of cord blood samples (n = 22) that were collected from fetuses who were born to HIV-positive mothers who received HAART. Levels of HIV-p24 antigen in cultures that were detected in randomly selected cord blood samples confirmed the presence of inducible infectious virus.

CONCLUSION: These findings suggest that some fetuses from HIV-infected mothers who receive HAART and who may be HIV-negative infants after delivery can harbor circulating leukocytes that are infected productively by intrauterine transmission of HIV.

Key words: cesarean delivery, HIV, intrauterine transmission, MTCT

Perinatal transmission of human immunodeficiency virus (HIV) is a serious problem worldwide, especially in developing countries. Based on statistics gathered through 2002, globally an estimated 19.2 million women and 3.2 million children (≤15 years old) were infected with HIV.1 Mother-to-child-transmission (MTCT) of HIV occurs generally by transplacental (IUT), during the intrapartum period (during labor), or after delivery (through breastfeeding). In the United States and other developed countries, effective clinical measures to limit MTCT, which include the administration of highly active antiretroviral therapy (HAART) to the mother during the antenatal period, cesarean deliveries that are scheduled before rupture of membranes or onset of labor (when IUT is most likely to occur), and avoidance of breast-feeding have reduced the overall incidence of MTCT to approximately 1%-2%.2 These transmission rates, however, are based primarily on measurements of neonatal plasma viral loads that were determined during the early postpartum period and may not reflect accurately or completely the status of fetal infection during the antenatal period.

Recent studies indicate that, after the administration of such effective clinical strategies, IUT accounts for approximately 50% of MTCT of HIV.3 However, the frequency of perinatal transmission, which results in the presence of cells in fetal circulation harboring potentially infectious integrated proviral DNA, has not been determined for pregnant women who undergo prenatal antiretroviral therapy. A discordance between the frequency of peripheral blood mononuclear cells that harbor integrated provirus in the fetus and the frequency of neonates who are positive for plasma viral RNA could raise important questions that relate to mechanisms of viral clearance and the establishment of either “occult” or “active” infection in the neonate. Without a better understanding of the status of infection (active vs latent or nonproductive) of circulating fetal cells, the timing, mechanism, and clinical significance of perinatal transmission during HAART remains unclear. We addressed these issues in a focused study to test the hypothesis that the frequency of evidence of transcriptionally active and potentially infectious, integrated proviral DNA in circulating mononuclear cells in the fetus corresponds with the frequency of evidence of detectable levels of HIV RNA in the plasma of infants who are born to infected women who undergo prenatal antiretroviral therapy to prevent MTCT.

MATERIALS AND METHODS

Subjects
Subjects from whom blood and tissue samples were collected were identified...
from parturients who were known to be HIV positive because of a positive enzyme-linked immunosorbent assay and confirmatory Western blot and who received prenatal care and were delivered at Grady Memorial Hospital in Atlanta, GA. Grady Memorial Hospital serves a high-risk, indigent population and is a regional referral center for high-risk obstetrics patients in the state of Georgia, which includes HIV-infected pregnant patients, and provides care for 60-75 patients per year in accordance with current guidelines for the care of the HIV-positive parturients.4,5

Twenty-two patients were recruited for this study over an approximate 2-year period between 2004 and 2006. Those who consented to participate in the study received standard initial prenatal laboratory tests plus hepatic and renal function tests, total CD4+ T-cell counts, HIV plasma viral loads, and ultrasound examination to determine the estimated gestational age and date of delivery. Once evidence of HIV infection and plasma viral loads had been determined, HAART was initiated for enrolled patients beyond their 14th week of pregnancy (n = 20) after detailed counseling on the risks and benefits. Two patients were already undergoing HAART before their most recent pregnancy. HAART consisted of lamivudine and zidovudine and hepatic functions were followed to ensure maternal tolerance to the antiretrovirals. Third-trimester viral loads were assessed for appropriate counseling on route of delivery.

Patients were screened during their antenatal course for evidence of genital tract infections that included bacterial vaginosisis, group B streptococcus, and sexually transmitted diseases other than HIV. None of the patients from whom tissue samples were collected demonstrated evidence of coexisting allergic diseases, chorioamnionitis, or other untreated genital tract infections at the time of delivery.

Routinely, cesarean deliveries are performed on HIV-positive mothers at Grady Memorial Hospital when their plasma viral load at term is >1000 copies/mL or there is an obstetric indication (eg, previous cesarean delivery or malpresentation). Elective cesarean deliveries, however, were scheduled for all patients who were enrolled in this study at 38 weeks of gestation, with 3 hours of intravenous zidovudine immediately before surgery. Maternal blood samples were obtained when the patient appeared for her preoperative evaluation the day before her scheduled surgery. Cord blood and placental tissues were collected meticulously in a sterile fashion during delivery. Portions of placental tissue were subjected to routine postoperative pathologic examination. No clinical or pathologic evidence of abruption was observed in any of the 22 patients. All blood and tissue specimens were collected, with informed consent, according to Emory University institutional review board–approved protocols. Plasma levels of HIV RNA in infants were recorded routinely during the first week after delivery with the Amplicor HIV-1 Monitor test (Roche Molecular Systems, Inc, Somerville, NJ).

Collection and preparation of human tissue samples

Single cell suspensions of maternal cells from collagenase digested placental tissues and fetal mononuclear cells from human umbilical cord blood, which were collected after delivery of term fetuses, were separated on density gradient medium to isolate cells of hematopoietic origin, as previously described.6,7

Real-time PCR amplification

Determining host cell nuclear integration of HIV. Infection of maternal and fetal tissues was determined at the level of HIV proviral nuclear integration into the host cell genome by measurement of the mean number of copies of integrated proviral DNA per 10⁶ cells. DNA was prepared from 2 x 10⁶ – 1 x 10⁷ cord blood mononuclear cells (CBMCs) and real-time polymerase chain reaction (PCR) amplification; measurements were performed with appropriate β-globin and HIV proviral complementary DNA (cDNA) copy-number standards as previously described7 and with the real-time PCR primers and conditions listed in Table 1. Briefly, 100 nmol/L each of Alu 1 and a compound primer, LM667, were incubated with 200 ng of sample DNA. PCR was performed (95°C for 3 minutes, then 23 cycles at 94°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; Apollo ATC 401 thermocycler; CLP-PGC Scientifics, San Diego, CA). Integrated provirus or HIV cDNA standard concentrations were determined by a modified absolute concentration nested real-time qualitative PCR assay (7). Preamplified samples and standards (2 μL/well) were subjected to qualitative PCR with 200 nmol/L each of a quasineested primer pair (Lambda-T; AA55M; 5’) with SYBR Green on a Bio-Rad iCycler (95°C for 3 minutes, then 50 cycles at 93°C for 1 minute, 55°C for 1 minute, and 72°C for 1 minute; Bio-Rad Laboratories, Hercules, CA). Efficiencies of the qualitative PCR assays were calculated from the slopes of the standard curves and the inverse natural logarithms of the cycle times (adjusted for efficiency) that were used to calculate starting copy numbers of integrated HIV provirus over a linear range between 6 and 6000 copies per 10⁶ cell equivalents.

Maternal microchimerism estimation in CBMC samples; measurements of Y chromosomal DNA. To rule out microtransfusion from mother to fetal cord blood in determining HIV transfer, male cord blood (n = 10) was examined for percentages of the Y chromosomal marker DYS 14 (fetal alone), compared with β-globin housekeeping standards (fetal + maternal).8 DNA was prepared from 2 x 10⁶ – 1 x 10⁷ CBMCs by standard methods, as described.7 The PCR conditions in Table 1 were used to quantify DYS 14 DNA by an absolute concentration assay. The conversion factor of 2 copies of β-globin and 1 copy of DYS 14 product per cell was used to compute the reference DYS 14/β-globin ratio. This ratio (0.5) was then determined from real-time PCR results for male CBMC samples (n = 10) and calculated to be 0.5 (P < .05).
Determining proviral transcription of full-length and multiply spliced HIV messenger RNA (mRNA). RNA was prepared from 1-2 × 10^6 cells with the RNeasy Mini Kit and protocol (Qiagen, Valencia, CA). Full-length and multiply spliced HIV mRNA transcript levels were determined as previously described, with the use of full-length and multiply spliced sense and antisense primers that are described in Table 1.

### Statistical methods

For this study the primary outcome was the determination of transcriptionally active integrated proviral DNA in fetal CBMCs by real-time PCR. In this case, no arbitrary divisions were used for subcategorizing outcomes, except for the quantitative limits of the “pseudo-nested” real-time qualitative PCR assay used. Both linear regression and analysis of variance were used along with multiple sample repeats, standard curves, and known positive and negative samples to test the validity of the PCR measures of primary outcomes. For each result, data from at least 3 wells from a representative experiment were used to calculate a single data point. Linear regression analyses (Excel; Microsoft Corporation, Redmond, WA) were performed on at least 6 data points, which produced correlation coefficients ≥0.9999 (P < .005).

### Evidence for infection of placental cells of maternal and hematopoietic origin

Detection of HIV DNA merely indicates that HIV has entered the host cell and reverse transcription has occurred. For productive infection to become established, successful viral integration must follow. Thus, measurements of integrated proviral DNA in placental cells were performed to determine the status of infection in these tissues. Evidence of infection in maternal cells of hematopoietic origin that were isolated from placental tissues from HIV-infected mothers who received HAART (n = 22) was detected in all tissue preparations. Real-time PCR

### Results

#### Age, parity, and infection characteristics of study volunteers

The maternal age of study volunteers ranged from 22-39 years; parity ranged from 0-4; CD4^+ T-cell counts ranged from 143 to 588 per mm^3, and plasma viral loads ranged from <400 to ≥12,800 copies/mL at term. As shown in Table 2, 80% of study volunteers (16 of 20 volunteers tested) who received

### Table 1. Real-time PCR primers and reaction conditions

<table>
<thead>
<tr>
<th>NA assessed</th>
<th>Primers</th>
<th>Sequence</th>
<th>Conc.</th>
<th>Conditions</th>
</tr>
</thead>
<tbody>
<tr>
<td>Strong-stop cDNA</td>
<td>AA55 M667</td>
<td>5′-CTGCTAGAGATTTCACACTGAC-3′/5′-GGCTAATACTAGGAAACCACCAG-3′</td>
<td>200 nM</td>
<td>95°C, 3 min; 50× (93°C, 1 min; 57°C, 1 min; 72°C, 1 min)</td>
</tr>
<tr>
<td>Y chromosome DNA or purified standards</td>
<td>DYS14F DYS14R</td>
<td>5′-CAACACAGCCAGCTCGG-3′/5′-TCCCCCGCCCTGCACAA-3′</td>
<td>200 nM</td>
<td>95°C, 3 min; 50× (93°C, 1 min; 57°C, 1 min; 72°C, 1 min)</td>
</tr>
<tr>
<td>Genomic DNA w/wo integrated provirus or purified standards</td>
<td>Alu2 (F) Alu1 (R) LM667</td>
<td>5′-GCTGACACCTCAAAATCCA-3′/5′-GACGCTCTTCTGCCCACTC-3′</td>
<td>100 nM</td>
<td>95°C, 3 min; 50× (93°C, 1 min; 55°C, 1 min; 72°C, 1 min)</td>
</tr>
<tr>
<td>Pre-amplified cDNA from above</td>
<td>AA55M Lambda T</td>
<td>5′-GCTAGAAGATTTTCACACTGACTA-3′/5′-ATGCGACGGTAAGGGAAACGTCA-3′</td>
<td>200 nM</td>
<td>95°C, 3 min; 50× (93°C, 1 min; 57°C, 1 min; 72°C, 1 min)</td>
</tr>
<tr>
<td>HIV mRNA full length transcript</td>
<td>La 8.1 La 9</td>
<td>5′-CTGAAGCCGCCGCACGGCAA-3′/5′-GACGCTCTGCACCACTC-3′</td>
<td>200 nM</td>
<td>50°C 10 min, 95°C 3 min (94°C 1 min, 56°C 1 min, 72°C 1 min) × 40 cycles, 4°C 10 min</td>
</tr>
<tr>
<td>HIV mRNA multiply-spliced transcripts</td>
<td>P659 P413Mod</td>
<td>5′-GACGCTCATCAGGTTTCTCATCAA-3′/5′-AGCTCTCCAAGCGTTGATG-3′</td>
<td>200 nM</td>
<td>50°C 10 min, 95°C 3 min (94°C 1 min, 56°C 1 min, 72°C 1 min) × 40 cycles, 4°C 10 min</td>
</tr>
<tr>
<td>Genomic β-globin or purified standards</td>
<td>β-globinF β-globinR</td>
<td>5′-CCCTTGACACAGAGTTGTTCT-3′/5′-CGAGCTTCTTCTGGCCATGA-3′</td>
<td>200 nM</td>
<td>95°C, 3 min; 50× (93°C, 1 min; 55/57°C, 1 min; 72°C, 1 min)</td>
</tr>
</tbody>
</table>
were determined by PCR. Quantitative measurements of the number of copies of integrated proviral DNA from matched fetal CBMC and maternal placental tissue samples identified 6 of 22 infected samples, which ranged from 8-35 copies of proviral DNA per $10^6$ cell equivalents, approximately 10% the level of infection measured for infected placental cells (Table 3). No apparent correlation between the IUT and the level of infection in corresponding placental cells was observed.

Concerns of possible contamination of cord blood samples with infected maternal cells were addressed by performing an analysis of Y chromosomal DNA in CBMCs from male fetuses ($n = 11$) by real-time PCR, as described earlier. The ratio of DYS 14/β-globin for all male samples tested was 0.5 ($P \leq 0.05$), which indicated no contamination of maternal cells (maternal microchimerism) within the linear range that was defined by the PCR conditions (approximately 1%).

### Evidence of transcriptional activity in infected fetal CBMCs

Detection of integrated proviral DNA still suggests only that the virus has the potential for replication but does not characterize whether viral replication is occurring. To characterize the level of infection precisely during IUT, real-time qualitative PCR analysis of RNA that was extracted from HIV-infected CBMCs was performed to measure relative levels of proviral transcription. Evidence of transcription of both full-length (early transcripional products) and multiply spliced (late transcription products) viral mRNA relative to uninfected controls was measured in all CBMC samples with integrated proviral DNA (Figure). These results indicate that transcription of proviral DNA in isolated CBMCs infected by IUT occurs even without previous activation in vitro or in vivo.

### Induction of infectious virus by alloactivation of infected fetal CBMCs

During posttranscriptional latency or HAART-infected fetal CBMCs harboring transcriptionally active integrated proviral DNA may be unable to produce detectable levels of infectious virus. To address this issue, 2 randomly selected HIV-positive CBMC samples were cocultured for 6 days with γ-irradiated matched maternal peripheral blood mononuclear cell stimulators in vitro to induce viral replication by allogeneic stimulation, as previously described.9 Measurement of significant levels of HIV p24 antigen ($\geq 40$ ng/mL) in culture supernatant fluids confirmed that (1) the expression of infectious virus could be induced in infected fetal CBMCs that harbor transcriptionally active integrated proviral DNA and (2) infected cells that are responsive to allogeneration by γ-irradiated maternal stimulator cells are of likely (MHC disparate) fetal origin.

### Comment

The efficacy of accepted measures to limit perinatal transmission of HIV during pregnancy, which includes the administration of HAART and scheduled cesarean delivery, has been determined primarily by evidence of a significant reduction in the frequency of MTCT of HIV infection in infants to 1%-2%.2 Clinical determinations of whether MTCT has occurred by either IUT or intrapartum transmission routinely are made indirectly by measuring how soon detectable levels of plasma-borne viral RNA appear after delivery.10-12 However, a rigorous examination for direct evidence MTCT and established infection in fetuses who are delivered to HIV-
infected mothers who undergo currently accepted prenatal clinical protocols to limit MTCT has not been conducted. Our findings that 6 of 22 CBMC samples (25%) that harbored transcriptionally active and potentially infectious integrated proviral HIV could be isolated from term fetuses that were selected randomly from a larger population of 150 fetuses that were delivered by HIV-positive women who received HAART with a rate of MTCT of 3% strongly suggest that IUT can occur in some fetuses that may present as HIV-negative postpartum.

Unlike intrapartum transmission or postpartum vertical transmission of HIV, little is known regarding how and when IUT of HIV occurs.\textsuperscript{12,13} For this study, only CBMCs that were collected from fetuses who were delivered during scheduled cesarean delivery and in the absence of clinical or pathologic evidence of abruption were assayed to minimize the confounding effects of maternal microchimerism. To determine more carefully whether infected cells exclusively of fetal origin were examined, PCR analysis of Y-chromosomal DNA in CBMCs from male fetuses was performed (Table 3), thus establishing a detection threshold (\(\leq 1.3\%\)) for contaminating maternal cells in all fetal cord blood samples. In addition, cellular viral loads of infected placental cells of maternal origin, which were determined to be \(\leq 0.004\%\) (Table 3), provide strong evidence that CBMCs that harbored integrated HIV proviral DNA were of fetal origin and were infected transplacentally.

Because of the routine administration of HAART, the presence of latently infected cells and cellular reservoirs that harbor low levels of persistent viral replication has become an important consideration in the pathogenesis of HIV infection in infected adults. However, the extent to which HAART may influence the establishment of “occult” fetal HIV infection during MTCT has received little attention. For infection to become established, the virus must do more than just enter susceptible cells; it must also successfully integrate and replicate within those cells. Studies that use PCR techniques that measure total HIV DNA in blood samples do not differentiate clearly between integrated and unintegrated proviral DNA in infected cells. Thus, the level of true infection determined by these methods potentially may be overestimated. Likewise, studies that rely solely on PCR determinations of number of copies of HIV RNA in plasma samples may be falsely negative, especially when HIV infection is undetectable because of pre- or postintegration latency. In support of this hypothesis is the finding that peripheral blood mononuclear cells that were isolated from 18% of infants who were born with undetectable plasma viral loads to HIV-infected mothers in a recent study harbored unintegrated virus.\textsuperscript{12} There is evidence that unintegrated virus can persist for a limited period in an integration-competent state and that integration of replication competent provirus will follow only after appropriate immune activation in the infected host cell.\textsuperscript{14} We confirmed infection by measuring levels of both proviral integration and postintegration transduction of HIV in cord blood. Our data therefore indicate that IUT may occur successfully before term and in the absence of clinical or pathologic evidence of abruptions, maternal microchimerism, intrauterine infection, or immune activation in fetuses with HIV-negative serologies at term.

### Table 3

<table>
<thead>
<tr>
<th>Sample set no.</th>
<th>Integrated provirus/10^6 isolated placental cells</th>
<th>Integrated provirus/10^6 CBMCs</th>
<th>DYS 14 ratio</th>
<th>Infant gender</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>142</td>
<td>N.D.</td>
<td>N.A.</td>
<td>M</td>
</tr>
<tr>
<td>2</td>
<td>144</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>3</td>
<td>157</td>
<td>8</td>
<td>N.A.</td>
<td>M</td>
</tr>
<tr>
<td>4</td>
<td>140</td>
<td>N.D.</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>5</td>
<td>102</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>6</td>
<td>182</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>7</td>
<td>323</td>
<td>N.D.</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>8</td>
<td>149</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>9</td>
<td>158</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
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<tr>
<td>10</td>
<td>123</td>
<td>7</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>11</td>
<td>181</td>
<td>N.D.</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>12</td>
<td>191</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>13</td>
<td>467</td>
<td>35</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>14</td>
<td>58</td>
<td>7</td>
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<td>F</td>
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<tr>
<td>15</td>
<td>168</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>16</td>
<td>29</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>17</td>
<td>266</td>
<td>12</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>18</td>
<td>19</td>
<td>N.D.</td>
<td>0</td>
<td>F</td>
</tr>
<tr>
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<td>N.D.</td>
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<td>M</td>
</tr>
<tr>
<td>20</td>
<td>N.D.</td>
<td>N.D.</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>21</td>
<td>15</td>
<td>N.D.</td>
<td>N.A.</td>
<td>F</td>
</tr>
<tr>
<td>22</td>
<td>285</td>
<td>19</td>
<td>0.5</td>
<td>M</td>
</tr>
<tr>
<td>Uninfected #1</td>
<td>0</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Uninfected #2</td>
<td>0</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
<tr>
<td>Uninfected #3</td>
<td>0</td>
<td>N.A.</td>
<td>N.A.</td>
<td>N.A.</td>
</tr>
</tbody>
</table>

N.A., not assayed; N.D., not detectable.
One trivial explanation for this result could be that the infected CBMCs from these fetuses harbor actively transcribing integrated provirus that is replication defective. We confirmed virus infection by measuring HIV p24 levels in 2 randomly selected cocultures of matched infected CBMCs and γ-irradiated maternal peripheral blood mononuclear cell stimulator cells, as previously described. Significant levels of HIV-1 p24 (≥40 ng/mL) could be measured in culture supernatant fluids from alloactivated (infected) CBMCs after 5 days (data not shown). Collectively, these data provide strong evidence that productive infection of circulating mononuclear cells can be established by IUT at time points significantly before term in certain fetuses that may appear serologically negative at birth.

Nevertheless, how IUT occurs, especially during HAART, remains unknown. Our results demonstrate the presence of cells that harbor integrated proviral DNA in all placental tissue samples that were examined, which indicates that the prenatal administration of HAART does not prevent completely the presence of infiltrating HIV-infected hematopoietic cells of maternal origin in the placenta. However, 80% of study volunteers who received HAART either had plasma viral loads <400 copies/mL or CD4+ T-cell counts >200/mm³. Furthermore, although levels of antiretroviral drugs in placental tissues or fetal circulation were not determined, evidence of HIV-infected CBMC samples was detected in 3 separate fetuses who were delivered by women who received HAART with plasma viral loads <400 per mL and CD4+ T-cell counts >200 per mm³ (Table 2).

These results suggest that, even in the presence of adequate prenatal HAART and an MTCT rate of ≤ 3%, HIV is transmitted transplacentally and infects circulating fetal mononuclear cells in >25% of pregnancies. This disparity between the rate of IUT in this report and rates reported by others may be due to at least 3 factors: (1) differences in study sample size, (2) possible contamination with infected maternal lymphocytes, or (3) differences in the methods used for determining HIV infection. Admittedly, the sample size (n = 22) of this study must be enlarged for accurate estimates of IUT. Nevertheless, because we were able to confirm (by Y-chromosomal DNA analysis) absence of maternal microtransfusion (≤1.3%) and because of the fact that γ-irradiated peripheral blood mononuclear cells of maternal origin could alloactivate and induce the release of infectious virus in infected CBMCs, IUT and antenatal fetal infections were presumed.

Our findings of an apparent increased frequency of infected circulating fetal CBMCs in HIV-infected pregnant women who received HAART were not confirmed in infants after delivery and therefore raise several important questions: How and when does IUT of HIV occur during pregnancy? Is the infection cleared? If so, how? Can an occult infection be established in the fetus that persists after delivery? Are circulating infected fetal cells able to establish extravascular sanctuaries of persistent or latent infection in cryptic fetal tissue compartments? If so, what is the significance of the timing of IUT on the creation of such reservoirs in utero, and which tissue compartments are involved potentially? More importantly, how should such infants be followed or treated clinically? Well-designed longitudinal studies of HIV-negative infants who are born to HIV-infected mothers are needed to appreciate fully the potential clinical consequences of intrauterine fetal HIV infection on the long-term immunologic and developmental health of these infants.

REFERENCES
First-trimester risk assessment for Trisomies 21 and 18 in twin pregnancy

Stephen T. Chasen, MD; Sriram C. Perni, MD; Robin B. Kalish, MD; Frank A. Chervenak, MD

**OBJECTIVE:** Our objective was to describe performance of first-trimester combined risk assessment in twin pregnancies.

**STUDY DESIGN:** Twin pregnancies that underwent risk assessment in our ultrasound unit from 2003-2006 were included. Adjusted risks for trisomies 21 and 18 were based on age, nuchal translucency (NT), and biochemistry were provided for each twin. Detection rates for Down syndrome and trisomy 18 were calculated for age/NT, and age/NT/biochemistry at a screen-positive rate of 5% of pregnancies.

**RESULTS:** Five hundred thirty-five pregnancies were included. Median maternal age was 34 years, with 47% of women ≥35 years old. There were 7 fetuses in 6 dichorionic pregnancies with Down syndrome and 3 fetuses in 3 pregnancies with trisomy 18. For a 5% false-positive rate, age/NT identified 83.3% of Down syndrome and 66.7% of Trisomy 18 pregnancies. Adding biochemistry resulted in 100% detection rates for both conditions.

**CONCLUSION:** The addition of biochemistry may enhance first-trimester risk assessment in twin pregnancies. Further studies with larger numbers of affected pregnancies are needed.

Key words: Down syndrome, risk assessment, twin pregnancy


The increasing rate of multiple pregnancy has coincided with the increasing age of the maternal population.¹ Advancing maternal age is associated with a higher rate of spontaneous multiple pregnancy and the use of assisted reproduction, which accounts for a large proportion of multiple pregnancies.¹ Because the rate of autosomal trisomy increases with maternal age, multiple pregnancies are at higher risk than the general population. Although amniocentesis and chorionic villus sampling (CVS) are recommended commonly in the older obstetric population, many women with multiple pregnancy are concerned about the risk of invasive testing.²

Maternal age is a poor screening tool for fetal aneuploidy, because most affected pregnancies are in women younger than the widely used cut off age of 35 years.³ Maternal serum screening is limited in twin pregnancies because of lower reported detection rates compared with singleton pregnancies.⁴ In addition, fetus-specific risks are not provided. Fetus-specific risk is important, because most affected pregnancies are dizygotic, with a normal cotwin. Risk assessment with nuchal translucency (NT) does provide fetus-specific risk. The detection rate with the use of maternal age and NT has been described as similar to that in singleton pregnancies, although the false-positive rate is higher.⁵

In singleton pregnancies, ultrasound scanning combined with biochemistry provides the best first-trimester risk assessment.⁶,⁷ In twin pregnancy, Spencer and Nicolaides⁸ described a Down syndrome detection rate of 75% (3 of 4) using a 1:300 cutoff, with 9% of pregnancies being false-positive. One theoretic model suggested a 70% Down syndrome detection rate, with a 5% false-positive rate, in dichorionic twin pregnancies.⁹ Our objective was to describe performance of first-trimester combined risk assessment in twin pregnancies.

**MATERIALS AND METHODS**

First-trimester combined risk assessment data from patients with twin pregnancies who were evaluated from January 2003-August 2006 in the New York Weill-Cornell Medical Center were reviewed. Biochemical risk assessment for Down syndrome and Trisomy 18 was performed between 9 and 13 weeks 6 days of gestation; NT was measured between 11 and 13 weeks 6 days of gestation (crown-rump length, 45-84 mm). Risk assessment was performed with maternal age, NT measurement, and levels of pregnancy-associated plasma protein A (PAPP-A) and free beta-human chorionic gonadotrophin (βhCG). When pregnancy was achieved with egg donation, the age of the egg donor was used. NT was measured by trained sonographers who are certified by the Fetal Medicine Foundation, with periodic review of images and distribution of NT measurements.

Gestational age-specific multiples of the median were generated for the serum analytes and adjusted for twin pregnancy. Values were also adjusted for maternal ethnicity and weight. Likelihood
Five hundred thirty-five twin pregnancies were included. The median maternal age was 34 years (interquartile range, 31-37 years), with 45.6% of the women ≥35 years at the estimated due date. There were 36 monochorionic pregnancies (6.7%). Two hundred eighty-five pregnancies (53.3%) were identified as being achieved with in vitro fertilization. In 61 pregnancies (11.4%), an egg donor was used, with a median age of 27 years (interquartile range, 24-31 years).

We obtained follow-up data in 519 pregnancies (97.0%). Down syndrome was identified in 7 fetuses in 6 pregnancies, all of which were dichorionic/diamniotic. Based on the distribution of maternal age, approximately 5 cases would be expected. The pregnancy with 2 affected fetuses was achieved spontaneously by a 43-year-old woman. Both fetuses were of the same gender, although zygotically testing was not done.

In the 6 affected pregnancies, median maternal age (39 vs 34 years; P = .01) and Delta-NT (+2.04 vs +0.97 mm; P < .001) were significantly higher than in pregnancies in which Down syndrome was not identified. There was a trend towards higher median free-βhCG (1.26 vs 0.97 multiples of the median [MOM]; P = .06) but no significant difference in median PAPP-A (0.92 vs 1.12 MOM; P = .53) between affected and unaffected pregnancies. A positive likelihood ratio based on biochemistry, which resulted in higher adjusted risk, was noted in 3 of 6 affected pregnancies (50%), compared with 9.8% of unaffected pregnancies (P = .01).

The Table summarizes the screening performance of risk assessment using NT with and without biochemistry in the detection of Down syndrome. Combined risk assessment, with age adjusted by biochemistry and NT, provided the highest detection rate. At a cut off of 1 in 300 (the approximate first-trimester risk of a 35-year-old woman), the use of age combined with just NT or with biochemistry included would identify all 6 affected pregnancies. The false-positive rate would be 11.2% with the use of age with NT, compared with 7.0% with biochemistry included.

The 6 affected twin pregnancies were compared with 45 singleton pregnancies in which Down syndrome was identified. Median PAPP-A was significantly higher in the twin pregnancies (0.92 vs 0.59 MOM; P = .03). Median free βhCG was lower in the twin pregnancies, although this was not statistically significant (1.26 vs 1.78 MOM; P = .15). There was no difference in median Delta-NT.

There were 3 cases of Trisomy 18 in 3 dichorionic twin pregnancies. Based on the distribution of maternal age, approximately 3 cases would be expected. In 1 affected fetus, a cystic hygroma was noted. In a second affected fetus, anencephaly and omphalocele were identified. In the third affected fetus, NT was normal, with no sonographic abnormalities. In this pregnancy, very low levels of both PAPP-A and free βhCG resulted in an adjusted Trisomy 18 risk of 1 in <5 for both twins. The Table summarizes the screening performance of risk assessment using NT with and without biochemistry in the detection of Trisomy 18.

In the pregnancies in which Trisomy 18 was identified, the median maternal age was higher than in unaffected pregnancies (40 vs 34 years; P = .03). Median free βhCG levels were significantly lower in affected pregnancies (.47 vs .98 MOM; P = .007). No significant difference was...
noted in median PAPP-A level (0.82 vs 1.12 MOM; \( P = .14 \)).

The 3 affected twin pregnancies were compared with 14 singleton pregnancies in which Trisomy 18 was identified. In the affected twin pregnancies, higher medians of both PAPP-A (0.82 vs 0.20 MOM; \( P = .20 \)) and free \( \beta \) hCG (0.47 vs 0.26 MOM; \( P = .77 \)) were observed; however, these differences were not statistically significant.

**Comment**

NT is clearly an effective marker for aneuploidy risk assessment in twin pregnancies. Even with our small number of affected Down syndrome pregnancies, the large difference in the largest Delta-NT between affected and unaffected pregnancies achieved statistical significance. Our detection rate of 83.3% with a false-positive rate of 5% is comparable with published data. With our small number of monochorionic pregnancies, we were unable to confirm the reported higher rate of increased NT in these pregnancies.

In our population, the addition of biochemistry did enhance risk assessment. With the use of combined risk assessment, all 6 affected pregnancies would be detected, with a 5% false-positive rate. With a cut-off of 1 in 300, the false-positive rate was lower than that when biochemistry was not included. Biochemical markers are clearly less effective than in singleton pregnancies, with values closer to the median in affected twin pregnancies compared with affected singleton pregnancies. Based on our limited data, free \( \beta \) hCG may be a more effective marker in twin pregnancies than PAPP-A.

In singleton pregnancies, in vitro fertilization has been associated with different values of PAPP-A and free \( \beta \) hCG, and adjustment for mode of conception has been described. Because data were not available to address this issue in twins, such adjustments were not made in our patients. In the future, adjustment based on mode of conception could enhance biochemical risk assessment.

The small number of affected pregnancies is the main limitation of this study. Although we describe a 100% detection rate with a 5% false-positive rate, we would anticipate a lower Down syndrome detection rate with a larger number of affected pregnancies. Based on the observed differences in biochemistry compared with affected singleton pregnancies, the detection rate would clearly be lower than in singleton pregnancies. A large number of affected pregnancies cannot be expected from a single institution over a relatively short period of time. A large, multicenter study in which follow-up evaluation can be obtained is needed to elucidate the proper role of biochemical markers in twin pregnancies.

Effective risk assessment for fetal aneuploidy is important in twin pregnancies. Women with twin pregnancies are at higher risk of miscarriage and at higher risk of autosomal trisomy. Those who have experienced infertility may be particularly reluctant to undergo invasive testing. Early risk assessment could provide women with valuable information about deciding whether to undergo CVS or amniocentesis. Most affected pregnancies will have a normal cotwin, and selective termination is frequently considered under these circumstances. Although it is not clear that selective termination is safer when performed earlier in gestation, some studies have suggested lower rates of complications when it is performed at earlier gestational ages.

In summary, we found first-trimester risk assessment to be effective in twin pregnancies, with high detection rates. Although NT is the most effective marker, the addition of biochemistry enhanced risk assessment. The use of early risk assessment can be used to help women determine whether invasive testing is warranted and may prove useful by minimizing invasive procedures while maintaining high detection rates.

**References**

Changes in pre-pregnancy body mass index between pregnancies and risk of primary cesarean delivery

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OBJECTIVE: The objective of the study was to examine whether the risk and indications for primary cesarean in the second pregnancy are influenced by changes in pre-pregnancy body mass index (BMI) between pregnancies.

STUDY DESIGN: We performed a cohort analysis using the Missouri maternally linked birth and infant death surveillance datasets (1989-1997), comprised of women with their first 2 consecutive live births (n = 113,789). BMI (kilograms per square meter) was categorized as underweight (less than 18.5 kg/m²), normal (18.5 to 24.9 kg/m²), overweight (25 to 29.9 kg/m²), and obese (30 kg/m² or greater). Indications for primary cesarean were categorized as breech, dystocia, fetal distress, and others. Timing of primary cesarean was categorized as elective (prior to labor) and emergent (after initiation of labor). Adjusted odds ratio (OR) was used to quantify the associations between changes in pre-pregnancy BMI and indications for primary cesarean.

RESULTS: The rate of primary cesarean in the second pregnancy was 9.2%. Compared with women with normal BMI in their first 2 pregnancies, women who increased their BMI between pregnancies had increased risk of primary cesarean for all indications. Women who remained obese or overweight in both pregnancies were at increased risk of primary cesarean following breech (OR 1.28 and 1.13, respectively); dystocia (OR 1.94 and 1.41, respectively); fetal distress (OR 1.43 and 1.26, respectively); others (OR 3.17 and 1.63, respectively); and elective (OR 2.31 and 1.43, respectively) and emergent (OR 1.66 and 1.30, respectively) cesarean section. Women who lowered their BMI from obese to overweight or overweight to normal between pregnancies had risks of primary cesarean comparable with those with normal BMI in both pregnancies. Any increase in BMI from underweight to overweight or obese between the first 2 pregnancies was associated with increased risk of primary cesarean (OR 1.20 to 3.04) in the second pregnancy.

CONCLUSION: Increases in pre-pregnancy BMI between first 2 pregnancies from normal to obese is associated with increased risk of indications for primary cesarean. The association between being overweight or obese or increases in pre-pregnancy BMI between pregnancies and primary cesarean in the second pregnancy suggests that counseling women with regard to their high BMI may be beneficial.

Key words: body mass index, cesarean delivery, indications, obesity, overweight


The prevalence of overweight and obesity has reached epidemic proportions in the United States. The prevalence of obesity among women has increased from 16.5% in 1976-1980 to 33.2% in 2003-2004 (a relative increase of 101%). About 70% of African-American and nearly half of Caucasian women in the United States aged 20-39 years were overweight or obese in 2003-2004, demonstrating a significant race disparity in body weight composition. The dramatic increase in obesity prevalence seen in recent years has been recognized as an important public health problem and incurs substantial health costs. It is well recognized that maternal overweight or obesity is associated with an array of adverse pregnancy outcomes.

Cesarean delivery is now considered to be the most common form of operative procedures in the United States, accounting for 29.1% of all deliveries in 2004. In particular, there has been a temporal increase in primary cesarean deliveries (from 14.6% in 1996 to 20.6% in 2004, a relative increase of 41%). Cesarean delivery is responsible for an important proportion of maternal morbidity including previa and abruptio in the subsequent pregnancy.
High prepregnancy body mass index (BMI) \(12,13\) and excessive weight gain during pregnancy \(14\) are associated with increased risk of cesarean delivery. Given the recent temporal increase in cesarean delivery and obesity in the United States, it is likely that maternal BMI status may have influenced cesarean delivery rates. Furthermore, whether changes in prepregnancy BMI between the first 2 pregnancies are associated with increased risk of primary cesarean delivery in relation to indication subtypes and timing of delivery also remain poorly studied.

The purpose of this study was to examine risk for cesarean delivery in a second pregnancy by change in BMI status across pregnancies among women with a vaginal delivery in the first pregnancy. We further assessed whether changes in prepregnancy BMI between the first 2 pregnancies influenced the risk for indications and timing of primary cesarean in the second pregnancy.

**Materials and Methods**

**Data source**

Subjects eligible for this study were identified from the Missouri longitudinally linked live birth and fetal and infant death files for the years 1989-1997, inclusive. These files are produced by longitudinally linking siblings to their biological mothers using unique identifiers. The methods and algorithm used in the linkage process and the validation of the linked data have been described in detail previously. \(15\) The Missouri vital record system is subjected to quality assurance checks and is considered highly reliable and serves as a gold standard to validate US national datasets that entail matching and linking procedures. \(16\) Information extracted from the linked datasets include: maternal sociodemographic and behavioral characteristics, medical history, labor, obstetric procedures and complications, and fetal outcomes.

The study was approved by the Ethics Committee of the Institutional Review Board of the University of Medicine and Dentistry of New Jersey–Robert Wood Johnson Medical School. All analyses were performed using SAS version 9.1 (SAS Institute, Cary, NC).

**Definition of variables**

Data on maternal characteristics were based on the study cohort that consisted of the first 2 successive pregnancies. Self-reported maternal race was grouped as non-Hispanic white (Caucasian), non-Hispanic black (African American), and others. Other variables considered as potential confounders included maternal age (younger than 25, 25-34, 35 years or older); education (less than 12, 12, and 13 years or more of completed schooling); marital status (married/unmarried); prenatal care (early or first-trimester care began and no or late care began); and smoking and alcohol use during pregnancy (yes/no). Prepregnancy weight and height, based on maternal self-report, were used to calculate BMI (kilograms per square meter) and were categorized as underweight (less than 18.5 kg/m\(^2\)), normal (18.5 to 24.9 kg/m\(^2\)), overweight (25 to 29.9 kg/m\(^2\)), and obese (30 kg/m\(^2\) or greater). Other variables considered in this analysis were interval between the first birth and the second pregnancy (interpregnancy interval of less than 1, 1 to 1.49, 1.5 to 1.99, 2 to 2.49, 2.5 to 2.99, 3 to 3.49, 3.5 years or longer) and pregnancy weight gain (40 lb or less and 41 lb or more). Weight gains of 15-40 lb are within the Institute of Medicine guidelines. \(17\)

Primary cesarean delivery in the second pregnancy was the principal outcome. Indications for primary cesarean were categorized as breech, dystocia (an abnormal or difficult labor), fetal distress (signs indicating fetal hypoxia), and other indications. The latter group comprised women with 1 or more maternal medical (chronic hypertension, diabetes mellitus) or obstetrical (preeclampsia, premature rupture of membranes, choioamnionitis, placental abruption, placenta previa, and excessive bleeding) complications. Elective and emergent cesarean deliveries were defined as cesarean deliveries that were performed before and after initiation of labor, respectively.

**Data exclusions**

Between 1989 and 1997, a total of 711,015 live births and fetal deaths were recorded in the state of Missouri. Because our objective was to examine the associations between changes in prepregnancy BMI between the first 2 pregnancies and the outcomes, we excluded the following categories: births to women who had only 1 pregnancy during the study period and those who had 1 or more pregnancies prior to 1989 (n = 526,983). Pregnancies with stillbirths (n = 2513), missing data on maternal weight and height (n = 11,703), births at less than 20 weeks’ gestation (n = 13,757), vaginal birth after cesarean (n = 10,391), repeated cesarean (n = 31,850), and missing data on cesarean (n = 29) were also excluded. Births with gestational age less than 20 weeks were excluded to avoid errors in gestational age estimation of births that were at borderline viability. After all exclusions, we were left with a total of 113,789 women who delivered their first 2 consecutive live births at 20 weeks of gestation or longer for analysis.

**Statistical analysis**

We conducted a retrospective cohort analysis to assess whether a change in prepregnancy BMI between the first 2 pregnancies is associated with primary cesarean in the second pregnancy and whether indications for primary cesarean in the second pregnancy are influenced by changes in prepregnancy BMI between pregnancies. Statistical analysis was performed in 3 steps: (1) we examined the distribution of maternal demographic and behavioral characteristics by mode of delivery in the second pregnancy; (2) a multivariate logistic regression model was fitted to examine the association between changes in prepregnancy BMI between the first 2 pregnancies and risk of primary cesarean and indication-specific primary cesarean in the second pregnancy; and (3) we repeated the analysis after stratifying the data by race/ethnicity in an attempt to clarify whether this risk varied by race/ethnicity.

All regression models were adjusted for maternal age, maternal race, mater-
Maternal characteristics in the second pregnancy by mode of delivery

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Deliveries in the second pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of births</td>
</tr>
<tr>
<td>Maternal age (y) (%)</td>
<td></td>
</tr>
<tr>
<td>Younger than 25</td>
<td>45,777</td>
</tr>
<tr>
<td>25-34</td>
<td>55,186</td>
</tr>
<tr>
<td>35 or older</td>
<td>9242</td>
</tr>
<tr>
<td>Missing</td>
<td>3584</td>
</tr>
<tr>
<td>Maternal race/ethnicity (%)</td>
<td></td>
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<tr>
<td>Non-Hispanic white</td>
<td>92,435</td>
</tr>
<tr>
<td>Non-Hispanic black</td>
<td>18,468</td>
</tr>
<tr>
<td>Other race/ethnicity</td>
<td>2886</td>
</tr>
<tr>
<td>Maternal education (y) (%)</td>
<td></td>
</tr>
<tr>
<td>Less than 12</td>
<td>22,801</td>
</tr>
<tr>
<td>12</td>
<td>41,442</td>
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<tr>
<td>More than 12</td>
<td>48,859</td>
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<tr>
<td>Missing</td>
<td>687</td>
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<tr>
<td>Single marital status (%)</td>
<td>31,613</td>
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<tr>
<td>Late initiate or no prenatal care (%)</td>
<td>20,343</td>
</tr>
<tr>
<td>Smoking during pregnancy (%)</td>
<td>24,855</td>
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<tr>
<td>Indications for cesarean</td>
<td></td>
</tr>
<tr>
<td>Breech</td>
<td>2996</td>
</tr>
<tr>
<td>Dystocia</td>
<td>4930</td>
</tr>
<tr>
<td>Fetal distress</td>
<td>4154</td>
</tr>
<tr>
<td>Other indications</td>
<td>744</td>
</tr>
<tr>
<td>Interpregnancy interval (y) (%)</td>
<td></td>
</tr>
<tr>
<td>Less than 1</td>
<td>5948</td>
</tr>
<tr>
<td>1 to 1.49</td>
<td>15,825</td>
</tr>
<tr>
<td>1.5 to 1.99</td>
<td>18,093</td>
</tr>
<tr>
<td>2 to 2.49</td>
<td>14,829</td>
</tr>
<tr>
<td>2.5 to 2.99</td>
<td>11,592</td>
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<tr>
<td>3 to 3.49</td>
<td>8221</td>
</tr>
<tr>
<td>3.5 or longer</td>
<td>18,239</td>
</tr>
<tr>
<td>Missing</td>
<td>20,988</td>
</tr>
<tr>
<td>Second prepregnancy BMI (kg/m²) (%)</td>
<td></td>
</tr>
<tr>
<td>Less than 18.5</td>
<td>9165</td>
</tr>
<tr>
<td>18.5 to 24.9</td>
<td>67,925</td>
</tr>
<tr>
<td>25 to 29.9</td>
<td>21,891</td>
</tr>
<tr>
<td>30 or greater</td>
<td>14,808</td>
</tr>
</tbody>
</table>

All comparisons were statistically significant (P < .001).

RESULTS

The incidence of primary cesarean in the second pregnancy was 9.2%. The primary elective (cesarean delivery prior to labor) and emergent (cesarean delivery after initiation of labor) cesarean delivery rates in the second pregnancy were 3.2% and 6.0%, respectively. Table 1 shows the distribution of maternal characteristics in relation to primary cesarean delivery in the second pregnancy. Advanced maternal age (35 years or older), African Americans, smoking during pregnancy, excessive weight gain during pregnancy, and shorter and longer interpregnancy intervals were associated with increased risks of primary cesarean in the second pregnancy.

Compared with women with normal BMI during their first and second pregnancies, increases in prepregnancy BMI between the first 2 pregnancies was associated with increased risk of primary cesarean in the second pregnancy (Table 2). A decrease in BMI from obese to normal or from overweight to normal between the first 2 pregnancies attenuated...
the risk of primary cesarean in the second pregnancy, although still present. Women who were overweight, obese, or obese in both their first and second pregnancies had higher rates of primary cesarean in the second pregnancy than those women with normal prepregnancy BMI in both pregnancies.

Figure 1 shows risk of primary cesarean delivery among whites (panel A) and African Americans (panel B) in the second pregnancy in relation to changes in prepregnancy BMI. Prepregnancy BMI in the first and second pregnancies showed stronger associations with primary cesarean among white women. Among African American women, prepregnancy BMI in the second pregnancy showed stronger associations than prepregnancy BMI in the first pregnancy with primary cesarean.

Compared with women with normal BMI during their first and second pregnancies, women who remained overweight or obese in both pregnancies were at increased risk of primary cesarean resulting from breech, dystocia, fetal distress, and other indications in the second pregnancy (Table 3). Increases in prepregnancy BMI from normal to obese between the first 2 pregnancies were associated with primary cesarean because of dystocia, fetal distress, and other indications. However, increased risk of primary cesarean in relation to

### Table 2

<table>
<thead>
<tr>
<th>Prepregnancy BMI</th>
<th>Deliveries in the second pregnancy</th>
<th>Primary cesarean</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number of births</td>
<td>Rate (%)</td>
</tr>
<tr>
<td>Obese in 1st</td>
<td>Overweight in 2nd</td>
<td>8235</td>
</tr>
<tr>
<td>Overweight in 1st</td>
<td>Overweight in 2nd</td>
<td>4760</td>
</tr>
<tr>
<td>Normal weight in 1st</td>
<td>Overweight in 2nd</td>
<td>1737</td>
</tr>
<tr>
<td>Underweight in 1st</td>
<td>Overweight in 2nd</td>
<td>76</td>
</tr>
<tr>
<td>Obese in 1st</td>
<td>Obese in 2nd</td>
<td>1958</td>
</tr>
<tr>
<td>Overweight in 1st</td>
<td>Overweight in 2nd</td>
<td>9580</td>
</tr>
<tr>
<td>Normal weight in 1st</td>
<td>Overweight in 2nd</td>
<td>10,182</td>
</tr>
<tr>
<td>Underweight in 1st</td>
<td>Overweight in 2nd</td>
<td>171</td>
</tr>
<tr>
<td>Obese in 1st</td>
<td>Normal weight in 2nd</td>
<td>842</td>
</tr>
<tr>
<td>Overweight in 1st</td>
<td>Normal weight in 2nd</td>
<td>4538</td>
</tr>
<tr>
<td>Normal weight in 1st</td>
<td>Normal weight in 2nd</td>
<td>57,824</td>
</tr>
<tr>
<td>Underweight in 1st</td>
<td>Normal weight in 2nd</td>
<td>4721</td>
</tr>
<tr>
<td>Obese in 1st</td>
<td>Underweight in 2nd</td>
<td>59</td>
</tr>
<tr>
<td>Overweight in 1st</td>
<td>Underweight in 2nd</td>
<td>97</td>
</tr>
<tr>
<td>Normal weight in 1st</td>
<td>Underweight in 2nd</td>
<td>3120</td>
</tr>
<tr>
<td>Underweight in 1st</td>
<td>Underweight in 2nd</td>
<td>5889</td>
</tr>
</tbody>
</table>

Rates are expressed in percent. Adjustments were made for maternal age, race, education, marital status, prenatal care, smoking, and interpregnancy interval.
breech was noted among women who increased BMI from underweight to overweight. A decrease in BMI from obese to normal weight between the first 2 pregnancies was associated with higher, albeit reduced, risks of primary cesarean for each indication subtypes.

Because pregnancy weight gain lies on the causal pathway between changes in prepregnancy BMI and primary cesarean, we examined the associations without adjustment for weight gain in the model. The results were essentially unchanged from the overall analysis (data not shown).

Changes in prepregnancy BMI from normal to obese between the first 2 pregnancies were associated with increased risks of both elective and emergent primary cesarean in the second pregnancy (Figure 2). There were no substantial differences in risks for women with decreasing prepregnancy BMI between the first 2 pregnancies as compared with women who retained normal BMI in both pregnancies.

### Comment

Our findings that overweight or obesity is associated with increased risk of cesarean is consistent with the growing body of research. This study indicates that overweight or obesity in the second pregnancy is significantly associated with increased risk for a primary cesarean in the second pregnancy. The magnitude of association, however, varies in relation to first prepregnancy BMI status.

Being overweight and obese in both pregnancies and increases in BMI from underweight to overweight or obese between the first 2 pregnancies may lead to abnormal maternal and fetal metabolism. In turn, this may lead to higher rates of complications, such as large for gestational age, dystocia, and fetal distress. Such pregnancies could potentially result in increased risk of cesarean delivery. Decreasing prepregnancy BMI from obese to normal between pregnancies attenuated the risk of primary cesarean.

However, the risk was still elevated with respect to those with normal BMI in both pregnancies. It is not clear to us by what mechanism the first prepregnancy BMI impacts cesarean risk in the second pregnancy.

The controversy surrounding elective cesarean has received considerable attention. Reliable data are needed as to how many of the elective cesareans are due to maternal request as opposed to physician preferences. Although the International Federation for Obstetrics and Gynecology opined that performing cesarean delivery in the absence of any medical indication remains unethical, there is some evidence to suggest that obstetricians prefer elective cesarean delivery of uncomplicated pregnancy out of fear of untoward maternal and/or infant outcomes. Our data suggest that changes in prepregnancy BMI from normal to overweight or obese between pregnancies are associated with 1.5- to 2.3-fold increased risk of elective cesarean, whereas changes in

### Table 3

<table>
<thead>
<tr>
<th>Prepregnancy BMI</th>
<th>Primary cesarean delivery in the second pregnancy</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Breech</td>
</tr>
<tr>
<td></td>
<td>Rate</td>
</tr>
<tr>
<td>Obese</td>
<td>3.4</td>
</tr>
<tr>
<td>Overweight</td>
<td>2.6</td>
</tr>
<tr>
<td>Normal weight</td>
<td>2.5</td>
</tr>
<tr>
<td>Underweight</td>
<td>2.6</td>
</tr>
<tr>
<td>Obese</td>
<td>1.7</td>
</tr>
<tr>
<td>Overweight</td>
<td>3.0</td>
</tr>
<tr>
<td>Normal weight</td>
<td>2.1</td>
</tr>
<tr>
<td>Underweight</td>
<td>5.3</td>
</tr>
<tr>
<td>Obese</td>
<td>2.9</td>
</tr>
<tr>
<td>Overweight</td>
<td>1.9</td>
</tr>
<tr>
<td>Normal weight</td>
<td>2.6</td>
</tr>
<tr>
<td>Underweight</td>
<td>2.4</td>
</tr>
<tr>
<td>Obese</td>
<td>1.7</td>
</tr>
<tr>
<td>Overweight</td>
<td>2.1</td>
</tr>
<tr>
<td>Normal weight</td>
<td>2.3</td>
</tr>
<tr>
<td>Underweight</td>
<td>3.6</td>
</tr>
</tbody>
</table>

Rates are expressed in percent. Adjustments were made for maternal age, race, education, marital status, prenatal care, smoking, and interpregnancy interval.
pregnancy BMI from obese to normal are not associated with risk of elective cesarean delivery. This may suggest that the obstetrician decision of elective cesarean might arguably be driven by temporal changes in increased maternal body composition.

The findings on emergent cesarean are of particular interest. The recent increasing trends of maternal overweight and obesity,1,2 coupled with our findings on the associations between changes in prepregnancy BMI between the first 2 pregnancies and increased risk of primary cesarean in the second pregnancy, may partly explain the reason for the rising primary cesarean rates. An explanation for the association between being overweight and obese in both pregnancies or increases in BMI from underweight to overweight or obese between the first 2 pregnancies and primary cesarean may be adverse fetal physiology (such as abnormal fetal heart rate tracings) requiring emergent intervention. Although clinician’s decision on emergent cesarean is driven by fetal and maternal medical and obstetrical complications, counseling of overweight and obese women about risks associated with cesarean delivery may help in reducing rates of primary elective cesarean.

Our rates of elective (3.2%) and emergent (6.0%) primary cesarean are smaller than those previously reported rates because of the fact that our study cohort consisted of women with their first 2 successive pregnancies who delivered liveborn babies. The vital statistics data are known for underreporting of some of the factors, notably those related to indications for cesarean. Because data on prepregnancy weight were self-reported, it is possible that the derived BMI may have been underestimated. However, self-reported prepregnancy weight was previously shown to be quite reliable for clinical studies.22 We were unable to examine the extent to which a clinician’s decision or maternal preference on elective cesarean may have affected our findings. Future studies will be needed to rigorously examine this issue. The large sample size, a population-based cohort design, and control for several confounding factors are strengths of this study.

These data have clinical and public health implications. The association between being overweight or obese in both the first 2 pregnancies or increases in prepregnancy BMI from normal to obese between the first 2 pregnancies and primary cesarean in the second pregnancy suggests that counseling women with regard to their high BMI may be beneficial.

ACKNOWLEDGMENT

We are grateful to the Missouri Department of Health and Senior Services for allowing us to utilize the maternally linked longitudinal data file. We are grateful to the Missouri Department of Health and Senior Services for allowing us to utilize the maternally linked longitudinal data file. We are grateful to the Missouri Department of Health and Senior Services for allowing us to utilize the maternally linked longitudinal data file. We are grateful to the Missouri Department of Health and Senior Services for allowing us to utilize the maternally linked longitudinal data file. We are grateful to the Missouri Department of Health and Senior Services for allowing us to utilize the maternally linked longitudinal data file.

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Prepregnancy body mass index and the length of gestation at term

Naomi E. Stotland, MD; A. Eugene Washington, MD, MSc; Aaron B. Caughey, MD, PhD

OBJECTIVE: The purpose of this study was to examine the relationship between prepregnancy body mass index (BMI) and length of gestation at term.

STUDY DESIGN: This was a retrospective study of 9336 births at the University of California, San Francisco, at ≥37 weeks' gestation. We performed univariate and multivariable analyses of the associations between prepregnancy BMI and length of gestation (≥40, ≥41, and ≥42 weeks' gestation).

RESULTS: Overweight women were more likely to deliver at ≥40, ≥41, and ≥42 weeks' gestation than were women who were underweight or normal weight. In multivariable analyses, higher prepregnancy BMI was associated with higher risk of progressing past 40 weeks. Obese women had 69% higher adjusted odds of reaching 42 weeks' gestation, compared with women of normal prepregnancy BMI (adjusted odds ratio, 1.69; 95% confidence interval, 1.23-2.31).

CONCLUSION: Higher BMI is associated with prolonged gestation at term. Achieving optimal BMI before conception may reduce the risk of postterm pregnancy and its associated complications.

Key words: obesity, postterm pregnancy, prepregnancy body mass index


Postterm (>42 weeks' gestation) and prolonged pregnancies (>41 weeks' gestation) have been associated with multiple adverse maternal and neonatal outcomes.1-4 Progression of pregnancy past 41 weeks is associated with increased costs related to antenatal fetal monitoring and induction of labor,5,6 and can be a source of anxiety for the pregnant woman.7 Risk factors for postterm pregnancy reported in the literature include dating error, primiparity, previous postterm pregnancy, male fetal sex, genetic predisposition, placental sulfatase deficiency, and fetal anencephaly.7-9 Except for dating error, none of these risk factors are modifiable.

Recently, a study of obese pregnant women found an increased risk of postterm pregnancy, compared with nonobese women.10 Low prepregnancy body mass index (BMI) has been associated with preterm delivery.11 These findings raise the question: does an elevated prepregnancy BMI increase the risk of postterm pregnancy and length of gestation at term? This question is particularly timely, given the current obesity epidemic in the United States and other industrialized nations.

Because recent research has suggested that perinatal complications, which include neonatal morbidity, perinatal infections, macrosomia, and meconium passage, begin to increase after 40 weeks' gestation (and in some cases after 37 weeks' gestation),3,12,13 our objective was to assess the role of prepregnancy BMI on the length of gestation at term and the risk of postterm pregnancy.

It is methodologically challenging to study predictors of prolonged pregnancy, because elective delivery at 39 or 40 weeks' gestation (by both cesarean and induction of labor) have become increasingly common.14,15 Until 2002, the usual practice at the University of California, San Francisco (UCSF) Medical Center had been to restrict the induction of labor and cesarean birth to obstetric indications; it has only been over the past 4 years that elective induction of labor before 42 weeks' gestation has been used. Therefore, this population of parturients is ideal to examine the effect of BMI on length of gestation.

MATERIALS AND METHODS

This retrospective cohort was selected from women who delivered singleton infants at the UCSF Medical Center between 1990-2001. The UCSF perinatal division maintains the research database that was used for this study. Demographic, antenatal, intrapartum, and delivery data are entered into a preprinted datasheet by the delivering physician or midwife immediately after every birth, and additional neonatal and discharge data are entered into the database by trained abstractors. For this study, we excluded births with the following characteristics: preterm birth (<37 weeks' gestation), multiple gestation, induction at <42 weeks' gestation, or elective cesarean without labor. Because the UCSF Medical Center is a referral center that accepts frequent maternal transports from community hospitals, transport patients were also excluded. This study...
received institutional review board approval from the Committee on Human Research at UCSF.

Gestational age was ascertained by last menstrual period, unless the last menstrual period was not consistent with ultrasound dating, either within 7 days for a first trimester ultrasound scan or 14 days for a second trimester ultrasound scan, in which case ultrasound dating was used. During the study period, ultrasound examinations were done routinely between 18-20 weeks' gestation.

Gestational age at delivery was the primary outcome variable and was examined as a continuous outcome between 37-43 weeks' gestation; categoric variables represented delivery at $\geq 40$ vs $<40$ weeks' gestation, $\geq 41$ vs $<41$ weeks' gestation, and $\geq 42$ vs $<42$ weeks' gestation.

Prepregnancy BMI was categorized according to the Institute of Medicine categories: low ($<19.8 \text{ kg/m}^2$), normal (19.8-26 kg/m$^2$), high (26.1-29.0 kg/m$^2$) and obese ($>29.0 \text{ kg/m}^2$). We performed bivariate analyses on the association between maternal demographic characteristics and length of gestation at term with $\chi^2$ analysis. We also used $\chi^2$ analysis to assess the association between prepregnancy BMI and delivery at 40, 41, and 42 weeks' gestation. We then performed multivariable logistic regression on the relationship between BMI category and risk of delivering after 40, 41, and 42 weeks' gestation. Covariates were selected on the basis of empiric importance and statistical significance in the bivariate analyses. Variables that were

### TABLE 1

**Characteristics of study cohort by gestational age at delivery**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>37-39 wk (%)</th>
<th>40-41 wk (%)</th>
<th>$\geq$42 wk (%)</th>
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<tr>
<td>Maternal age (y)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$&lt;20$</td>
<td>8.8</td>
<td>9.4</td>
<td>9.7</td>
</tr>
<tr>
<td>20-29</td>
<td>45.2</td>
<td>46.1</td>
<td>45.4</td>
</tr>
<tr>
<td>30-39</td>
<td>43.0</td>
<td>42.0</td>
<td>42.0</td>
</tr>
<tr>
<td>$&gt;40$</td>
<td>3.0</td>
<td>2.5</td>
<td>3.0</td>
</tr>
<tr>
<td>$P$ value</td>
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<td></td>
<td></td>
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<tr>
<td>Race/ethnicity</td>
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<tr>
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<td>15.8</td>
</tr>
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<td>11.8</td>
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<td>Asian</td>
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<td>8.7</td>
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<tr>
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<td>13.0</td>
<td>12.7</td>
<td>11.6</td>
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<td>Below guidelines</td>
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</tr>
<tr>
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<td>97.0</td>
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<tr>
<td>$P$ value</td>
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<td>10.3</td>
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<tr>
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<td>91.0</td>
<td>92.4</td>
<td>89.7</td>
</tr>
<tr>
<td>$P$ value</td>
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</tr>
</tbody>
</table>

37-39 weeks: $n = 4425$; 40-41 weeks: $n = 4404$; $\geq$42 weeks: $n = 507$. 

$P < .001$ for log-rank test.

*Scotland. Prepregnancy body mass index and the length of gestation at term. AJOG 2007.*
included in the multivariable models were maternal age, maternal race/ethnicity, parity, gestational weight gain (kg/week), insurance status, hypertension (pregestational or gestational), diabetes mellitus (pregestational or gestational), and smoking. A “cluster” command was used to adjust the confidence intervals (CIs) of the odds ratios to account for lack of independence between births to the same mother within the cohort. We constructed survival curves for each BMI category, with delivery as the failure event, and compared the curves by the log-rank test. Statistical significance was defined as a P value of .05. Statistical analyses were performed with Stata software (version 9.1; Statacorp, College Station, TX).

**Results**

The study cohort consisted of 9336 term births; 47.4% of the pregnancies were delivered between 37-39 weeks’ gestation; 47.2% of the pregnancies were delivered at 40-41 weeks’ gestation, and 5.4% of the pregnancies were delivered at ≥42 weeks. Characteristics that were associated with longer gestation at term were white race/ethnicity, lower parity, higher weight gain, and private insurance. Asian women were less likely to progress past 40 weeks’ gestation, compared with other groups (Table 1).

In bivariate comparisons between maternal prepregnancy BMI and length of gestation at term, higher BMI was associated with longer gestation at delivery (Table 2), with 28.5% of obese women progressing to ≥41 weeks’ gestation vs 18.3% and 21.9% of underweight and normal weight women, respectively (P < .001). Higher BMI was also associated with higher rates of progress beyond 42 weeks’ gestation (P < .001), although subjects in the overweight and obese categories had similar rates (7.3% and 7.2%).

In the multivariable analysis (Table 3), compared with subjects with normal prepregnancy BMI, those women in the high or obese categories had increased odds of progressing past 40, 41, and 42 weeks’ gestation. Subjects in the low BMI category had decreased odds of progressing past 41 weeks’ gestation, when compared with those of normal BMI (adjusted odds ratio, 0.84; 95% confidence interval [CI], 0.75-0.97). In multivariable logistic regression with gestational age of ≥41 weeks as the outcome and with the use of BMI as a continuous predictor, an increase of 1 BMI unit was associated with an adjusted odds ratio of 1.29 (95% CI, 1.21-1.38).

Survival analysis revealed statistically significantly different survival curves for the 4 BMI categories (Figure), with the curves for higher BMIs shifted toward later delivery, whether or not we adjusted for confounding variables (P value for log-rank test, <.001).

**Comment**

In this cohort of women who delivered at ≥37 weeks’ gestation, we found that higher prepregnancy BMI was associated with longer gestation at term and higher risk of postterm pregnancy. Not only are overweight and obese women at increased risk of prolonged pregnancy, but underweight women are at a slightly decreased risk, compared with women with a normal prepregnancy BMI.

Our findings are consistent with those of Usha Kiran et al., who found that obese women were more likely to have a postterm pregnancy compared with nonobese women. We extend these previous findings by demonstrating that this relationship spans the 4 BMI categories that were studied, with progressively higher prepregnancy BMI associated with progressively longer gestation.

The possible mechanisms for this association remain unclear. Because adipose tissue is hormonally active and because obese women may have an altered metabolic status, it is possible that endocrine factors that are involved in the initiation of labor are altered in women with an increased BMI. The long-noted associations between lower prepregnancy BMI and increased spontaneous preterm birth and higher BMI with lower rates of spontaneous preterm birth are consistent with our findings and may be explained by a common, as yet unknown, mechanism regarding parturition.

Women who are overweight or obese may be more likely to be induced or delivered by elective cesarean delivery before they reach 40, 41, or 42 weeks’ gestation, because of gestational hypertension, diabetes mellitus, or suspected macrosomia. If a true physiologic, causal relationship exists between high BMI and prolonged pregnancy, it may be masked or attenuated by these earlier elective deliveries. The finding of an association between higher BMI and longer gestation at term, despite the higher rates of antepartum complications among obese women, suggests that this association may be even stronger than we are able to measure in this cohort. Confirmatory studies in other populations, perhaps in countries where elective delivery is less common, would help to validate our findings.

The clinical ramifications of this study are important to women who are preparing to become pregnant. For those in the overweight category, if they can lower their BMI to the normal category, they may decrease the chance that they will have a postterm pregnancy and the associated complications and need for induction of labor. The effect magnitude can be conceived with a number needed.
to treat calculation. Given that the adjusted odds ratio is 1.29 for these women (with a baseline risk of reaching 41 weeks’ gestation of 24.5%), if 14 women moved down 1 weight category, 1 less woman would go past 41 weeks’ gestation. For obese women with an adjusted odds ratio of 1.81, only 4 women would need to achieve a normal BMI to prevent 1 woman going beyond 41 weeks’ gestation.

Limitations of this study are inherent to its retrospective, nonrandomized design. Although a randomized trial of obesity is unlikely to occur, our study design may lead to potential confounding bias. We attempted to control for these confounders with multivariable analysis, but some residual confounding may remain. We excluded induced labors of <42 weeks’ gestation but included those at ≥42 weeks of gestation. Because it has been our standard practice to induce labor for the indication of postdates at 42 weeks’ gestation, we chose not to exclude those inductions at ≥42 weeks’ gestation. Ideally, we would be able to separate out inductions for postterm gestation vs inductions for other indications, but indication for induction is not included in our database. As a sensitivity analysis, we reran the multivariate models, excluding all inductions, but we lost statistical power to examine birth at ≥42 weeks’ gestation because nearly one-half of births at that late gestation were induced. However, the directions of the odds ratios were similar and supported the robustness of our findings.

Another limitation of our study is that it is restricted to births at ≥37 weeks’ gestation. The literature is conflicting on the influence of obesity on the risk of spontaneous preterm birth, although, as noted earlier, most studies suggest an increasing risk with decreasing prepregnancy BMI. Obese women may be at increased risk for medically indicated preterm birth that is related to pre eclampsia, diabetes mellitus, and other complications. One study showed an increased risk of overall preterm birth among obese women. The relationship between BMI and overall length of gestation is complex; therefore, we restricted our analysis to term births.

Longer gestation at term is associated with increased risks of macrosomia, pre eclampsia, cesarean birth, postpartum hemorrhage, severe lacerations, and infant morbidity. Multiple reports have suggested that a policy of induction of labor at 41 instead of 42 weeks’ gestation does not increase the cesarean rate and may reduce maternal and infant morbidity. However, induction of labor is expensive and may be riskier, compared with spontaneous labor. If women can optimize their BMI before conception, it is possible that both the need for labor induction and adverse perinatal outcomes that are related to prolonged term gestation would be reduced. Although many pregnancies are unplanned, primary care clinicians can take the initiative to discuss weight loss with overweight reproductive age women and inform them of the risks that are related to childbearing at a higher BMI. Our findings may also provide a hint as to the mechanisms of parturition initiation. Future research should explore the potential role of adipose tissue in the timing of labor.

### TABLE 3

<table>
<thead>
<tr>
<th>Gestational age (wk)</th>
<th>Prepregnancy BMI</th>
<th>Adjusted odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>≥40</td>
<td>Low</td>
<td>0.96 (0.86-1.07) Reference</td>
</tr>
<tr>
<td>≥41</td>
<td>Normal</td>
<td>1.17 (1.01-1.34) 1.63 (1.39-1.92)</td>
</tr>
<tr>
<td>≥42</td>
<td>High</td>
<td>0.83 (0.72-0.95) Reference</td>
</tr>
<tr>
<td></td>
<td>Obese</td>
<td>1.29 (1.10-1.52) 1.81 (1.50-2.18)</td>
</tr>
</tbody>
</table>

Analyses controlled for the following variables: maternal age, maternal race/ethnicity, parity, gestational weight gain (kg/week), insurance status, hypertension (pregestational or gestational), diabetes mellitus (pregestational or gestational), smoking.

### REFERENCES


Pulmonary arteriole muscularization in lambs with diaphragmatic hernia after combined tracheal occlusion/glucocorticoid therapy

Marcus Davey, PhD; Shincy Shegu; Enrico Danzer, MD; Eduardo Ruchelli, MD; Scott Adzick, MD; Alan Flake, MD; Holly L. Hedrick, MD

OBJECTIVE: A morphometric study was performed to examine the effects of prenatal glucocorticoids, which were administered 48 hours before birth, on muscularization of small pulmonary arterioles (<60 μm diameter) in lambs with diaphragmatic hernia (DH) after fetal tracheal occlusion (TO).

STUDY DESIGN: DH was created in 23 fetal sheep at 65 days gestation; 9 of the fetuses were exposed prenatally to beta-methasone (0.5 mg/kg body weight) 48 hours before delivery. Six sham-operated animals served as controls. Sections of paraffin that were embedded in lung tissues were stained with Elastin-Van Gieson, and the percentage of medial wall thickness (MWT) was determined.

RESULTS: The percentage of MWT in DH lambs (29.6% ± 1.9%) was increased compared with sham animals (18.1% ± 1.3%) and was not different from that of DH/TO animals (30.3% ± 1.7%). In DH/TO + glucocorticoid lambs, the percentage of MWT (24.6% ± 1.2%) was significantly lower than in the DH/TO group but was higher than the sham group.

CONCLUSION: In fetuses who underwent prolonged TO therapy for severe DH, prenatal glucocorticoid treatment decreased medial hypertrophy of pulmonary arterioles by approximately 19%. We speculate that such structural changes may have contributed to improve gas exchange that was observed in this model.

Key words: diaphragmatic hernia, fetus, glucocorticoid, sheep, tracheal occlusion

C ongenital diaphragmatic hernia (DH) occurs in 1 in 2500 live births and is associated with significant mortality and morbidity rates because of severe lung hypoplasia and pulmonary hypertension. Morphologically, the lungs of infants with DH contain fewer airways and alveoli,1-7 which reduces the gas exchange surface area; the decrease in the cross-sectional area of the pulmonary vascular bed contributes to the development of early pulmonary hypertension.8-12 Excessive muscularization of pulmonary arterioles and extension of the arterial muscle sheath into normally nonmuscularized acinar regions of the lung exacerbate pulmonary hypertension and may make vessels hyperreactive to vasoconstrictor stimuli.8-12

Fetuses with liver herniation into the chest cavity and a lung-to-head ratio of <1.0 have the worst outcome13 and are often unresponsive to the current postnatal therapeutic armamentarium. In this subset of fetuses with congenital DH, tracheal occlusion (TO) is considered a potential therapy to restore lung mass and improve neonatal gas exchange.4-6 During fetal life, the lungs are expanded with fluid that is secreted actively by the pulmonary epithelium.14 Normally, this fluid leaves the lungs through the trachea and is either swallowed or contributes to amniotic fluid volume.14 When the fetal trachea is occluded, fluid accumulates within the future airspaces, increasing basal levels of tissue stretch, which ultimately accelerates lung growth.15-18 In experimental DH, the lung growth response after TO is characterized by an increased number of alveoli and pulmonary arterioles,16,19 reduced muscularization of small pulmonary arterioles,20,21 and a reduction in surfactant synthesis22,23 because of increased differentiation of type II into type I pneumocytes.24 Consequently, neonatal lung compliance is low after prolonged TO, and neonatal gas exchange is abnormal25 but may improve with exogenous surfactant therapy.26

In fetal sheep that underwent prolonged TO therapy for severe DH, prenatal exposure to glucocorticoids dramatically improved gas exchange and ventilation efficiency index.27 In our previous study, pregnant ewes received a single dose of betamethasone (0.5 mg/kg body weight) 48 hours before cesarean delivery of lambs at near term. We speculated that enhanced structural maturation of alveoli (as evidenced by thinning of alveolar walls), increased lung tissue...
compliance, and surfactant protein synthesis were likely to have contributed to improved respiratory outcome of glucocorticoid-treated DH/TO lambs.²⁸ It is also conceivable that glucocorticoid-induced changes in pulmonary vascular structure may have contributed to improved gas exchange in DH/TO lambs. In fetal rats with DH, medial wall thickness (MWT) of pulmonary arterioles was ameliorated by prenatal glucocorticoid treatment.²⁹,³⁰ In our present study, we have used established morphometric techniques³¹,³² to determine the effects of prenatal glucocorticoid treatment on the degree of pulmonary arteriole muscularization in fetal sheep with severe DH that underwent TO therapy. In the current study, lung samples were obtained after inflation fixation through the airways,²⁵,²⁸ which caused compression/distortion of pulmonary arterioles that were >60 µm external diameter and prevented accurate measurements of MWT. Therefore, our analysis focused on muscularized arterioles that were <60 µm external diameter.

### Study design

Experimental protocols were approved by the local Institutional Animal Care and Use Committee and followed guidelines set forth in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

### Surgical procedures

Left-sided DHs were surgically created in 24 fetal sheep at 65 days’ gestation;²⁷ full term is approximately 145 days. A second fetal surgery was performed at 110 days of gestation to occlude the fetal trachea in 17 of 24 fetuses with DH; the remaining 7 fetuses with congenital DH underwent a sham surgical procedure in which Prolene suture was passed under the trachea and not tied.²⁷ In the DH/TO group, 10 of 17 fetuses were exposed to prenatal glucocorticoids by maternal intramuscular administration (Celestone Chronodose, 0.5 mg/kg maternal body weight; Schering Plough, Baulkham Hills, Australia). Ewes were injected 48 hours before planned cesarean delivery of lambs; this dose of glucocorticoid has consistently improved pulmonary arteriole structure and function in preterm sheep.³²,³³ A group of animals that underwent sham fetal surgery for DH (thoracotomy without diaphragm incision) and TO (passing suture under the trachea) served as controls (n = 6). Hence, there were 4 animal groups: sham (n = 6), DH (n = 7), DH/TO (n = 7), and DH/TO + glucocorticoid (n = 9). It was necessary to use a greater number of glucocorticoid-treated fetuses because the average number of vessels per animal was significantly lower in this group compared with the sham (P = .17) and DH (P = .01) groups. At 140 days, gestational age, fetuses were delivered partially by cesarean section, instrumented with vascular catheters and an endotracheal tube, then fully delivered and mechanically ventilated for 2 hours after birth; studies of neonatal pulmonary function have been published.²⁷

### Postmortem analysis

At the end of the resuscitation study, lambs were killed painlessly by intravenous sodium pentobarbital, and the bodyweight for each lamb was recorded. After the lungs were removed and weighed, the right (contralateral lung) lung was inflation fixed through the trachea with 4% paraformaldehyde-2% glutaraldehyde in phosphate-buffered saline solution (pH 7.3) to a distending pressure of 20 cm H₂O. When the fixation pressure had reached a plateau (typically within 1 hour), the trachea was occluded, and the expanded lungs were submerged in buffered fixative and stored at 4°C overnight. The left (ipsilateral) lungs did not undergo morphometric analysis in this study and were used to determine dry lung weight and surfactant protein expression; these data have been previously reported.³⁴

### Pulmonary arteriole analysis

A cross-sectional portion of lung tissue was obtained from the central zone of the lower right lobe, dehydrated through a series of graded alcohol solutions, and embedded in paraffin. Lung blocks were sectioned at 3 µm and stained with Elastin–Van Gieson to facilitate identification of pulmonary arterioles, based on the presence of both internal and external elastic laminae. Pulmonary arterioles were examined with a light microscope (Leica DMRD; Leica, Wetzler, Germany) interfaced with a digital camera (Retiga EXi; QImaging Corporation, Surrey, BC, Canada) connected to a computer (Apple G5 Mac; Apple Computers, Inc, Cupertino, CA). Digital images were acquired (×400 final magnification) and analyzed with QCapture software (QImaging Corporation).

The degree of pulmonary arteriole muscularization was assessed by measurements of percent MWT. Our intention was to analyze all muscularized vessels within each section to obtain measurements of MWT for a range of different size arterioles.²¹,³¹ However, after initial examination, it was evident that both fully and partially muscularized arterioles with an external diameter of >60 µm were collapsed and that measurements of MWT within individual vessels varied considerably. This was not an unexpected observation, because the lungs were fixed through the trachea, which causes compression of vessels.³⁵ To measure MWT accurately in pulmonary arterioles of >60 µm external diameter, the pulmonary vasculature should be fixed under consistent conditions of pressure and flow³⁶ (personal communication, Dr Paul Davies; January 2002). For the reasons described, our morphometric analysis was limited to pulmonary arterioles with an external diameter of <60 µm. In addition, to facilitate consistent measurements of MWT, vessels that were included for analysis were circular in shape and had an intact muscular wall of consistent circumferential thickness. External diameter was measured across the shortest luminal profile between external elastic laminae; across the same diameter, MWT was measured on both sides of the vessel (Figure 1). To obtain accurate estimates of average MWT, vessels were excluded if their 2 measurements of MWT differed by >30%.²⁰ For each pulmonary arteriole, the 2 MWT measurements were averaged. As first described by Davies and Reid,³⁷ percent MWT was calculated as in the following manner: %MWT = (2 × MWT/external diamet-
ter) × 100. Adventitial thickness of individual vessels varied widely and could not be estimated accurately. The observer (S.S.) was blinded to animal identification. Randomly selected slides (n = 4) were reanalyzed to calculate observer error between duplicate analysis (<1.6% for measurements of external diameter and MWT).

The number of fully muscularized pulmonary arterioles per unit area of lung (ie, density) was calculated by dividing the total number of vessels that were included for measurements of MWT by area of lung section (square centimeter). Statistically, the latter parameter was measured from scanned images of slides with the flood-fill function of an image analysis program (SigmaScan; Systat Software, Inc, San Jose, CA).

Statistical analysis
For each animal, an average value for the percentage of MWT was calculated and used in analysis (n = 29). Because there were more animals in the DH/TO + glucocorticoid group (n = 9) compared with the sham group (n = 6), nonparametric statistical tests were used. A Kruskal-Wallis test for independent samples was used to determine significant differences in the percentage of MWT among groups; Mann-Whitney post hoc testing was used to determine individual differences between mean values. Reported probability values are for 2-tailed tests.

Previous morphometric studies of experimental DH have demonstrated that aberrant muscularization is predominant in smaller, rather than larger, pulmonary arterioles. To determine whether a similar relationship existed in this study, arterioles were categorized into 2 groups based on external diameter; again, an average value was obtained for each animal and for each size category. The size categories, which were arbitrarily chosen, were (1) <20 μm (n = 338 vessels) and (2) between 20 and 60 μm (n = 268 vessels). A 4 × 2 chi-square frequency distribution (with degrees of freedom = 3) produced a value of 1.05; because a chi-square value of >7.82 would be required to achieve significance at the probability level of .05, distribution of vessels within groups was not significantly different. In addition, the number of vessels in each of the 2 size categories (<20 μm and 20-60 μm, respectively) were each analyzed by 1-way analysis of variance (ANOVA), with the animal group as the factor and 29 animals. The average number of vessels that were analyzed was not significantly different between animals groups for vessels of <20 μm (P = .247) or vessels 20-60 μm (P = .065) external diameter.

Percentage of MWT was calculated and compared in each group. Within each vessel size category, mean values that do not share a common letter are significantly different (P < .5) from each other are given as mean ± SEM.

Results
Lung growth
Surgical creation of DH in fetal sheep at 65 days of gestation resulted in a 58% reduction in dry lung-to-body weight ratio (sham group, 4.1 ± 0.5 g/kg; DH group, 1.7 ± 0.1 g/kg; P = .001). The dry lung-to-body weight ratio for the DH/TO (4.4 ± 0.2 g/kg) and DH/TO + glucocorticoid (4.4 ± 0.4 g/kg) groups were higher (P = .001) than the DH group and not statistically different from the sham group (P = .568 and .839, respectively).

Pulmonary arteriole morphometry
The number of arterioles that were analyzed in each group were: sham, 146; DH, 203; DH/TO, 131; DH/TO + glucocorticoid, 126. The average number of vessels (14 ± 2.3) that were obtained per animal was significantly lower in the DH/TO + glucocorticoid group, compared with the sham (24.3 ± 3.0 vessels; P = .017) and DH (29.0 ± 4.2 vessels; P = .001) groups, but not different from the DH/TO group (18.7 ± 1.3 vessels; P = .234). The percentage of MWT in DH animals (29.6% ± 1.9%) was significantly higher than the sham group (18.1% ± 1.3%; P = .010) and not different from the DH/TO group (30.3% ± 1.7%; P = .848; Figure 2). The percentage of MWT for the DH/TO + glucocorticoid group (24.6% ± 1.2%) was significantly lower than the DH/TO (P = .039) and DH (P = .017) groups and remained significantly higher than that of the sham group (P = .025; Figure 2). Representative photomicrographs from an animal from each of the 4 groups are presented in Figure 3.
Percentage of MWT in vessels of different size (<20 μm vs 20-60 μm arterioles)

For pulmonary arterioles of <20 μm external diameter, the average number of vessels analyzed per animal for each group was (1) sham group, 13.0 ± 3.2; (2) DH group, 14.1 ± 1.4; (3) DH/TO group, 11.7 ± 1.6; and (4) DH/TO + glucocorticoid group, 8.8 ± 1.7. For vessels with an external diameter between 20 and 60 μm, the number of vessels that were analyzed for each group was (1) sham group, 11.3 ± 1.5; (2) DH group, 14.9 ± 5.0; (3) DH/TO group, 7.0 ± 1.3; and (4) DH/TO + glucocorticoid group, 5.2 ± 3.7. The repeated measures ANOVA for the percentage of MWT detected significant differences among groups (P = .001), although there was no significant interaction between group and vessel size category (P = .327). Differences among groups were the same as those described for nonparametric analysis: percentage of MWT was higher in DH and DH/TO groups, compared with the sham group, and the percentage for MWT for the DH/TO + glucocorticoid group was lower than that of the DH/TO group and higher than that of the sham group.

Density of small pulmonary arterioles

The average area of lung tissue analyzed in DH/TO (3.1 ± 0.1 cm²) and DH/TO + glucocorticoid (2.9 ± 0.2 cm²) was not different from each other (P = .453) but were both significantly higher than that of the DH group (2.2 ± 0.2 cm²; P = .008 and .032, respectively). Lung area analyzed in the sham group (2.4 ± 0.2 cm²) was not different from the DH (P = .596) or DH/TO + glucocorticoid (P = .122) groups. The density of pulmonary arterioles (ie, number of vessels per square centimeter of lung) was not different between the sham and DH groups (10.7 ± 1.9 vs 13.9 ± 2.0 vessels/cm², respectively; P = .135). Mean values in the DH/TO (6.1 ± 0.52 vessels/cm²) and DH/TO + glucocorticoid (4.8 ± 0.77 vessels/cm²) groups were lower than both the sham (P = .032 and .006, respectively) and DH (P = .001 and .001, respectively) groups.

Comment

The ability of the lung to exchange respiratory gases adequately at birth is largely dependent on its structural development during fetal life. Severe fetal lung growth deficits that are associated with congenital DH translate into serious respiratory illness that is often unresponsive to the most supportive care. As such, their study design did not incorporate a group of non-glucocorticoid-treated DH/TO fetuses; as such, their study design did not incorporate a group of non-glucocorticoid-treated DH/TO fetuses. An intriguing finding in their study was that prolonged TO reduced MWT of small (<60 μm external diameter) pulmonary arterioles. Although the reduction in medial hypertrophy parallels the profound improvement in neonatal gas exchange, we can speculate only that glucocorticoid-induced changes in pulmonary arteriole structure contribute to improved respiratory outcome in glucocorticoid-treated DH/TO lambs.

To our knowledge, this is the first study that has examined the effects of prenatal glucocorticoids on pulmonary arteriole muscle mass in the DH/TO model. A study by Bratu et al measured the percentage of MWT in fetal sheep with DH that underwent prolonged TO and prenatal glucocorticoid treatment 24 hours before birth. However, the purpose of their study was not to evaluate differences between glucocorticoid-treated and nonglucocorticoid-treated DH/TO fetuses; as such, their study design did not incorporate a group of non-glucocorticoid-treated DH/TO fetuses. An intriguing finding in their study was that prolonged TO reduced medial hypertrophy in pulmonary arterioles of <75 μm external diameter but not arterioles between 75 and 500 μm. These data confirm earlier work by Luks et al, who found that fetal lambs that underwent temporary TO between 108 and 122 days of gestation and that were delivered at 136 days of gestation had a decrease in the percentage of MWT, predominantly in smaller (<90 μm) but not larger (>120 μm) arterioles. Fetal TO has also been shown to reduce the percentage of MWT in small pulmonary ar-
pulmonary arteriole muscularization.

In our current study, we did not have access to lung tissues from a group of DH lambs (ie, non-TO) that underwent TO therapy. In stillborn and early neonatal death cases, Heerema et al. found that the percentage of MWT of both small (<50 μm) and larger (50-150 μm) pulmonary arterioles in the DH group (30.4% ± 1.8% and 23.8% ± 1.8%, respectively) was not significantly changed by TO therapy (33.3% ± 1.7% and 21.8% ± 1.6%, respectively). Results from the human TO study should be interpreted carefully, because the duration of TO varied widely (11.25 ± 4.75 days; median, 3.5 days; range, 0-29 days); in 5 of 8 cases, the duration of TO was ≤6 days, which may be an insufficient time to reduce pulmonary arteriole muscularization.

In our current study, we did not have access to lung tissues from a group of DH lambs (ie, non-TO) that underwent glucocorticoid treatment; however, there is some evidence that brief exposure to glucocorticoid shortly before birth may improve pulmonary function. In a study by Suzuki et al, fetal lung hypoplasia was induced by continuous drainage of fetal lung fluid and amniotic fluid between 105 and 140 days of gestation. In their model, the dry lung-to-body weight ratio was reduced by approximately 28%, from 3.7 ± 0.2 g/kg to 2.7 ± 0.1 g/kg. One group of fetuses with lung hypoplasia was exposed to prenatal glucocorticoid by maternal intramuscular injection of betamethasone (0.5 mg/kg maternal body weight) 48 hours before delivery. In the first 2 hours after delivery, pulmonary blood flow was increased, and pulmonary vascular resistance was reduced significantly in lambs with lung hypoplasia that were prenatally treated with glucocorticoid, compared with nonglucocorticoid-treated lambs. In contrast to results from our current study, Suzuki et al did not observe significant differences in the percentage of MWT of pulmonary arterioles (25-75 μm external diameter) between glucocorticoid-treated and nonglucocorticoid-treated lambs with lung hypoplasia. Discrepancies in findings between studies may be explained by differences in (1) experimental models used (ie, surgical DH vs lung liquid drainage), (2) degree of lung hypoplasia at term (the lung-to-birthweight ratio was reduced by 58% in the current study compared with 28%), or (3) severity of medial hypertrophy in animals (percentage of MWT of pulmonary arterioles averaged 30% in our DH group, whereas the mean value in lambs with lung hypoplasia was only 23%). In fetal sheep with severe DH, intravenous infusions of cortisol between 133 and 135 days of gestation increased arterial PO2 (DH group, 20 ± 3 mm Hg; DH-cortisol group, 38 ± 6 mm Hg) and dynamic lung compliance and reduced septal wall-thickness. Kapur et al also reported that lung compliance was increased in lambs with DH that underwent prenatal glucocorticoid therapy, although improvement in gas exchange was not observed. In a report of 3 infants with DH that underwent repetitive prenatal glucocorticoid-therapy, Ford et al concluded that "prolonged steroid administration over 10 weeks of pregnancy may have contributed to ease of ventilation, absence of pulmonary hypertension, and survival." In a randomized trial, prenatal glucocorticoid therapy (n = 17) did not improve neonatal survival, length of stay, ventilator days, or oxygen use at 30 days, compared with placebo-treated infants (n = 15); lung compliance was not assessed in the study.

Medial hypertrophy of pulmonary arterioles in infants with DH is thought to increase pulmonary vascular resistance abnormally in response to vasoconstrictor stimuli. It is tempting therefore to speculate that the improved gas exchange in our glucocorticoid-treated DH/TO lambs was contributed, in part, by the observed reduction in the percentage of MWT. However, prenatal exposure to glucocorticoid can also modulate molecular mediators of pulmonary vascular tone, which would promote vasodilation and enhance gas exchange. In normally grown fetal sheep, repetitive glucocorticoid leads to the up-regulation of endothelial nitric oxide synthase. In another sheep study, prenatal glucocorticoid treatment enhanced pulmonary vasodilation in response to endogenous catecholamines. In fetal rats with DH, prenatal glucocorticoid ameliorated the increase in angiotensin-converting enzyme activity, which may reduce the risk of pulmonary hypertension. In previous work, we have shown that prenatal glucocorticoid treatment can restore surfactant protein expression partially in lambs with DH that undergo prolonged TO. The ability of prenatal glucocorticoid to correct the endogenous surfactant deficiency that was incurred by prolonged TO may also enhance neonatal gas exchange.

Taken together, data from this and our previous studies demonstrate the beneficial effects of a single dose of prenatal glucocorticoids, administered shortly before birth, in fetuses that undergo prolonged TO therapy for severe congenital DH. Our results should be interpreted carefully, and the limitations of our study should be realized if a combined TO/glucocorticoid strategy is considered for clinical application. Results from our current study were obtained from near-term fetal sheep. Fetal surgery in humans, including minimally invasive fetoscopic strategies, is associated with an increased risk of preterm labor. It is unknown whether the reduction in medial hypertrophy of small pulmonary arterioles that was observed in near-term animals would occur in the immature fetus. Our analysis focused on structure of small (<60 μm) muscularized pulmonary arterioles in the right contralateral lung (ie, the lung with less severe hypoplasia in left-sided DH). We were unable to examine the left ipsilateral lung because these tissues were used exclu-
sively for biochemical analysis and measurements of pulmonary wet and dry weights. As already stated, measurements of the percentage of MWT in larger arterioles/arterioles could not be performed because of compression/distortion of vessels caused by fixation of the lungs through the trachea. Another limitation of the current study was that we were unable to identify partially and nonmuscularized arterioles, which would allow us to profile the distribution of these vessels with fully muscularized arterioles in the different animal groups. In the current study, we found that the density of muscularized arterioles was decreased in the DH/TO and DH/TO + glucocorticoid groups, compared with the sham group. Although this result may suggest abnormal vascularization of lung parenchyma, it is possible that the density of partially and/or nonmuscularized vessels was increased and that regional blood flow is normal in these animals. A more comprehensive study, in which the lungs are fixed through the vasculature, is required to overcome the limitations of our current study.

The effects of prenatal glucocorticoid treatment on neonatal gas exchange in infants who have undergone TO therapy have not been examined in a randomized study (ie, DH/TO vs DH/TO + glucocorticoid). Keller et al compared pulmonary function of untreated (ie, non-TO) infants with DH with a group of infants with DH who underwent prolonged TO and received antenatal glucocorticoids. Although oxygenation did not differ between groups, alveolar-arterial oxygenation difference was lower in the TO/glucocorticoid group, and respiratory system compliance within the first week of life (24 hours before congenital DH repair) was higher (0.28 vs 0.17 mL × cm H2O⁻¹ kg⁻¹) than that of infants in the DH group. Without a nonglucocorticoid-treated DH/TO group, it is impossible to determine whether improvement in alveolar-arterial oxygenation difference and compliance was the direct result of prenatal glucocorticoid treatment.

In fetal sheep that undergo TO therapy for DH, a single dose of prenatal glucocorticoid that was administered 48 hours before birth reduced muscular hypertrophy of small (<60 μm) pulmonary arterioles. We speculate that such structural changes may improve pulmonary blood flow at birth and potentially decrease hyperreactivity to vasoconstrictor stimuli.

REFERENCES

9. Geggel RL, Murphy JD, Langleben D, Crone F, Alcorn D, Adamson TM, Lambert TF, Ma-
A cost decision analysis of 4 tocolytic drugs

Edward Hayes, MD, MSCP; Leslie Moroz, BS; Laura Pizzi, PharmD, MPH; Jason Baxter, MD, MSCP

OBJECTIVE: The purpose of this study was to determine the optimal tocolytic agent, based on a cost decision analysis.

STUDY DESIGN: A PubMed search of commonly used tocolytics was performed to determine the probability of adverse events. Cost for an agent was determined by acquisition cost and the probability and cost of adverse events. A decision tree was constructed to determine which tocolytic had the lowest total costs, with subsequent sensitivity analysis.

RESULTS: A total of 19 clinical trials combined for a cohort of 1073 patients (indomethacin, 176 patients; magnesium sulfate, 451 patients; nifedipine, 176 patients; and terbutaline, 270 patients). The probability of adverse events was 57.9% for terbutaline, 22.0% for magnesium sulfate, 27.2% for nifedipine, and 11.4% for indomethacin. Nifedipine ($16.75) and indomethacin ($15.40) were the least expensive treatment options, compared with magnesium sulfate ($197.90) and terbutaline ($399.02) because of the cost of monitoring and treating adverse events.

CONCLUSION: If one elects a tocolytic, both nifedipine and indomethacin should be the agents of choice, based on a cost decision analysis.

Key words: indomethacin, magnesium sulfate, nifedipine, preterm labor, tocolytic


Physicians have treated premature labor with the goal of decreasing neonatal morbidity and death. Despite a variety of interventions that range from bed rest to tocolytics, the rate of preterm delivery has continued to increase. The United States rate rose 2% in 2004 to 12.5%, which corresponds to an overall increase of 18% since 1990.1 Physicians in the United States have been trained to use tocolytics to stop preterm labor to prolong pregnancy. Greater than 98.1% of obstetric care providers surveyed at a recent meeting in the United States reported that they would use tocolytics in a patient in preterm labor at <32 weeks of gestation, despite the questionable efficacy of tocolytics.2 In this survey, magnesium sulfate (MgSO4) was chosen as the first-line agent by 69% of respondents, followed by terbutaline (13%), nifedipine (11%), and indomethacin (6%).2 This strong predilection to choose magnesium sulfate is remarkable because no tocolytic has been shown definitively to have superior effectiveness in sufficiently prolonging gestation and improving neonatal outcome.3-6 Based on a lack of superior effectiveness, the American College of Obstetricians and Gynecologists (ACOG) stated that “there is no clear first-line tocolytic drug”7 for the treatment of preterm labor. ACOG acknowledges the limitations of these agents in prolonging gestation and endorses their use for 48 hours to provide for the maternal administration of antenatal corticosteroids.7 More than 81,000 pregnant women in the United States were subjected to tocolytics in 2004.4 Because there are several agents available to the practitioner, all with comparable efficacy, if one elects to use a tocolytic for the treatment of preterm labor, the choice of agent should be based on the occurrence of adverse events, ease of administration, and cost. Our goal was to determine, through a decision analysis, which of the 4 tocolytics that are used most commonly in the United States over the past decade (MgSO4, terbutaline, indomethacin, or nifedipine) should be considered the agent of choice, based on the risk and costs of adverse events.

Materials and Methods
Inclusion and review process
To determine the incidence of side effects, a systematic literature search of the 4 most commonly used tocolytics was performed. The query was conducted in PubMed using the terms tocolytics and preterm labor, restricted to (1) English language, (2) human, (3) published in core clinical journals, and (4) having a clinical trial design. Two independent reviewers examined each article to see whether they met inclusion criteria: (1) the study was a randomized prospective clinical trial that examined the treatment of preterm labor; (2) the tocolytic dose was within accepted practice guidelines7; (3) the subjects were not receiving tocolytics at or before enrollment; (4) the tocolytic of interest was used as a monotherapy, and (5) the adverse event rates were reported. For trials that met these inclusion criteria, the total number of patients who were exposed to the tocolytic in each study was recorded.

Probability of adverse events
The maternal adverse events that were included were those that are clinically severe and demonstrated to result in mea-
### TABLE 1

#### Probability of adverse events, based on clinical trial data (n = 19 studies)

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<th>Tocolytic agent</th>
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<th>Patients (n)</th>
<th>Chest pain (n)</th>
<th>Palpitations (n)</th>
<th>Arrhythmia (n)</th>
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<td>I</td>
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<tr>
<td></td>
<td>Chau et al&lt;sup&gt;16&lt;/sup&gt;</td>
<td>52</td>
<td>0</td>
<td>3 (6%)</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td>European Atosiban Study Group&lt;sup&gt;28&lt;/sup&gt;</td>
<td>129</td>
<td>3 (2%)</td>
<td>12 (9%)</td>
<td>0</td>
<td>10 (8%)</td>
<td>22 (17%)</td>
<td>40 (31%)</td>
<td>I</td>
<td>I</td>
</tr>
<tr>
<td></td>
<td><strong>Probability of adverse event (range)</strong>&lt;sup&gt;14&lt;/sup&gt;</td>
<td><strong>270</strong></td>
<td>7.4% (0-28%)</td>
<td>6.7% (0-9%)</td>
<td>0.8% (0-3%)</td>
<td>8.8% (0-14%)</td>
<td>10.0% (0-17%)</td>
<td>25.0% (0-31%)</td>
<td>I</td>
<td>I</td>
</tr>
</tbody>
</table>

* Studies were reviewed and evaluated for quality, according to the method outlined by the US Preventive Services Task Force.

† Probability of adverse events refers to the percentage of patients who experience the event in the combined study cohort.

‡ Range refers to the percentage range in the individual studies.
Cost calculations

Costs included in the model were (1) drug acquisition costs, (2) laboratory and diagnostic tests required in the monitoring of patients who received each agent, and (3) diagnostic procedures and treatments that were required to evaluate and treat each type of adverse event. Although the discontinuation of agents was reported commonly in the studies, discontinuation was excluded from the model because it had not been documented to result in quantifiable healthcare costs.

The time horizon used in the model was the 48 hours after the diagnosis of preterm labor, based on the premise that this would allow for the administration of corticosteroids; thus, there would be no significant difference in baseline cost per occupied bed on a labor and delivery unit. The specific treatment regimen (dosage and route of administration) and monitoring procedures for each tocolytic were based on recommendations in standard obstetrics texts, which reflect common practice patterns in the United States.

The analysis was performed from the hospital perspective. Cost attributable to each type of adverse event was calculated on the basis of diagnostic and treatment practices in the hospital setting that was based on the clinical trials cohort. The cost of laboratory tests and procedures that were required to monitor for and treat each type of adverse event was based on the median Medicare reimbursement levels for 2005. Drug acquisition costs were obtained from the pharmacy department at a tertiary academic medical center.

A decision tree that compared the 4 tocolytic agents of interest was constructed with TreeAge Pro, 2006 Health-care software (TreeAge Software, Inc, Williamstown, MA). A “do-nothing” arm was not included because the model was formulated with the assumption that a physician was electing to choose a tocolytic. The tree was then populated with the probability of experiencing each type of adverse event for each drug and the costs that are associated with each treatment arm and for each type of adverse event. A rollback analysis, which calculated the expected value at each chance node by the combination of branches arising from that node from right to left, was performed to determine which agent was dominant. A sensitivity analysis was completed subsequently to test the robustness of findings. The sensitivity analysis was designed to determine whether the agent of choice (eg, dominant) would change with the use of the lowest rate of each type of adverse events that were reported (in any individual study of each dominated agent) along with the

<table>
<thead>
<tr>
<th>Agent</th>
<th>Administration protocol*</th>
<th>Cost (range)†‡§</th>
</tr>
</thead>
<tbody>
<tr>
<td>Terbutaline</td>
<td>0.25 mg Subcutaneously for 3 doses then 0.25 every 4 hours for 48 hours</td>
<td>$6.30</td>
</tr>
<tr>
<td></td>
<td>Baseline electrocardiogram</td>
<td>$70.59 ($70.59-91.18)</td>
</tr>
<tr>
<td></td>
<td>Foley catheter placement</td>
<td>$116.05 ($116.05-158.60)</td>
</tr>
<tr>
<td></td>
<td>Potassium every 6 hours for 24 hours</td>
<td>$81.12 ($81.12-113.40)</td>
</tr>
<tr>
<td></td>
<td>Glucose every 6 hours for 24 hours</td>
<td>$86.88 ($86.88-115.92)</td>
</tr>
<tr>
<td></td>
<td>Total administration cost for terbutaline</td>
<td>$420.08 ($420.08-544.54)</td>
</tr>
<tr>
<td>Magnesium sulfate</td>
<td>Loading dose of 6 grams then 2 grams per hour for 48 hours</td>
<td>$8.19</td>
</tr>
<tr>
<td></td>
<td>Foley catheter placement</td>
<td>$116.05 ($116.05-158.60)</td>
</tr>
<tr>
<td></td>
<td>Magnesium level</td>
<td>$21.84 ($21.84-30.53)</td>
</tr>
<tr>
<td></td>
<td>Total administration cost for magnesium sulfate</td>
<td>$146.08 ($146.08-197.32)</td>
</tr>
<tr>
<td>Indomethacin</td>
<td>50 Mg every 6 hours for 48 hours</td>
<td>$11.10</td>
</tr>
<tr>
<td></td>
<td>Total administration cost for indomethacin</td>
<td>$11.10</td>
</tr>
<tr>
<td>Nifedipine</td>
<td>30 Mg load then 20 mg every 6 hours for 48 hours</td>
<td>$6.96</td>
</tr>
<tr>
<td></td>
<td>Total administration cost for nifedipine</td>
<td>$6.96</td>
</tr>
</tbody>
</table>

* Monitoring requirements during the first 48 hours of treatment based on data from references 8 and 9.
† Drug costs valued with the use of hospital acquisition costs.
‡ Administration costs valued at the median fee for the United States in 2005.
§ Fee range is the median to the 75th percentile national fee in 2005.
highest reported rates of each adverse event for the dominant agent. Furthermore, the costs of monitoring procedures for each adverse event caused by the dominated agents was valued increased to the 75th percentile of 2005 Medicare reimbursement, while holding the costs of treating events from the other agents constant.

**RESULTS**

The literature search yielded 127 studies, of which 19 trials met inclusion criteria. The total number of patients who were exposed to the tocolytics across all trials was 1073 (176 patients in the indomethacin cohort, 451 patients in the MgSO4 cohort, 176 patients in the nifedipine cohort, and 270 patients in the terbutaline cohort). The probability of experiencing each type of adverse event for each agent and the range of adverse events rates that were reported in the individual studies is shown in Table 1.

The monitoring requirements for each tocolytic and associated costs are reported in Table 2. These costs were greatest for terbutaline ($354.64), and lowest for nifedipine ($6.96). Costs that were attributable to the evaluation and treatment of each adverse event are described in Table 3. Because there were no deaths or myocardial ischemic events reported in any study, these events were excluded from the model.

The resulting decision tree is shown in the Figure. Rollback analysis revealed the total cost of the terbutaline arm to be $399.02 per patient, MgSO4 to be $197.90 per patient, indomethacin to be $15.40 per patient, and nifedipine to be $16.75 per patient. Indomethacin was determined to be dominant, so the sensitivity analysis was performed to evaluate the impact if the highest reported event rates for this drug that was priced at the 75th percentile were used in the model along with the lowest reported event rates for nifedipine, MgSO4, and terbutaline were left at the median fee. The sensitivity analysis revealed that nifedipine ($6.36) became the dominant agent over indomethacin ($33.59), which suggests equivalency, but each was superior to terbutaline ($354.64) and MgSO4 ($209.61).

**COMMENT**

By conducting a decision analysis to account for the overall costs of administering established tocolytics, we were able to determine that nifedipine and indomethacin are the least expensive agents currently used in the United States. Differences in cost were not driven by drug acquisition costs, rather by the monitoring requirements for each option. For example, in the case of terbutaline, the added costs of cardiac evaluation, electrolyte monitoring, and strict fluid status by means of a Foley catheter are required because of the potential for maternal adverse events that are associated with β-mimetics (myocardial ischemia, arrhythmia, hypokalemia, hyperglycemia, and pulmonary edema). MgSO4 requires close monitoring of fluid status (given the risk of pulmonary edema) and periodic magnesium levels (because of its affect on muscular function). Indomethacin and nifedipine, which are associated with a good maternal safety profile, do not require costly monitoring, which results in significant savings.

A common critique of decision analytic models is that they are based on assumptions and therefore do not reflect real-world situations. To construct a model that was consistent with clinical practice, established protocols served as...
the basis for our assumptions regarding the administration and monitoring requirements for each tocolytic and the management of adverse events. We also strived to achieve real-world applicability by basing adverse event probabilities on a sizeable cohort of prospective trials. The authors purposely avoided performing a metaanalysis on adverse events because of the tendency to eliminate rare but serious (and costly) adverse events. To determine the robustness of our findings, a sensitivity analysis was performed to better gauge the impact of modifying assumptions over a range of costs and adverse event rates.

The strongest opposition to this model will come from those who argue that 1 agent has superior efficacy in prolonging gestation, compared with the others. If this were true, the superior agent would improve neonatal outcome corresponding to considerable reduction in hospital costs. However, we assumed equal effectiveness in our model because this is a view that is endorsed by ACOG and supported by the Cochrane Library of systematic reviews. The latter reported that (1) there is not enough data to show any difference between magnesium, placebo, or alternative therapies, (2) there is insufficient information on COX inhibitors (of which indomethacin was the agent in 10 of 13 studies that were analyzed) to determine superiority, and (3) calcium channel blockers (of which nifedipine is a member) appear to be at least as good as betamimetics, which affirms noninferiority.

Another area of critique for this model may be the limited size of each agent’s cohort, which may be viewed as a source of selection bias. This was primarily due to the strict limitations on study inclusion that resulted in a large number of studies being eliminated. For example, in the case of terbutaline, most studies in the literature examined maintenance tocolysis with this agent, which is no longer considered a valid indication. Of the 7 studies that examined acute tocolysis with terbutaline, only 4 studies met inclusion criteria (3 studies involved the use of the medication intravenously and 1 study involved the use of it subcutaneously). Because of the small number of studies, the authors needed to combine the 2 dosing forms (both methods are considered valid to treat preterm labor) to obtain adequate sample size to estimate the incidence of adverse events.

Another limitation that may result from the small numbers in the studies to date on the commonly used tocolytics is that the model may not capture rare, but cost-generating adverse events, both maternal and fetal. In the past, significant adverse events with the continuous subcutaneous infusion of terbutaline caused the Food and Drug Administration to issue a warning about this regimen. In the case of nifedipine, given the limited experience with this as an acute tocolytic agent, some of the rare complications that might be associated with its use may not have been described yet or their frequency appreciated. With greater use and experience, these less frequent complications may become better known, and the result may be increased intrapartum monitoring, which may drive up
costs. The size of the cohort and few studies that examined neonatal adverse events inhibited this outcome from being differentiated in the model between agents and therefore was excluded in attributable costs. Appreciating these limitations, we still believe that the model adds a substantial contribution to the literature in the field of obstetrics.

If our conclusions are accepted as valid and bring about change in US practice, the impact of the use of nifedipine and indomethacin as the initial agents would be significant. We estimate that $14.2 million could be saved in hospital costs per year, based on the number of women who received tocolytics in 2004 (81,391 women).1 This estimate was determined by converting the approximately 69% of women who received MgSO4 and 13% of patients, as the initial agents would bring about change in US practice, the impact of the use of nifedipine and indomethacin as the initial agents would be significant. We estimate that $14.2 million could be saved in hospital costs per year, based on the number of women who received tocolytics in 2004 (81,391 women).1 This estimate was determined by converting the approximately 69% of women who received MgSO4 and 13% of women who received terbutaline first2 to nifedipine or indomethacin. Corresponding morbidity decreases would be 730 fewer cases of tocolytic-induced pulmonary edema, and 85 fewer cases of tocolytic-induced arrhythmias per year.

In conclusion, this decision analysis revealed that nifedipine and indomethacin are superior to other common tocolytics, based on safety and cost. This finding is of clinical importance because previous research has shown that nifedipine and indomethacin are not popular alternatives that are "at least as good"5 in prolonging pregnancy. It is our hope that these results will serve to inform hospital decision-makers when they develop clinical treatment guidelines on tocolysis and will assist clinicians in understanding the costs and risks that are associated with tocolytics.

REFERENCES

Associations between 2 polymorphisms in the methylenetetrahydrofolate reductase gene and placental abruption

Cande V. Ananth, PhD, MPH; Morgan R. Peltier, PhD; Celeste De Marco, RN, BSN; Denise A. Elsasser, MPH; Darios Getahun, MD, MPH; Rima Rozen, PhD; John C. Smulian, MD, MPH; for the New Jersey–Placental Abruption Study Investigators

OBJECTIVE: Heritable thrombophilias have been implicated as a potential cause of abruption by vascular disruption at the uteroplacental interface. Polymorphisms of the methylenetetrahydrofolate reductase (MTHFR) gene have been linked to vascular complications outside of pregnancy, which includes stroke. Given the underlying thrombotic nature of abruption, we hypothesized that polymorphisms in the MTHFR gene are associated with abruption.

STUDY DESIGN: We examined 2 variants in MTHFR: 677C→T and 1298A→C in genomic DNA extracted from maternal blood from the New Jersey–Placental Abruption Study, an ongoing, multicenter case-controlled study. We identified 195 women with a clinical diagnosis of abruption (cases) and 189 control subjects who were matched on race/ethnicity and parity. We assessed allele and genotype frequencies and their associations with abruption risk after adjusting for confounders through multivariable logistic regression analysis.

RESULTS: The wild-type allele (C) frequency of the 677C→T variant of MTHFR among cases and control subjects was 69.0% and 64.3%, respectively; the wild-type allele (A) of the 1298A→C variant was 75.9% and 79.4%, respectively. Distributions of the 677C→T alleles among control subjects violated the Hardy–Weinberg equilibrium (P = .007); distributions of the 1298A→C alleles were in equilibrium (P = .825). In comparison to the wild-type genotype (C/C), the homozygous mutant form (T/T) of 677C→T was not associated with abruption (odds ratio, 0.60; 95% confidence interval [CI], 0.33-1.18). Similarly, the homozygous mutant form (C/C) of the 1298A→C polymorphism was distributed equally between cases and control subjects (odds ratio, 2.28; 95% CI, 0.82-6.35). Plasma homocysteine and vitamin B12, but not folate, concentrations were elevated in cases compared with control subjects among women with the wild-type genotype of MTHFR 677C→T (P = .039 for homocysteine; P = .048 for B12; P = .224 for folate).

CONCLUSION: In this population, neither heterozygosity nor homozygosity for the 677C→T and 1298A→C variants in MTHFR was associated with placental abruption.

Key words: case-control, DNA, linkage disequilibrium, MTHFR, placental abruption


Placental abruption is a serious obstetric complication that occurs in approximately 1 in 100 pregnancies.1-3 Although its occurrence is relatively uncommon, it is a major cause of third-trimester bleeding and accounts for a disproportionately high rate of preterm birth, low birthweight, stillbirth, and infant death.4-8 The cause of abruption is poorly understood, but epidemiologic studies have observed advanced maternal age, multiparity, smoking, crack and cocaine use, intraamniotic infections, prolonged rupture of membranes, chronic hypertension, preeclampsia, and folate deficiency to be associated with increased risk.8-18 The strongest risk factor is placental abruption in a previous pregnancy.1,2,19-21

Recent studies have suggested a genetic predisposition to placental abruption.19-21 5,10-methylenetetrahydrofolate reductase (MTHFR), an important metabolic enzyme, is required in the conversion of homocysteine to methionine. A mutation in the MTHFR gene is associated arguably with thrombotic events. Homozygosity for the cytosine-to-thymine substitution at nucleotide 677 (677C→T) and for the adenine-to-cytosine substitution at nucleotide 1298...
(1298A→C) in the MTHFR gene has been suggested to be associated with increased risk for abruption. The results from these studies are, however, inconclusive. Although some studies have reported the presence of the mutant genotype of the 677C→T polymorphism to be associated with increased risk for abruption, other studies have not. Association between the 1298A→C variant and abruption risk is less well-examined. Whether a gene–gene interaction in the MTHFR gene on the risk of placental abruption exists also remains uncertain. Given the underlying thrombotic nature of abruption, we examined the association between MTHFR polymorphisms (677C→T and 1298A→C) and the risk of placental abruption.

**Materials and Methods**

**The New Jersey–Placental Abruption Study**

Data for this study were obtained from an ongoing case-control study that was conducted in Robert Wood Johnson University Hospital, New Brunswick, NJ (since July 2003) and Saint Peter’s University Hospital, New Brunswick NJ (since August 2002). Both hospitals serve as large tertiary, level III centers (located within a mile of each other), with a total of approximately 8000 deliveries annually. The ethics review committee of the institutional review boards of both institutions approved this investigation. Further details of the New Jersey–Placental Abruption study have been described in detail elsewhere.

**Placental abruption cases and control subjects**

Placental abruption cases that were eligible for inclusion included women with a clinical diagnosis of abruption before or during delivery by the attending obstetrician. The definition of placental abruption included the classic signs and symptoms of painful vaginal bleeding or hemorrhage that was accompanied by documented fetal distress, uterine pain or tenderness, or uterine hypertonicity. In the absence of these clinical hallmarks for abruption, if the delivered placenta showed visual signs of retroplacental bleeding or retroplacental clot/hematoma on the placental surface, then such patients were eligible for inclusion as potential cases. In addition, if an abruption was diagnosed visually on sonographic examination during routine prenatal care, such cases were considered for inclusion as abruption cases. Women with an abruption were identified by a review of the daily hospital delivery logs at both hospitals and/or by referral by the physician, nurse, or obstetrics and gynecology residents. Medical and obstetric labor and delivery charts were reviewed carefully for all abruption cases for confirmation before enrollment. The criteria for eligibility included patients with an abruption that delivered at ≥20 weeks’ gestation and those patients who provided consent to participate in the study.

Control subjects were comprised of women that did not have a placental abruption, who were identified from daily delivery logs in both hospitals. Control subjects were matched to cases on parity during pregnancy (yes/no), prenatal care (nulliparous, primiparous, parity 2, or parity ≥3) and maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other race/ethnicity). After the recruitment of a case, a control patient was sought for recruitment from the same hospital as the abruption cases. From the potential pool of eligible control subjects, we excluded women with a diagnosis of placental previa or stillbirth in the current pregnancy or with history of placental abruption in any of their previous pregnancies.

**Polymorphisms in the 5,10-methylenetetrahydrofolate reductase gene**

The 677C→T variant of MTHFR occurs in exon 4 and results in an alanine-to-valine substitution at codon 222. A second MTHFR polymorphism, 1298A→C in exon 7, results in a glutamate-to-alanine substitution at codon 429. Both these polymorphisms in the MTHFR gene have been linked with reduced enzyme activity. The 677C→T mutation is also associated with altered distribution of intracellular folate metabolites.

Genomic DNA was extracted from maternal peripheral blood, precipitated with ethanol, washed, dissolved in a Tris-EDTA buffer, and stored at -20°C. The extracted DNA was then assayed for the 2 mutations in the MTHFR gene with the use of the polymerase chain reaction for DNA amplification and restriction digestion of polymerase chain reaction products with *Hinfi* for the 677C→T and *MwoI* 1298A→C, as previously reported.

**Biochemical assays**

For the total plasma homocysteine, folate, and vitamin B12 assays, 1.0 mL of blood was drawn into EDTA tubes and transported on dry ice to the laboratory for assays. The plasma was then separated and stored in Eppendorf tubes and stored at -70°C. These specimens were processed for nonfasting homocysteine and vitamin metabolism with the Abbott IMX technology (Abbott Laboratories, Abbott Park, IL), which is based on a fluorescence polarizing immunoassay technique. Plasma folate and vitamin B12 were determined with the Abbott Diagnostic IMX (Abbott Laboratories), which is based on a microparticle enzyme immunoassays, according to the manufacturer’s protocols, and the coefficients of variation for these assays were <4%.

**Statistical analysis**

We examined the distributions of placental abruption cases and control subjects in relation to study center, year recruited, maternal age (<19, 19-34, and ≥35 years), maternal education (<12, 12, 13-16, and ≥17 completed years of schooling), prepregnancy body mass index, smoking and alcohol use before and during pregnancy (yes/no), prenatal care (or any care), the matching factors of parity (nulliparous, primiparous, parity 2, and parity ≥3), and maternal race/ethnicity (non-Hispanic white, non-Hispanic black, Hispanic, or other race/ethnicity). Body mass index was calculated as the ratio of weight (in kilograms) over squared-height (in meters).
We tested differences in these factors between cases and control subjects on the basis of either the \( t \) test (for continuous variables) or the Fisher exact or \( \chi^2 \) test (for categoric variables).

We derived allele and genotype frequencies of the 677C→T and 1298A→C variants in MTHFR with bootstrap generated 95% confidence intervals (CIs). The bootstrap estimates were based on 10,000 replications. In addition, we evaluated whether the observed allele frequencies for the 2 MTHFR polymorphisms were in Hardy–Weinberg equilibrium. Linkage disequilibrium was assessed for the cosegregation of the 677C→T and 1298A→C variants.

The association between the 2 MTHFR polymorphisms and abruption was based on the unadjusted odds ratio with 95% CIs. This was a matched case control study by design; all preliminary analyses were based on a matched analysis. However, because the result of these matched analyses (not shown) did not differ from analyses that were based on an unmatched analysis, we report only the results of the unmatched analysis.

We adjusted the associations between the MTHFR polymorphisms and abruption for several confounders through a multivariable logistic regression analysis. We adjusted the analyses for study site, year recruited, parity, maternal race/ethnicity, maternal age, smoking and alcohol use during pregnancy, and prepregnancy body mass index. In addition, we examined gene–gene interactions in the 677C→T and 1298A→C mutations in MTHFR and risk of abruption. Finally, the entire analysis was replicated after stratification of the data on maternal race/ethnicity.

Differences in total plasma homocysteine, folate, and vitamin \( B_{12} \) distributions were examined between cases and control subjects. For this analysis, we applied the Box-Cox transformation to the analytes to ensure that the variance stabilization and normality assumptions of the analysis of variance methods were met. General linear models were fitted to the posttransformed analyte data to examine differences between abruption cases and control subjects and within genotypes of the 2 MTHFR polymorphisms.

This case control study was designed primarily to detect a 2-fold increased frequency of the homozygous mutant genotype (T/T) of the 677C→T polymorphism. We based this on an assumed genotype frequency of 6% among control subjects and 12% among abruption cases and a type II error rate of 10% (1 - \( \beta \) = 90%). The sample size that was required to detect an association between the MTHFR 677C→T polymorphism and abruption, with an odds ratio of 2.0, were 180 cases and 180 control subjects.

**RESULTS**

A total of 195 abruption cases and 189 control subjects had complete MTHFR analysis for both the 677C→T and 1298A→C variants. As previously reported, distributions of maternal race/ethnicity and parity were similar between cases and control subjects, and abruption cases were more likely to be less educated and to be smokers.

Approximately two-thirds of cases and control subjects carried the wild-type allele of the 677C→T polymorphism (Table 1). In comparison with the wild-type genotype, the frequencies of the heterozygous \( C/T \) and homozygous \( T/T \) mutant genotypes of 677C→T were distributed relatively equally between abruption cases and control subjects. Distributions of the 677C→T alleles violated the Hardy–Weinberg equilibrium both in cases (\( P = .019 \)) and control subjects (\( P = .007 \)). As with the 677C→T mutation, the 1298A→C variant of the MTHFR was also not associated with an overall increased abruption risk, and adjustments for confounders had little effect on these associations. Distribution of the 1298A→C alleles were in equilibrium in both cases (\( P = .078 \)) and control subjects (\( P = .825 \)).

We examined the joint effects of the 677C→T and 1298A→C variants on the risk of placental abruption (Table 2). Compound heterozygosity for the 677C→T and 1298A→C variants of MTHFR \( (C/T-A/C) \) was not associated with increased risk of abruption.

We compared the distributions of plasma total homocysteine, folate, and vitamin \( B_{12} \) between abruption cases and control subjects within the different genotypes of the MTHFR mutations (Table 3). Among the wild-type 677C→T polymorphism \( (C/C) \), mean homocysteine and vitamin \( B_{12} \) concentrations were higher among abruption cases than control subjects (\( P = .039 \) and .048, respectively). Among women carrying the homozygous mutant genotype of the 1298A→C polymorphism \( (C/C) \), mean folate levels were lower among abruption cases than control subjects (\( P = .046 \)).

The associations between MTHFR \( (677C→T \text{ and } 1298A→C) \) and placental abruption that were stratified on maternal race/ethnicity did not reveal any significant associations (data not shown).

**COMMENT**

MTHFR, an important metabolic enzyme, is required in the conversion of homocysteine to methionine. Although the association between an increase in homocysteine level and venous thrombotic events remains equivocal, if there were indeed a thrombotic tendency, then it might be expected to affect placental function. We examined the associations between 2 variants of MTHFR, 677C→T and 1298A→C, in relation to abruption. We found no association between the 2 variants of MTHFR and abruption, nor was there any evidence of a joint association between the 2 MTHFR polymorphisms and abruption risk.

The association between increased risk of placental abruption and variant forms of MTHFR remains unclear. In a study by Kupferminc et al., the authors found no association between MTHFR 677C→T and abruption, with subsequent studies corroborating these findings. A metaanalysis reported an increased risk for abruption among women carrying the 677C→T polymorphism of MTHFR (pooled odds ratio, 2.3; 95% CI, 1.1-4.9). However, a study of (black) South African Zulu women reported that the individual 677C→T variant of MTHFR was not associated with increased risk of abruption but that...
### TABLE 1
Allele and genotype frequencies of MTHFR 677C→T and 1298A→C mutations and associations with placental abruption: the New Jersey-Placental Abruption study

<table>
<thead>
<tr>
<th>MTHFR allele and genotypes</th>
<th>Abrupt cases (n = 195)</th>
<th>Control subjects (n = 189)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>%</td>
<td>Unadjusted</td>
</tr>
<tr>
<td><strong>677C→T</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>C</td>
<td>269</td>
<td>69.0</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>T</td>
<td>121</td>
<td>31.0</td>
<td>0.77 (0.44-1.33)</td>
</tr>
<tr>
<td>Genotype frequency</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>C/C</td>
<td>100</td>
<td>51.3</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>C/T</td>
<td>69</td>
<td>35.4</td>
<td>0.87 (0.56-1.35)</td>
</tr>
<tr>
<td>T/T</td>
<td>26</td>
<td>13.3</td>
<td>0.69 (0.38-1.23)</td>
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<tr>
<td><strong>1298A→C</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Allele frequency</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>296</td>
<td>75.9</td>
<td>1.00 (Reference)</td>
</tr>
<tr>
<td>C</td>
<td>94</td>
<td>24.1</td>
<td>2.32 (0.93-5.78)</td>
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<tr>
<td>Genotype frequency</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>A/A</td>
<td>117</td>
<td>60.0</td>
<td>1.00 (Reference)</td>
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<tr>
<td>A/C</td>
<td>62</td>
<td>31.8</td>
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<tr>
<td>C/C</td>
<td>16</td>
<td>8.2</td>
<td>2.31 (0.92-5.81)</td>
</tr>
</tbody>
</table>

Probability values for the test of Hardy–Weinberg equilibrium among cases and control subjects were .019 and .007, respectively, for the 677C→T and .078 and .825, respectively, for the 1298A→C variants of the MTHFR gene.

* Adjusted for study site, year recruited, maternal race/ethnicity, parity, maternal age, education, prenatal care, pregnancy body mass index, and smoking during pregnancy.

### TABLE 2
Interaction between MTHFR 677C→T and 1298A→C genotypes and the risk of placental abruption: the New Jersey-Placental Abruption study

<table>
<thead>
<tr>
<th>MTHFR 677C→T</th>
<th>MTHFR 1298A→C</th>
<th>Placental abruption (n)</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Cases (n = 195)</td>
<td>Control subjects (n = 189)</td>
</tr>
<tr>
<td>C/C</td>
<td>A/A</td>
<td>45 (23.1%)</td>
<td>39 (20.6%)</td>
</tr>
<tr>
<td>C/C</td>
<td>A/C</td>
<td>39 (20.0%)</td>
<td>41 (21.7%)</td>
</tr>
<tr>
<td>C/C</td>
<td>C/C</td>
<td>16 (8.2%)</td>
<td>7 (3.7%)</td>
</tr>
<tr>
<td>C/T</td>
<td>A/A</td>
<td>46 (23.6%)</td>
<td>46 (24.3%)</td>
</tr>
<tr>
<td>C/T</td>
<td>A/C</td>
<td>23 (11.8%)</td>
<td>23 (12.2%)</td>
</tr>
<tr>
<td>C/T</td>
<td>C/C</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>T/T</td>
<td>A/A</td>
<td>26 (13.3%)</td>
<td>33 (17.5%)</td>
</tr>
<tr>
<td>T/T</td>
<td>A/C</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>T/T</td>
<td>C/C</td>
<td>0 (0.0%)</td>
<td>0 (0.0%)</td>
</tr>
</tbody>
</table>

* Adjusted for study site, year recruited to study, maternal race/ethnicity, parity, maternal age, education, prenatal care, prepregnancy body mass index, and smoking during pregnancy.
combined heterozygosity for 2 MTHFR mutations (677C→T and 1298A→C) was present in 22% and 3.5% of abruption cases and control subjects, respectively (odds ratio, 5.2; 95% CI, 1.1-24.5). These authors demonstrated that increased risk of abruption was driven largely by an association with the MTHFR 1298A→C variant (odds ratio, 3.2; 95% CI, 1.0-10.4) and speculated that this variant may serve as a susceptibility factor that can be triggered in the presence of the homozygous mutant form of the 677C→T polymorphism.

Our results are at variance with these findings. In fact, a subanalysis that was restricted to black women did not reveal such an association. Furthermore, none of our cases or control subjects was homozygous for both the 677C→T and 1298A→C MTHFR polymorphisms. These findings corroborate those of a study of Irish women that showed that combined heterozygosity for 2 MTHFR mutations (677C→T and 1298A→C) was present in 22% and 3.5% of abruption cases and control subjects, respectively (odds ratio, 5.2; 95% CI, 1.1-24.5). These authors demonstrated that increased risk of abruption was driven largely by an association with the MTHFR 1298A→C variant (odds ratio, 3.2; 95% CI, 1.0-10.4) and speculated that this variant may serve as a susceptibility factor that can be triggered in the presence of the homozygous mutant form of the 677C→T polymorphism.

Despite strong epidemiologic associations between maternal race and abruption risk, genetic variations by race in our study were accounted by population stratification by design. Although the study had sufficient power to detect associations between MTHFR and abruption risk, our study may have lacked sufficient power to detect associations that were stratified by maternal race/ethnicity. Our patient population was also comprised of largely high-risk women, as previously reported. Laboratory personnel carrying out the assays for homocysteine, folate, and vitamin B12 were blinded to case control status, and all assays were performed with automated systems so that the potential for a diagnostic bias is unlikely. All analyses also incorporate adjustments for a variety of confounders, but bias because of residual confounding that were due to unmeasured factors is likely. Finally, the Hardy-Weinberg equilibrium was violated for the 677C→T genotype of MTHFR in our study. This may have resulted because of our patient population was fairly heterogeneous and comprised of a relatively larger proportion of women who were at high-risk for placental abruption and related obstetric complications. Thus, some caution in interpretation of our findings for this particular genotype is warranted. Because placental function is determined by both maternal and fetal genes, future studies may benefit from the examination of associations between fetal MTHFR genotypes, homocysteine pathways, and abruption. In addition, whether women carrying the mutant genotypes of the MTHFR polymorphisms are at increased risk for recurrent placental abruption remains unknown and may be a topic worthy of future investigation.

In summary, our study shows no evidence for an association between the MTHFR polymorphisms (677C→T and 1298A→C) and risk of abruption. These data also suggest a lack of distributional changes in the profiles of plasma homocysteine and folate between placental abruption cases and control subjects.
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partment of Pathology, Saint Peter's University 
Hospital, New Brunswick, NJ), and Vinay Prasad, MD (Department of Pediatric Pathol- 
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Arkansas Medical Sciences, Little Rock, AR).

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A comparison of a new rapid real-time polymerase chain reaction system to traditional culture in determining group B streptococcus colonization

Michael Gavino, MD; Eileen Wang, MD

OBJECTIVE: The objective of the study was to evaluate a rapid real-time polymerase chain reaction (PCR)–based assay for intrapartum detection of group B streptococcus (GBS).

STUDY DESIGN: This prospective, observational study enrolled outpatients after GBS screening at 35-37 weeks’ gestation. At admission for delivery, paired rectovaginal swabs were obtained for the GBS GeneXpert (Cepheid, Sunnyvale, CA) assay and standard culture. Using the intrapartum culture as the gold standard, sensitivities, specificities, and predictive values of the rapid assay and the antenatal screen were determined. Statistical significance was determined by Fisher’s exact test.

RESULTS: Fifty-five subjects had both rapid test and intrapartum culture results. The intrapartum GBS colonization rate was 43.6%. The sensitivity and specificity of the PCR test were 95.8% (95% confidence interval [CI], 76.9-99.8%), and 64.5% (95% CI, 45.4-80.2%), respectively, whereas the antenatal culture sensitivity was 83.3% and specificity was 80.6%.

CONCLUSION: The GeneXpert rapid GBS point-of-care assay was highly sensitive for GBS detection in our sample population. Confirmation of these data is needed on a large population basis.

Key words: group B streptococcus, real-time polymerase chain reaction, point-of-care assay


In 2002, the Centers for Disease Control and Prevention issued revised guidelines recommending universal prenatal screening for vaginal and rectal group B Streptococcus (GBS) colonization of all pregnant women at 35-37 weeks’ gestation. Approximately 10-30% of pregnant women are colonized with GBS in the vagina or rectum.1,2 GBS colonization risk is increased in health care personnel, black women, and those with high body mass index.3 Despite preventive strategies, GBS remains the number 1 cause of infectious morbidity and mortality among newborns in the United States.1

It is accepted that women with known negative GBS screening cultures within 5 weeks of delivery do not require prophylaxis to prevent GBS disease. The positive and negative predictive values of the 35-37 week culture for GBS status at the time of delivery have been estimated to range between 71-92% and 93-99%, respectively, depending on prevalence.4 This implies that either some women change their GBS status in the interval between screening and actual delivery or that the test characteristics can be improved. Some women may either be unnecessarily treated with antibiotics or receive inadequate GBS prophylaxis. Although current preventive methods have been fairly effective, screening results are not always known or available at the time of delivery. This may be because of insufficient prenatal care or prenatal care elsewhere without a documented GBS screen. Rapid intrapartum tests for GBS have been developed.5,6 A meta-analysis of several studies has shown that previous commercially available tests lacked both sensitivity and specificity for the detection of GBS colonization.7

Polymerase chain reaction (PCR)–based assays have fared better in the detection of GBS. One multicenter study demonstrated that a molecular-based assay for GBS colonization during labor was highly sensitive and specific (94.0% and 95.9%, respectively).8 However, that testing process required multiple laboratory steps and may not be easily implemented in a clinical setting. With newer technology, rapid molecular tests can allow for point-of-care testing. GeneXpert GBS (Cepheid, Sunnyvale, CA) is a new, rapid, real-time PCR-based assay for the intrapartum detection of GBS colonization. This pilot study was undertaken to establish the analytical performance of this novel, point-of-care assay in an active labor and delivery unit.

MATERIALS AND METHODS
This prospective, observational, pilot study was conducted at the University of Chicago Hospitals after institutional review board approval. During prenatal visits to the University of Chicago obstetrics clinics, patients were informed about the study and asked about participation. The following inclusion criteria for selection of potential subjects were used: prenatal care at the University of...
Chicago and a culture-based GBS screen at 35-37 weeks’ gestation. Subjects provided informed consent to participate in the study; these term participants were later identified on their admission to the hospital’s labor and delivery (L&D) unit for delivery. Reasons for admission included labor, rupture of membranes, nonreassuring fetal testing/well-being, and scheduled induction of labor or cesarean section.

In L&D, 2 rectovaginal swabs were collected from participants at admission, before the start of intrapartum antibiotics for those with a positive antepartum GBS culture. One swab was used for the real-time PCR GeneXpert GBS assay, the other for standard culture. Vaginal/rectal specimen collection was performed in a uniform fashion using Copan Venturi Transystem (Copan Diagnostics, Corona, CA) swabs. The lower one third of the patient’s vagina was sampled with both swabs, followed by rectal sampling. The 2 swabs were brushed together for a more uniform sample distribution. One swab was placed into the GeneXpert cartridge for immediate PCR processing; the other was placed into the liquid media for culture, which was transported to an outside laboratory (LabCorp, Burlington, NC) within 24 hours and kept at room temperature. These results were returned in approximately 36-48 hours.

The real-time PCR system, GeneXpert, included a desktop machine, laptop computer, and preloaded software, on loan from Cepheid. This automated system allows for sample DNA purification, nucleic acid amplification, and detection of the target sequence in the biological samples using real-time PCR. Single-use GeneXpert cartridges hold the sample and PCR reagents and host the PCR process. The GBS assay detects the presence of *Streptococcus agalactiae* (GBS) DNA, based on the principle that GBS is distinguished from other streptococci by the cell-wall–associated group B carbohydrate. Each cartridge includes internal controls to validate sample processing and PCR performance. The sample swab and the 2 reagent solutions are placed into the appropriate chambers of the GeneXpert GBS cartridge, which is then loaded into the machine, a 2-minute process.

In a completely automated fashion, the GeneXpert Dx system elutes the bacteria from the swab, mixes the sample reagent with a sample-processing control (ie, *Bacillus globigii*) and treatment reagent, and captures and lyses the bacterial cells with subsequent elution of the DNA. The DNA solution is mixed with dry PCR reagents and transferred into the reaction tube for real-time PCR. The process is completed in less than 75 minutes while the computer collects cycle data to detect the presence or absence of the GBS target.

The GBS carriage rate was calculated for the study population by intrapartum culture and PCR data. Using the intrapartum culture results as the gold standard, the sensitivities, specificities, predictive values, and likelihood ratios of both the rapid GBS test and the prenatal culture were obtained. Statistical analysis on the collected data was performed using Fisher’s exact test, and 95% confidence intervals (CIs) were calculated for the sensitivities and specificities.

**RESULTS**

The rapid test and intrapartum culture were completed on a total of 55 subjects. The racial distribution of the subjects was as follows: 92.7% black (51/55); 7.3% Hispanic (4/55). By intrapartum culture, the GBS colonization rate for the study population was approximately 43.6% (24/55) vs 61.8% (34/55) by rapid PCR. The 35-37 week GBS screen was as follows: 92.7% black (51/55); 67.6% Hispanic (4/55). The positive and negative likelihood ratios were 2.7 and negative likelihood ratio 0.065.

**Table 2** compares both the prenatal and intrapartum culture-based tests. Twenty subjects had both a positive prenatal GBS and a positive intrapartum culture. Twenty-five subjects had both a negative prenatal GBS and a negative intrapartum culture. Of the remaining 10, 6 had a positive prenatal GBS but a negative intrapartum culture; 4 had a negative prenatal GBS but a positive intrapartum culture. The sensitivity and specificity of the prenatal GBS culture were 83.3% (95% CI, 61.2-94.5) and 80.6% (95% CI, 62.0-
96%, respectively. The positive likelihood ratio was 4.3 and the negative likelihood ratio, 0.21.

**Comment**

This pilot study was among the first to assess the GeneXpert rapid GBS assay in a clinical setting. In our sample population, this real-time PCR assay appears highly sensitive (95.8%) for the intrapartum detection of GBS in a point-of-care testing environment, compared with the antenatal culture, which had a sensitivity of 83.3%. Previous rapid tests based on PCR and optical immunoassay were not as sensitive overall (62.5% and 7.1%, respectively). The previous iteration of the rapid PCR assay, the IDI-Strep B, using the SmartCycler (Cepheid) demonstrated a 94% sensitivity for GBS detection. The disadvantage of that assay is that it is laboratory based, requiring technical personnel to perform multiple steps.

The GeneXpert GBS assay was recently approved by the US Food and Drug Administration based on data showing a sensitivity and specificity of 91% and 96%, respectively. Our rapid PCR data demonstrated a comparable sensitivity, but the specificity was lower at 64.5%. This may be explained by contamination in processing, because PCR is generally very sensitive and a variable culture technique or a sample procurement. Notably, 2 different swabs were used for the specimen collection. Although these were obtained in a fairly uniform fashion, in the future formal standardized training for all personnel using the rapid test should be implemented with proper cleansing of the work surfaces between assays.

Ten of the 55 patients had discordant antepartum and intrapartum cultures (18%), with 6 having a positive antenatal culture but a negative intrapartum culture. Unfortunately, 1 of these subjects received intrapartum antibiotics prior to swabbing. Although labeled as false positives or false negatives, the discordant results could be due to a change in GBS colonization status between prenatal testing and the time of delivery. A recent investigation revealed that 10% of women with negative 35-37 week GBS cultures were positive intrapartum. Discordancy between antepartum and intrapartum cultures may also reflect variation in culture technique and processing. PCR-based assays, on the other hand, are typically very sensitive and may detect slight amounts of bacterial antigen; it remains to be determined whether the presence of minute concentrations of GBS is clinically significant. The PCR sensitivity may explain the increased GBS carriage in our sample when compared with the routine culture.

Because prevalence affects predictive values, particularly in our study in which the GBS colonization rate was higher than is expected in the general population, likelihood ratios were calculated. The PCR and antenatal cultures had similar positive likelihood ratios of 2.7 and 4.3, respectively; the negative likelihood ratios were 0.065 for PCR and 0.21 for the antepartum culture. This suggests that the very low value of the PCR-negative likelihood ratio would lead to a large shift toward the probability of the patient not carrying GBS.

The study population was predominantly comprised of African American, Medicaid patients, which may explain our high GBS carriage rate. In the population studied by Yancey et al., the overall colonization rate was 26.5%, more akin to the 10-30% reported. The ethnic distribution of that study’s population, however, was diverse, including 61% white and 13% African American.

Despite the sample size and inherent limitations of this pilot study, the data are promising. This study demonstrates the feasibility of using the easy-to-use rapid GeneXpert GBS assay in a L&D setting. The system fully automates the traditionally complex molecular technique of PCR within the assay cartridges, with simple preparation and results in less than 75 minutes, compared with at least 36 hours for standard culture results. At our institution patients with an unknown GBS status (because of lack of prenatal care or care elsewhere) comprise at least 20% of our deliveries. These women are demographically similar to our study population. This rapid PCR could allow for appropriate GBS antibiotic prophylaxis by converting the unknown to known, particularly given a very low negative likelihood ratio. There will always be certain women who will not receive antibiotic prophylaxis, regardless of known GBS status, particularly those with precipitous or emergency delivery.

Further study is needed to fully validate the performance of this particular rapid GBS test in a large clinical setting. There is also a need to determine the cost-effectiveness of this intrapartum assay in comparison with a risk-based protocol when the GBS status is unknown, and in comparison with traditional antenatal culture with the ultimate goal of reducing GBS associated neonatal morbidity and mortality.

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**Duration of antimicrobial prophylaxis for group B streptococcus in patients with preterm premature rupture of membranes who are not in labor**

Jesus R. Alvarez, MD; Shauna F. Williams, MD; Vijaya L. Ganesh, MD; Joseph J. Apuzzio, MD

**OBJECTIVE:** The purpose of this study was to determine the duration of the time that is needed to eradicate group B Streptococcus (GBS) in pregnant women with preterm premature rupture of membranes (PPROM).

**STUDY DESIGN:** A retrospective cohort study was performed of pregnant women with PPROM from January 1, 2000, through December 31, 2005. Vaginal/rectal cultures were performed on admission and repeated daily. Patients received antibiotics until cultures were negative for 3 consecutive days.

**RESULTS:** Two hundred fourteen women were identified with PPROM; 169 of the women met the inclusion criteria. Thirty-three patients were GBS positive on admission and had negative cultures by day 3. Neonatal sepsis occurred in 19 neonates (11.2%); 3 neonates (16%) were from mothers who tested positive for GBS on admission, and 16 neonates (84%) were from mothers who tested negative on admission. There were no cases of neonatal sepsis because of GBS.

**CONCLUSION:** A 3-day regimen of antibiotic prophylaxis appears to be adequate to eradicate GBS from the genital tract of patients with PPROM.

**Key words:** group B streptococcus, neonatal sepsis, PPROM

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Group B streptococcus (GBS) remains a leading cause of serious neonatal infection, despite great progress in perinatal GBS disease prevention. It colonizes approximately 10%-30% of all pregnant women. GBS may be transmitted vertically to the newborn infant by either ascending infection after rupture of the fetal membranes or by acquisition during vaginal delivery. GBS may cause both an early-onset neonatal disease within the first 5 days of life and a late-onset disease, which occurs after the first week of life. The incidence of early-onset GBS disease among neonates who are born to colonized mothers is approximately 0.5%. Late-onset GBS disease is not believed to be associated with obstetric factors.

The neonatal mortality rate from GBS infections was reported to be as high as 50% before 1980 and has decreased to 4% as the result of intrapartum chemoprophylaxis and advances in neonatal care. The American College of Obstetricians and Gynecologists and the Centers for Disease Control and Prevention (CDC) developed recommendations in 1996 for intrapartum chemoprophylaxis to prevent perinatal GBS disease. The CDC later updated these guidelines in 2002.

Preterm premature rupture of membranes (PPROM) refers to rupture of membranes (ROM) before 37 weeks of gestation and accounts for close to one-fourth of all cases of ROM. PPROM accounts for nearly 40% of all preterm births. Multiple studies in the 1980s reported an increased association between PPROM, low birthweight neonates, and early-onset GBS neonatal disease.

A multicenter case-control study by Schuchat et al reported that a risk-based strategy for chemoprophylaxis potentially could prevent a substantial portion of GBS cases. One of the risk factors identified by Schuchat et al that increased the incidence of early-onset GBS disease was ROM of >18 hours.

The CDC algorithm for GBS prophylaxis for women with threatened preterm delivery that included those with PPROM includes prescribing intravenous penicillin for at least a total of 48 hours; at the physician’s discretion, the antibiotic prophylaxis may be continued beyond 48 hours.

Our hypothesis is that GBS can be cleared from the genital tract with <3 days of antimicrobial therapy. Daily cultures for GBS were obtained from the lower genital tract of women with PPROM. We reviewed the culture results to determine the duration of time that antimicrobials were needed to eradicate GBS from the lower genital tract of women with PPROM.

**Materials and Methods**

A retrospective cohort study was performed for all women who were admitted with PPROM to University Hospital, Newark, NJ, from Jan. 1, 2000, through Dec. 31, 2005. The study was approved by the institutional review board of UMDNJ–New Jersey Medical School.
Patients with a complaint of leakage of fluid per vagina were evaluated in the labor and delivery triage area by a sterile speculum examination. ROM was confirmed with visualization of fluid from the cervical os, a positive fern, and nitrogen test. Patients with confirmed PPROM at ≥34 weeks of gestation were delivered. Patients with PPROM that was confirmed at a gestational age of <34 weeks of gestation and without signs of fetal distress, infection, or labor were admitted to the antepartum service. One specimen was monitored for any signs of infection until 48 hours after admission. All such patients who were admitted to the antepartum service received a course of antenatal corticosteroids, either betamethasone or dexamethasone, if β1-agonist occurred. Intraamniotic infection was diagnosed by the presence of uterine tenderness, leukocytosis, and temperature >100.4°F without another source of infection. Every patient received intrapartum antimicrobials, except in the setting of a patient having 3 consecutive negative GBS cultures and without a suspicion of intraamniotic infection.

We reviewed the incidence of neonatal sepsis in our PPROM population. Bacterial identification and sensitivity were obtained for the culture-positive neonates. Neonatal blood cultures were done on admission to the neonatal intensive care unit as per departmental protocol, and neonatal sepsis was considered if these were positive. The presence of GBS colonization in mothers was assessed for neonates who were diagnosed with sepsis. Placenta were sent to the pathology department for a histologic examination after delivery, as ordered by the attending physician. We also compared intraamniotic infection with histologic chorioamnionitis of the placenta.

Results
A total of 214 patients were identified as having PPROM at <34 weeks of gestation. We excluded 45 patients from the study group because 23 women were in active labor and 22 other patients did not have GBS cultures done on admission (day 0). A total of 169 patients met the inclusion criteria of having GBS cultures on admission who were admitted for expectant treatment to the antepartum service. Of the 169 patients, 33 women (19.5%) were GBS positive, and 136 women (80.5%) were GBS negative on admission (Table). There were 6 multifetal pregnancies; therefore, a total of 140 neonates were born from the GBS-negative group and 35 neonates were born to the GBS-positive group.

In the GBS-positive carrier group, the daily genital tract cultures for GBS were negative in 29 patients (88%) by day 1, in 32 patients (97%) by day 2, and in all 33 patients (100%) by day 3. The median latency period until delivery for this group was 6 days (range, 3-17; Table 1). Of the 33 patients in this group, 31 women received chemoprophylaxis with penicillin G, and 2 women received clindamycin.

From the group of PPROM mothers with negative GBS cultures on admission, 2 of 187 patients reverted to being positive on day 1. They both had 3 consecutive genital cultures after day 1. No cultures in the GBS-negative group re-
verted to positive after 2 consecutive days of being negative from the admission cultures.

In the GBS-positive group, 7 of 33 patients (21.2%) were diagnosed with intraventricular infection, and 11 of the 33 placenta (33.3%) had histologic evidence of acute chorioamnionitis. Intraventricular infection was diagnosed in 14 of 136 patients (10.3%) in the GBS-negative group with PPROM. Histologic evidence of acute chorioamnionitis was found in 48 of 136 placentas (35.3%).

Overall, the incidence of positive blood cultures from neonates who were born to mothers with PPROM was 19 of 175 (10.9%). The incidence of positive blood cultures in neonates who were born to GBS-positive mothers was 3 of 35 (8.6%) and 16 of 140 (11.4%) in neonates who were born to GBS-negative mothers.

There were no cases of neonatal GBS sepsis. Nineteen neonates had positive blood cultures with staphylococci: *Staphylococcus hominis* (n = 2), *S aureus* (n = 3), *Enterobacter aerogenes* (n = 1), *Enterococcus faecalis* (n = 1), *Enterococcus faecium* (n = 1), *Proteus mirabilis* (n = 1), *E coli* (n = 1), *Candida albicans* (n = 1), *C. hominis* (n = 1), and *Enterococcus faecalis* (n = 2).

**Comment**

The neonatal and maternal benefits of GBS prophylaxis after PPROM have been described. Some studies have reported similar neonatal outcomes when a 3-day intravenous penicillin protocol is used, compared with a 7-day protocol. However, there are no data available to recommend the optimal duration of antibiotic prophylaxis for GBS-positive patients with threatened preterm delivery. A Medline search was performed, and no studies were found that have recommendations regarding the duration of antimicrobial prophylaxis for GBS in PPROM. The CDC has suggested 48 hours of intravenous antibiotics when threatened preterm delivery included PPROM. The treatment strategy has been left to the discretion of the physician.

To our knowledge, there are no studies that used daily genital tract cultures for GBS to answer the question of how long prophylactic antibiotics should be used in patients with PPROM to eradicate GBS from the lower genital tract. We found that, after 2 days of prophylactic antimicrobials, 97% of the genital cultures for GBS were negative and that, after 3 days of antimicrobials, 100% of the genital cultures were negative for GBS. We also noted that the latency period in our study was close to 6 days, which is consistent with other reports. In this study, there appears to be no difference in the incidence of neonatal sepsis between neonates who were mothers who were GBS carriers and those who were not GBS carriers. An interesting finding was that there were no cases of neonatal GBS septicemia identified. Of concern, the bacteria that were identified in neonates with sepsis appear to have greater resistance to antibiotics. Two neonates who were infected with *S hominis* had a strain that was penicillin resistant, which was the antibiotic used as chemoprophylaxis for GBS. A strain of *S hominis* was reported to be 100% resistant to penicillin, oxacillin, and erythromycin; 95.2% were resistant to gentamicin and clindamycin. *E coli* strains are becoming more resistant to antibiotics. A recent study demonstrated that, in 1142 *E coli* isolates from urinary tract infections, the ratio of the ampicillin resistance was reported to be 37.7%. New strains of extended-spectrum beta-lactamase–producing *E coli* are emerging also. Compared with beta-lactam/beta-lactamase–inhibitor and carbapenems-based regimens, empirical therapy with cephalosporins or fluoroquinolones is associated with a higher mortality rate because of sepsis by this new strain of *E coli* (9% vs 35%, respectively).

The limitations of our study include its retrospective design and the small sample size of GBS-positive patients with PPROM. However, the low incidence of early onset neonatal disease has been described to be approximately 0.5%; therefore, we would need 6271 in each group for sufficient power, as determined by post hoc power analysis. Because most patients delivered by day 6, we do not know how long patients would remain GBS-negative after having negative cultures for 3 consecutive days. Despite these limitations, our data suggest that it may take up to 3 days of antibiotics to obtain negative GBS cultures from the lower genital tract after PPROM occurs. However, further studies are needed to confirm these findings and to explore whether shorter courses of prophylactic antimicrobials are effective.

**References**


The twin-twin transfusion syndrome: spectrum of cardiovascular abnormality and development of a cardiovascular score to assess severity of disease

Jack Rychik, MD; Zhiyun Tian, MD; Michael Bebbington, MD; Feng Xu, MD; Margaret McCann, BA; Stephanie Mann, MD; R. Douglas Wilson, MD; Mark P. Johnson, MD

OBJECTIVE: Current means of grading twin-twin transfusion syndrome do not characterize cardiovascular aspects adequately. We sought to develop a score that describes the magnitude of cardiovascular severity in twin-twin transfusion syndrome.

STUDY DESIGN: Fetal echocardiograms of 150 monochorionic/diamniotic twins were reviewed. Blinded to Quintero stage, we applied a cardiovascular score to each twin set and compared it to the Quintero grade. The score is a composite of variables that include ventricular hypertrophy, dilation, function, valve regurgitation, great artery size, and diastolic properties in the recipient and umbilical artery flow in the donor. Doppler indices of vascular and ventricular function were measured.

RESULTS: Mean age was 21 ± 3 weeks. Discrepancy was noted in degree of severity between Quintero and cardiovascular stages. The score correlated well with myocardial performance index of the recipient right ventricle ($r^2 = .65$).

CONCLUSION: We describe the spectrum of cardiovascular abnormalities that are seen in twin-twin transfusion syndrome and propose a scoring paradigm for assessment of severity.

Key words: cardiomyopathy, fetal echocardiography, twin-twin transfusion syndrome, twins


Twin-twin transfusion syndrome (TTTS) is a major contributor to morbidity and death in monochorionic twins.1 The pathophysiologic cause of the disorder is presumed to be the result of a complex cascade of events that is derived from the presence of abnormal intertwin placental vascular connections. These connections lay the groundwork for an inequitable exchange of volume between the 2 fetal circulatory systems with an impact on cardiovascular loading conditions.2 The “donor” manifests hypovolemia with a responsive release of endogenous vasoactive agents to compensate for volume depletion; the “recipient” experiences the burdens of a volume load in addition to the deleterious effects of transferred vasoactive agents that originate from the donor.3

A wide spectrum of cardiac findings can occur in TTTS.4,8 In mild forms, subtle differences in the recipient (such as ventricular dilation and wall thickening) can be seen. As the disease severity progresses, the recipient can manifest severe cardiac enlargement, hypertrophy, and valvular and ventricular dysfunction that often lead5 to fetal death. Postnatal sequelae in fetal survivors include persistence of right ventricular outflow tract obstruction in recipients9 and abnormalities of arterial distensibility in the donor.10,11 Cardiovascular abnormalities significantly contribute to the overall morbidity and death seen in TTTS.

Currently, disease severity in TTTS is estimated by Quintero’s staging system, which describes progressive changes that begin with intertwin differences in amniotic fluid and progress to an absence of visualization of the urinary bladder as a surrogate for hypovolemia in the donor, abnormalities of Doppler ultrasonography, and ultimately the findings of hydrops and fetal death.12 Although this staging system provides an important basic framework for choosing between various management strategies, it ignores the fundamental cardiovascular elements of the disease, which in subtle forms are likely present at the earliest stages.

The purpose of this study is to describe the spectrum of cardiovascular abnormalities seen in TTTS, to propose a scoring system that accurately characterizes the cardiovascular aspects of the disease, and to compare this new scoring paradigm to the conventional Quintero staging system. Quantifying the magnitude of cardio-
vascular derangement through a cardiovascular score (CV Score), that is designed specifically for TTTS, can be useful in a number of ways. First, it can more precisely grade the severity of disease in a manner that is true to the pathophysiologic origins of the disorder and thereby improve decision-making for type of treatment. Second, serial application of a cardiovascular score can assist in measuring the degree of success or failure of various treatment modalities. Finally, it can serve as a tool to quantify the magnitude of cardiovascular abnormality that is seen in fetal life to prognosticate for possible late cardiovascular sequelae in childhood or beyond.

**Materials and Methods**

We performed a retrospective review of fetal echocardiograms in patients who were referred with the diagnosis of TTTS to The Center for Fetal Diagnosis and Therapy at The Children’s Hospital of Philadelphia between January 2003 and July 2006. Twin pairs had evidence of oligohydramnios in the smaller twin (maximum vertical pocket <2 cm) and polyhydramnios in the larger twin (maximum vertical pocket >8 cm). Twin pairs were graded by the Quintero staging system in the following manner: stage I = polyhydramnios/oligohydramnios sequence alone; stage II = absent bladder filling in the donor; stage III = “critically abnormal” Doppler studies defined as umbilical artery absent/reversed diastolic blood flow, ductus venosus absent/reversed diastolic blood flow, or umbilical venous pulsations; stage IV = fetal hydrops. Fetal echocardiograms that were performed at the initial evaluation were analyzed, and parameters were graded for each twin set. The Quintero stage assignment was determined by an individual who was blinded to the results of the cardiac score. Subjects were excluded if there was (1) congenital heart disease or significant arrhythmia in either twin, (2) only 1 twin alive at the time of evaluation, or (3) inadequate images for analysis. Studies were performed with a Siemens Acuson Sequoia (Mountain View, CA) 256 system that was coupled with a 6C2 curvilinear transducer. All studies were recorded digitally in DICOM format and reviewed on a Siemens KinetDx workstation. Institutional Review Board permission was granted for the study.

**Cardiovascular assessment and rationale for the elements of the CV Score**

We chose parameters for the proposed CV Score that reflected the underlying physiologic processes in TTTS. Each parameter was given a numeric value that was based on its presence/absence or degree of severity of the finding with a total maximum of 20 points (Table 1).

**Donor**

In the donor, the predominant process is that of increased placental vascular resistance, reflected as either diminished (1 point) or absent or reversed diastolic blood flow (2 points) on Doppler evaluation of the umbilical artery (Figure 1). No other cardiac findings are typically present in the donor twin, hence, no other cardiovascular parameters are included.

**Recipient**

In the recipient, the cardiovascular parameters measured reflect changes in appearance of the heart and the diastolic and systolic performance:

- Ventricular hypertrophy or wall thickening is determined by qualitative assessment or by measurement. Ventricular hypertrophy is present (1 point) if the free walls of the ventricle or ventricular septum appear thickened or if the right ventricle free wall or ventricular septum thickness exceeds 2 SD from the expected mean for gestational age.
- Cardiac dilation/enlargement is graded as a normal heart size (0 points) when the cardiothoracic ratio is ≤1/3, as mild (1 point) when the cardiothoracic ratio is >1/3 but <50%, or more than mild (2 points) when cardiothoracic ratio is ≥50%.
- Systolic function is evaluated by qualitative assessment of ventricular contractility or by calculation of the percentage of shortening fraction = [ventricular end-diastolic dimension − systolic dimension]/end-diastolic dimension. No dysfunction (0 points) is the percentage of systolic function at ≥30%; mild dysfunction (1 point) is the percentage of systolic function at SF <30% but ≥20%; more than mild dysfunction (2 points) is the percentage of systolic function at ≤20%.
- Tricuspid and mitral valve regurgitation are graded as none (0 points), mild (1 point) when the regurgitant jet area is ≤25% of the atrial area, or more than mild (2 points) when the regurgitant jet area is >25% of the atrial area (Figure 2).
- Decreased blood flow in the ductus venosus (1 point) or absent/reversed blood flow with atrial contraction (2 points) suggests decreased right ventricular compliance (Figure 4).
- Umbilical venous blood flow pulsations suggest decreased ventricular compliance and are abnormal (1 point).
- Right ventricular outflow tract obstruction has been identified in recipient twins with TTTS; however, we have observed very early signs of this phenomenon that manifested as an abnormality of the size ratio of the pulmonary artery to the aorta. Normally the pulmonary artery is larger in diameter than the aorta; as TTTS progresses, the pulmonary artery may not grow as well as expected. Hence, the pulmonary artery may be equal to the aorta in size (1 point), smaller than the aorta (2 points), or there may be frank right ventricular outflow tract obstruction that is manifested as subpulmonic or pulmonic stenosis (3 points).
- Pulmonary insufficiency, which is a finding that is observed rarely in the normal fetus, is a reflection of abnormal right-sided mechanics, as was seen in some recipient twins (1 point).
TABLE 1
Cardiovascular parameters that were used to make up the CV Score and the number of fetuses with specific cardiovascular findings

<table>
<thead>
<tr>
<th>Variable</th>
<th>Parameter</th>
<th>Finding</th>
<th>Numeric score</th>
<th>Fetuses with this finding (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor</td>
<td>Umbilical artery</td>
<td>Normal</td>
<td>0</td>
<td>96 (64%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Decreased diastolic blood flow</td>
<td>1</td>
<td>34 (23%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent/reversed diastolic blood flow</td>
<td>2</td>
<td>20 (13%)</td>
</tr>
<tr>
<td>Recipient</td>
<td>Ventricular hypertrophy</td>
<td>None</td>
<td>0</td>
<td>77 (51%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>1</td>
<td>73 (49%)</td>
</tr>
<tr>
<td>Cardiac dilation</td>
<td>None</td>
<td>Mild</td>
<td>1</td>
<td>47 (31%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;mild</td>
<td>2</td>
<td>25 (17%)</td>
</tr>
<tr>
<td>Ventricular dysfunction</td>
<td>None</td>
<td>Mild</td>
<td>1</td>
<td>12 (8%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;mild</td>
<td>2</td>
<td>21 (14%)</td>
</tr>
<tr>
<td>Tricuspid valve regurgitation</td>
<td>None</td>
<td>Mild</td>
<td>1</td>
<td>31 (21%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;mild</td>
<td>2</td>
<td>22 (15%)</td>
</tr>
<tr>
<td>Mitral valve regurgitation</td>
<td>None</td>
<td>Mild</td>
<td>1</td>
<td>131 (87%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>&gt;mild</td>
<td>2</td>
<td>13 (9%)</td>
</tr>
<tr>
<td>Tricuspid valve inflow</td>
<td>Double-peak</td>
<td>0</td>
<td>113 (75%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single-peak</td>
<td>1</td>
<td>37 (25%)</td>
</tr>
<tr>
<td>Mitral valve inflow</td>
<td>Double-peak</td>
<td>0</td>
<td>135 (90%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Single-peak</td>
<td>1</td>
<td>15 (10%)</td>
</tr>
<tr>
<td>Ductus venosus</td>
<td>All antegrade</td>
<td>0</td>
<td>114 (76%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Absent diastolic blood flow</td>
<td>1</td>
<td>13 (9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reverse diastolic blood flow</td>
<td>2</td>
<td>23 (15%)</td>
</tr>
<tr>
<td>Umbilical vein</td>
<td>No pulsations</td>
<td>0</td>
<td>136 (91%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulsations</td>
<td>1</td>
<td>14 (9%)</td>
</tr>
<tr>
<td>Right-sided outflow tract</td>
<td>Pulmonary artery &gt; aorta</td>
<td>0</td>
<td>126 (84%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulmonary artery = aorta</td>
<td>1</td>
<td>13 (9%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pulmonary artery &lt; aorta</td>
<td>2</td>
<td>8 (5%)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Right ventricle outflow obstruction</td>
<td>3</td>
<td>3 (2%)</td>
</tr>
<tr>
<td>Pulmonary regurgitation</td>
<td>None</td>
<td>0</td>
<td>145 (97%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Present</td>
<td>1</td>
<td>5 (3%)</td>
</tr>
</tbody>
</table>

Quantitative indices
Cardiovascular indices were calculated for each individual twin and included (1) peak S (systolic) wave and peak D (diastolic) wave for the middle cerebral and umbilical arteries; (2) pulsatility indices of the middle cerebral and umbilical arteries; (3) degree of reduced forward blood flow in the ductus venosus with atrial contraction by calculation of the ratio of peak velocity at atrial contraction (A wave) to peak velocity at systole (S wave); when there was atrial reversal the A wave was assigned a negative value; and (4) myocardial performance index (MPI, or Tei Index), which is a geometry-independent Doppler-derived measure of combined diastolic and systolic ventricular performance (Figure 5). The greater the MPI value, the greater the degree of global ventricular dysfunction. Angle of insonation was maintained at <20 degrees for all assessments.

Data analysis
Values are expressed as mean ± SD. Cardiovascular score values were summed,
and each twin pair was graded for severity of cardiovascular abnormality: CV Score $0-5$ (cardiovascular grade 1); CV Score $6-10$ (cardiovascular grade 2); CV Score $11-15$ (cardiovascular grade 3); CV Score $16-20$ (cardiovascular grade 4). The percentage of twin-pairs with varying cardiovascular grade at each Quintero stage was plotted. Quantitative indices were compared between recipient and donor twins with the paired $t$-test. Correlation between the CV Score and MPI was evaluated with logistic regression. A probability value of $<.05$ was considered significant.

Results

Data for 150 twin pairs were available for analysis. The distribution of each cardiovascular finding in the CV Score is listed (Table 1). The most common cardiovascular abnormalities were ventricular hypertrophy (49%), ventricular dilation (48%), and abnormal donor umbilical artery diastolic blood flow (36%). Right-sided abnormalities were more common than left (tricuspid regurgitation [35%] vs mitral regurgitation [13%]; tricuspid valve single-peak Doppler inflow [25%] vs mitral valve single-peak Doppler inflow [10%]).

Tables 2 and 3 list the number of twin pairs in each category of severity for the Quintero stage and for the cardiovascular grade. Although weak correlation was present between Quintero stage and cardiovascular score ($r^2 = .46$), there was a wide degree of variation in severity designation noted between the Quintero grade and cardiovascular grade. More than 12% of Quintero I, 35% of Quintero II, and 20% of Quintero III twin pairs had a higher cardiovascular grade than that designated by the Quintero system (Figure 6).

Table 4 lists the differences in quantitative measures between the donor and recipient twins. Umbilical artery pulsatility index was higher in the donor twin with no difference in middle cerebral artery pulsatility index. Ductus venosus A wave peak velocity and the peak A wave-to-peak S wave velocity were lower in the recipient, which suggests impaired forward blood flow relative to the donor. Both the right ventricle and left ventricle MPI values were significantly higher in the recipient twin than in the donor, respectively. In the recipient, right ventricle MPI was significantly greater than left ventricle MPI ($P < .01$). Right ventricle MPI in the recipient twin correlated well with the overall cardiovascular score ($r^2 = .65$) but correlated poorly with the Quintero stage ($r^2 = .14$). Left ventricle MPI correlated more weakly than did the right ventricle MPI with the cardiovascular score ($r^2 = .44$).

Comment

Our current understanding of the pathophysiologic condition of TTTS suggests that the disease begins with subtle cardiovascular phenomena at its earliest stage with progression to more severe derangements over time.1-3 These phe-
nomena should be observable clearly through the imaging tools of echocardiography. We report the development of a TTTS-specific cardiovascular score that characterizes the spectrum of disease. We found our cardiovascular grade of severity to be frequently discrepant with the Quintero staging system. Significant cardiovascular abnormalities may be present at early Quintero stages, which suggests that the Quintero paradigm does not characterize the cardiovascular aspects of this disease adequately. In our cohort, a substantial number of twin pairs had a higher grade of disease severity that was based on cardiovascular assessment than that designated by the Quintero system. There are no cardiovascular variables to be considered in the Quintero staging system until stage 3. The absence of cardiovascular variables at each stage may explain the reason that some investigators have found the Quintero system to be of limited prognostic value.

The frequency of findings of the components of our CV Score mimics the natural development of the disease. Early on, the recipient heart experiences volume loading and ventricular hypertrophy, and the donor heart experiences elevated placental vascular resistance. Recipient ventricular dilation, hypertrophy, and tricuspid regurgitation (likely related to right ventricular dilation and an undue afterload) and abnormal donor umbilical artery diastolic blood flow are the most common findings in our CV Score. Pulmonary insufficiency was seen in only 3% and was present in twins with multiple other findings and at least cardiovascular grade 3 severity of cardiovascular disease. Ventricular hypertrophy leads to, a stiff, noncompliant ventricle; hence, specific abnormalities of diastolic performance are seen as commonly as abnormalities of systolic performance. Noncompliance of the right ventricle is exhibited by the finding of

TABLE 2

<table>
<thead>
<tr>
<th>Quintero stage</th>
<th>Twin pairs (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Stage I</td>
<td>75 (50%)</td>
</tr>
<tr>
<td>Stage II</td>
<td>46 (31%)</td>
</tr>
<tr>
<td>Stage III</td>
<td>23 (15%)</td>
</tr>
<tr>
<td>Stage IV</td>
<td>6 (4%)</td>
</tr>
</tbody>
</table>
diminished forward blood flow with atrial contraction in the duc-
tus venous. In addition, a poorly compliant ventricle alters diastolic filling character-
istics that leads to a reduction in the early passive filling phase of diastole,
with greater dependence on late active atrial contraction.\textsuperscript{22} As the degree of
noncompliance and diastolic dysfunction progresses, the 2 diastolic wave
forms fuse, with regression of the Doppler inflow pattern to that typi-
cally seen in the first trimester of gestation. Although other scores for the
evaluation of fetal cardiovascular dis-
 ease (namely, congestive heart failure)
have been proposed,\textsuperscript{23} these include
generic parameters such as the pres-
ence of hydrops fetalis, which is a rela-
tively late, end-stage finding in TTTS.

These scores do not include a richly de-
scriptive and progressive assessment of
diastolic dysfunction, ventricular in-
flow characteristics, and pulmonary
outflow tract abnormality, which are
features that are unique and specific to
TTTS that we believe are well charac-
terized by our CV Score.

Of note, we found the right ventricle in
the recipient twin to be affected much
more commonly than the left ventricle.
This may be explained by the fact that
the right ventricle performs the majority of
circulatory perfusion work during fetal
life and has been shown to respond dif-
derentially to loading conditions than does
the left ventricle.\textsuperscript{24}

Relatively uncommon, but of interest,
are the findings of right-ventricular out-
flow tract abnormalities in the recipi-
 ents. The phenomenon of right ventric-
ular outflow tract obstruction has been
reported previously and was seen in 3 of
our fetuses;\textsuperscript{2} however, we describe the
new finding of a precursor to overt ob-
struction. Pulmonary artery diameter at
the valve level normally should be larger
than the aorta. We noticed that, in 21 of
our recipient twins (14%), pulmonary
artery size was equal to or smaller in size
than the aorta in the absence of any gra-
dient, discrete obstruction, or valvar ste-
nosis across the pulmonary valve. This
finding supports the notion that right-
ventricular outflow tract obstruction
may be a consequence of diminished for-
ward blood flow across the right side of
the heart. As the right side hypertrophies
and stiffens, there is less ventricular fill-
ing and decreased blood flow across the
pulmonary valve, which leads to poor
growth of the right-sided outflow struc-
tures that include the pulmonary an-
nulus. In its early, most subtle, form, the
pulmonary artery annulus fails to grow
and is equal to or smaller than the aorta;
however, as the ventricle continues to
hypertrophy, the outflow tract becomes

Note the presence of significant discrepancy in
categorization of severity in particular for Quin-
tero stages II and II, in which a wide variety of
cardiovascular grades are found.

Rychik. The twin-to-twin transfusion syndrome: spectrum of
cardiovascular abnormality and development of a cardiovascular
score to assess severity of disease. AJOG 2007.
more crowded and narrow, which leads to the end result of “acquired” congenital heart disease of pulmonary stenosis or atresia. This phenomenon further supports the notion of diastolic abnormalities laying the groundwork for the progressive pathophysiologic condition of recipient cardiomyopathy in TTTS. The reason that some recipient fetuses respond to the cardiovascular burdens of TTTS by developing progressive ventricular dilation and systolic dysfunction and that other fetuses experience right-ventricular outflow tract obstruction and pulmonary stenosis is still unclear. We speculate that differences in myocardial response to loading conditions may be due to genetic variability, as has been shown similarly in other conditions that affect the fully developed mature heart.\textsuperscript{25}

The quantitative parameters that were measured in our study provide further insight into the pathophysiologic condition of TTTS. We found an increased umbilical arterial pulsatility index in the donor in comparison with the recipients, which supports the concept of inequitable placental vascular resistances, as previously described.\textsuperscript{13} Analysis of blood flow in the ductus venosus reveals a significantly lower velocity waveform with atrial contraction, which suggests impaired filling of the right ventricle. Analysis of the MPI lends further support to the importance of diastolic dysfunction in the recipient twin in TTTS. The MPI is a geometry-independent Doppler-derived measure of combined systolic and diastolic dysfunction. The higher the value, the greater the degree of dysfunction that is present. Calculation of the MPI has been demonstrated previously to be useful in discerning early myocardial changes in recipient twins in TTTS.\textsuperscript{26} Our CV Score values for each twin pair correlated well with the myocardial performance index for the recipient right ventricle. Myocardial performance indices were significantly higher for the recipient in comparison with the donor for both the right and left ventricles. Although our donor MPI values fell mostly within the range of normal,\textsuperscript{19} both right and left ventricle recipient MPI values were markedly abnormal. Analysis of the components of the MPI reveals that there is very little difference in the systolic ejection times between donor and recipient; however, the times between atrioventricular valve closure and opening were prolonged for the recipient right and left ventricles. This finding is consistent with our CV Score parameter observation of fusion of the normal 2-peak inflow pattern into a single peak. As the 2 peaks of diastolic fuse, overall diastolic filling time is shortened, and the times between atrioventricular valve closure and opening is prolonged, which increases the MPI value. Diastolic dysfunction is likely the predominant contributor to the abnormal MPI values that are seen in the recipient twin in TTTS.

We believe that a detailed fetal echocardiographic evaluation with the use of the Children’s Hospital of Philadelphia CV Score is of value and should be incorporated into an integral part of the assessment of TTTS. One of the limitations may be the time commitment and training necessary to acquire the high-quality images that are required to grade the various parameters in a timely manner. We allot 60 minutes for the evaluation of twins in our laboratory; however, with experience, we are currently able to obtain all variables of the CV Score in 30-45 minutes. The cardiovascular parameters that are used in our CV Score provide an identifiable pattern or imprint of the clinical appearance of TTTS, which can be diagnostically helpful in distinguishing true TTTS from monochorionic twins in whom there may be poor growth of 1 fetus for other reasons. Both intrauterine growth retardation and TTTS may share the finding of abnormal diastolic blood flow in the umbilical artery of the smaller twin; the absence of cardiovascular parameters, as we have described in the larger twin, should raise the suspicion of a disease process other than TTTS.

In summary, we describe the spectrum of cardiovascular abnormalities seen in TTTS and propose a detailed cardiovascular scoring system for the assessment of disease severity. The Children’s Hospital of Philadelphia CV Score assigns a grade of disease severity that is often times discrepant with that suggested by the traditional Quintero staging system but that may be more representative of the mechanistic origins of the disease. The Children’s Hospital of Philadelphia CV Score incorporates parameters of diastolic dysfunction in the recipient that are a fundamental aspect of the pathophysiologic cause of the disease. In this study, we do not evaluate the impact of the application of our CV Score on outcome but rather focus on description and rationale of its elements and leave the investigation of impact and serial evaluation for future study. Quantifying the burden of prenatal cardiovascular abnormality in a detailed manner will allow us to correlate fetal and postnatal cardiovascular findings accurately in future studies and provides a useful research tool to investigate the fetal origins of childhood or adult disease in survivors of TTTS.\textsuperscript{27}

ACKNOWLEDGMENT

We thank Danise D. Donaghue, RN, for her coordinating efforts at The Fetal Heart Program at The Children’s Hospital of Philadelphia and for her contributions to this study.

REFERENCES

Evolving trends in 2000 cases of multifetal pregnancy reduction: a single-center experience

Joanne Stone, MD; Victoria Belogolovkin, MD; Andrea Matho; Richard L. Berkowitz, MD; Erin Moshier, MS; Keith Eddleman, MD

OBJECTIVE: The purpose of this study was to examine changes in multifetal pregnancy reduction (MPR) procedures in 2000 cases and to evaluate evolving trends within the last 1000 MPRs.

STUDY DESIGN: Two thousand patients who underwent MPR were identified. Data were collected from a computerized database. Comparisons were made between the first 1000 patients (group 1) and the second 1000 patients (group 2). In addition, changing trends within group 2 were also analyzed. Differences in proportions were evaluated by chi-square test and Fisher’s exact test, as appropriate.

RESULTS: There was a significant difference in the starting and finishing number of fetuses and a significant increase in the use of chorionic villus sampling before MPR in group 2 vs group 1 (43.7% vs 1.5%; P < .0001). The incidence of monochorionicity was significantly higher in group 2 (5.7%), compared with group 1 (2.1%; P < .001).

CONCLUSION: Recent trends in MPR demonstrates significant increases in overall reductions to a singleton fetus, the use of chorionic villus sampling, and the presence of monochorionicity.

Key words: chorionic villus sampling, multifetal pregnancy reduction, trends


The incidence of higher order multiple gestations (triplets or more) has increased exponentially over the last 3 decades, mainly because of advances in assisted reproductive technologies (ARTs). Between 1971 and 1997, triplet, quadruplet, and quintuplet or higher births increased by >400%, 1100%, and 500%, respectively.1 Although twin births have continued to increase, the rise has been less dramatic, increasing 60% between 1980 and 2001.2,3 Multiple gestations are at higher risk for complete pregnancy loss and fetal, neonatal, and maternal complications, when compared with singleton pregnancies. Additionally, long-term complications are of particular concern and relate to both the increased rates of prematurity and fetal growth restriction. In fact, in 1 study the incidence of cerebral palsy at the age of 1 year was found to be 1.6 per 1000 survivors in singletons, 7.3 per 1000 in twins and 28 per 1000 in triplets.4

Multifetal pregnancy reduction (MPR) is a technique that was developed 20 years ago to decrease the incidence of preterm delivery in multiple gestations by reducing the number of live fetuses who were present within the uterus. Over this 20-year period, changes in both ARTs and patient demographic characteristics have led to changes in the MPR procedure. In addition, the realization of the increased risks that are inherent in multiple gestations, including twins, has influenced patient and physician attitudes towards reduction. Many centers, including our own, did not offer “elective” reduction to a singleton pregnancy in the early years. However, the increased recognition of adverse outcomes that are associated with twins has led to a change in opinion and practice among patients and physicians.5 In addition, increasing success rates of ART with advancing maternal age has affected the use of invasive prenatal diagnostic modalities, including chorionic villus sampling (CVS).

Our center last published our experience with 1000 consecutive cases of MPR in 20026 and currently has performed MPR in >2000 patients. The purpose of this current series is to evaluate the evolving trends, such as starting and finishing numbers, the use of CVS before reduction, and the incidence of monochorionicity in the second 1000 consecutive cases of MPR and compare various characteristics to the first 1000 cases.

Materials and Methods

A computerized database was used to identify 2000 consecutive patients who underwent MPR. This database contains detailed information about patient demographic data, procedural details, and outcome information. Specific variables that are contained in the database have changed over time, because ART practices and our own practices have changed. Any publications from our institution on MPR are derived from this database, although the information may be supplemented with chart review. Over approximately a 13 year time pe-
period between 1986 and early 1999, 1012 cases of MPR were performed at the Mt. Sinai Medical Center. The outcome for 1000 of these cases has been reported previously. Over the next 7 years, an additional 1212 patients underwent MPR, the first 1000 of whom are included in this analysis. All cases of MPR were performed for the intent of reducing fetal number. Cases in which a pregnancy was referred because of a known fetal anomaly (ie, selective termination) were not included in this analysis. However, patients in whom a chromosomal abnormality or structural defect were found incidentally during evaluation before MPR were included in this series. All MPR procedures were performed by the transabdominal injection of potassium chloride into the region of the fetal thorax under ultrasound guidance. Details relating to the technique of performing this procedure have been reported previously.8 The technique remained the same, except that in mid 2003 the prophylactic use of antibiotics was discontinued. In most cases, CVS was performed by either a combination of transcervical and transabdominal approach or transabdominally alone. In rare cases, 2 fetuses were sampled both by the transcervical method. In patients in whom a CVS was performed before reduction and a chromosomal abnormality was found that fetus was targeted for reduction. In addition, a detailed sonographic evaluation was performed on all fetuses before reduction and, if any growth lag or fetal anomaly was present, that fetus was also targeted. In addition in 2004, nuchal translucency was measured routinely before reduction; if an enlarged nuchal translucency was present, that fetus was reduced. All patients underwent counseling before reduction. The counseling was nondirective, unless a clear medical indication for reduction was present (ie, history of preterm delivery, uterine abnormality). Data were collected from the database and by chart review with Institutional Review Board approval. Comparisons were made between the first 1000 cases (group 1) and the second 1000 cases (group 2). In addition, changing trends within group 2 were also analyzed by dividing patients into chronologic groups of 200. Differences in proportions were evaluated by chi-square and Fisher’s exact test, as appropriate. In addition, group 2 was divided into 5 groups that each consisted of 200 patients, with group membership ascribed by time of entry into the study. The Cochran-Armitage trend test and Jonckeeere-Terstra trend test were used to assess linear trends across chronologic group categories.

**RESULTS**

There was a significant difference in starting and finishing numbers of fetuses between the 2 groups (Tables 1 and 2; \( P < .0001 \)). In the last 1000 patients, more patients started with twins, and fewer patients started with ≥4 fetuses. Interestingly there was almost a 3-fold increase in the number of patients reducing to a singleton pregnancy (11.8% vs 31.8%; \( P < .001 \)). When group 2 was analyzed by dividing cases into chronologic groups of 200, there was a significant trend in the proportions of patients whose pregnancy was reduced to singleton across groups (\( P < .001 \)), with >40% of patients in the last group of 200 reducing to 1 fetus (\( P < .001 \); Table 3).

Because certain variables were added to the database over time, detailed information on particular maternal characteristics were available for group 2, but not for group 1. The mean maternal age in group 2 was 35 years; this increased over time from 34 years in the first 200 patients to 35.4 years for the last 200 patients (\( P = .009 \)). Within group 2, 67% of patients conceived through in vitro fertilization; 26% of patients conceived through intrauterine insemination; 4% of patients conceived with ovarian stimulation and coitus, and 2% of patients conceived spontaneously. The mean number of embryos that were transferred was 3.8, and mean day of transfer was 3.5. There was a significant trend toward a decrease in the number of embryos that were transferred and an increase in day of transfer over time (\( P < .05 \)). Within group 2, 99% of the MPR procedures were performed by 1 of the 3 authors.

A significant increase in the number of patients who underwent CVS before MPR was also seen. In group 1, only 1.5% of patients underwent CVS before reduction, compared with 43.7% in group 2 (\( P < .001 \)). The average gestational age at CVS was 10.6 weeks, and the average delay between CVS and MPR was 1.6 weeks. The MPR was performed significantly earlier in those patients who did not undergo CVS before MPR (11.8 vs 12.7 weeks of gestation; \( P < .0001 \)). When trends over time were observed within group 2, a significant increase in the use of CVS before MPR was seen (Figure); 25.5% of the first 200 patients in group 2 underwent CVS, and 69.6% of the last 200 patients had CVS before MPR (\( P < .001 \)). Interestingly, the number of fetuses who were sampled also steadily increased over time. Within group 2, only 5% of the first 200 cases had 3 fetuses sampled, although 31% of patients in the last 200 cases had 3 fetuses sampled. In this last group of 200, 44% of all patients who underwent CVS had 3

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**TABLE 1**

Comparison of cases, based on starting numbers between the first and second 1000 cases of MPR

<table>
<thead>
<tr>
<th>Starting no.</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>4.0</td>
<td>15.6</td>
</tr>
<tr>
<td>3</td>
<td>54.9</td>
<td>60.8</td>
</tr>
<tr>
<td>4</td>
<td>29.4</td>
<td>17.6</td>
</tr>
<tr>
<td>5</td>
<td>8.6</td>
<td>3.3</td>
</tr>
<tr>
<td>6+</td>
<td>3.1</td>
<td>2.5</td>
</tr>
</tbody>
</table>

**TABLE 2**

Comparison of cases, based on finishing numbers between the first and second 1000 cases of MPR

<table>
<thead>
<tr>
<th>Finishing no.</th>
<th>Group 1 (%)</th>
<th>Group 2 (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>1.8</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>86.4</td>
<td>67.6</td>
</tr>
<tr>
<td>1</td>
<td>11.8</td>
<td>31.8</td>
</tr>
</tbody>
</table>
fetuses sampled. This trend most likely reflects increasing operator experience over time and confidence in the technical ability to sample a greater number of fetuses. Within group 2, of those patients who underwent CVS before MPR, 9% of patients were found to have a chromosomal abnormality. Of this 9%, 30% had mosaic abnormalities; 13% were abnormalities of sex chromosomes, and 21% were autosomal trisomies. In total, 3.5% of all fetuses who were sampled had a chromosomal abnormality. In 0.4% of fetuses who were sampled, a result was not obtained because of a failure of cultured cells to grow. Interestingly, there was a significant increase in the incidence of pregnancies carrying a monochorionic component. In group 1, 2.1% of patients had a monochorionic twin/triplet pair vs 5.7% in group 2. When the data for group 2 were analyzed by chronologic groups of 200, there was a steady increase in monochorionicity over time ($P < .001$; Table 4).

**COMMENT**

Since the inception of the MPR procedure in 1986, significant changes in the procedure have evolved over time. Although some of these changes reflect modifications in assisted reproductive techniques, other modifications reflect changes in patient demographic factors and in patient and physician attitudes. In this series of 2000 cases of MPR, the latter 1000 cases had fewer patients carrying quadruplets or higher. This most likely represents improvements in ART and the increasing practice of transferring fewer embryos.

We were also able to demonstrate a trend toward reduction to a singleton gestation. In the most recent years, approximately 40% of patients who underwent MPR had CVS before reduction, which only 11.8% chose this route. In our earlier series in which only 11.8% chose this route. The 4-fold increase in patients who reduced from twins to singletons also demonstrates changing opinions regarding reduction for twins. It is now recognized that perinatal morbidity and death is far greater in twins compared with singletons. Delivery at $<32$ weeks of gestation and birthweight of $<1500$ g is almost 5-fold increased in twins, compared with singles.8 Additionally, the risks of intrauterine death and neonatal death within a week and 1 month of life are 2- to 3-fold greater in twins, compared with singletons. In addition, the risks of complete pregnancy loss are also greater than previously suspected, with complete loss rates for twins after identification of fetal heart activity reported to be 6%-16%.9-11 This is greater than the reported loss rate of 3.4% after MPR to a singleton.$^6$ In a survey of 801 patients who attended a fertility clinic, approximately 60% expressed their belief that the ideal number to conceive is 1.$^{12}$ Interestingly, they found that patients with children were less likely to want a multiple birth. Also, patients who recognized the increased fetal risks that are associated with multiple were 70% less likely to desire a multiple pregnancy.

The almost 40-fold rise in the use of CVS before MPR in the second 1000 patients, compared with the first 1000 patients, is dramatic. In fact in the most recent years, close to 70% of the patients who underwent MPR had CVS before the procedure. The ability to be selective and to avoid leaving chromosomally abnormal fetuses is an important advantage. The increasing acceptance of CVS before MPR may be related to the documented safety and efficacy of CVS in multiple pregnancies before MPR.$^{13,14}$ In addition, we found a significant increase in maternal age over the last 1000 patients, which may also have contributed to the use of prereduction CVS. Additionally, the increased risks of chromosomal abnormalities among multiple gestations is well-documented.$^{14}$ In a study of 424 multiple pregnancies that underwent CVS before reduction, Brambati et al$^{14}$ found a 7.3% incidence of chromosomal abnormalities per pregnancy, with a rate of Down syndrome of 2.3% (expected rate, 0.47%). This was significantly higher than in the control group of singleton pregnancies. This is similar to our data, in that we found a 9% incidence of chromosomal abnormalities per patient and a 3.5% incidence of

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**TABLE 3**

Percentage of cases in group 2 (second 1000 cases), based on finishing numbers and divided into chronologic groups of 200

<table>
<thead>
<tr>
<th>Finishing no.</th>
<th>1001-1200</th>
<th>1201-1400</th>
<th>1401-1600</th>
<th>1601-1800</th>
<th>1801-2000</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>4</td>
<td>1.0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>3</td>
<td>1.0</td>
<td>0</td>
<td>0</td>
<td>0.5</td>
<td>0</td>
<td>0.3</td>
</tr>
<tr>
<td>2</td>
<td>76.0</td>
<td>71.0</td>
<td>72.0</td>
<td>60.5</td>
<td>58.5</td>
<td>67.6</td>
</tr>
<tr>
<td>1</td>
<td>22.0</td>
<td>29.0</td>
<td>27.5</td>
<td>39.0</td>
<td>41.5</td>
<td>31.8</td>
</tr>
</tbody>
</table>

---

**FIGURE**

Utilization of CVS prior to MPR in group 1 vs. group 2, and a comparison within group 2, divided into chronologic groups of 200

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The number of fetuses sampled over time also increased, which likely represents increasing operator experience. Finally, our study also demonstrated the significantly increased risk of monochorionicity that occurred in the last 1000 patients who underwent MPR, compared with the first 1000 cases. In fact, even within the last 1000 cases, the incidence of monochorionicity increased 6-fold from 2.5%-12.5%. The association between ART and monozygosity is now well-established.15-17 This is most likely not only due to the increasing prevalence of blastocyst transfers but also is implicated in the cause of monochorionic twins are intracytoplasmic injection, assisted hatching, and preimplantation genetic diagnosis. Although we do not have specific information as to the prevalence of these techniques among our cases, it is likely that these modalities were used more frequently in the last 1000 patients, compared with the first 1000 patients. The increase in monochorionicity is of particular concern, because of the increased risks that are associated with this type of twinning. Most patients with a monochorionic component chose to reduce the monochorionic pair, thereby eliminating the possibility for twin-twin transfusion and other associated complications.

In conclusion, this large series of 2000 patients who underwent MPR demonstrates the evolving trends with this procedure over time and also reflects changes in ART techniques and patient demographics. We have been able to document the trends towards reduction to a singleton, reduction from twins, and an overwhelming preference for undergoing prenatal diagnosis with CVS before MPR. This information is relevant when patients are counseled for reduction. The option for reduction to a singleton pregnancy, the possibility for reduction of twins, and the common practice of the performance of CVS before MPR is extremely important information to provide to patients.

REFERENCES


<table>
<thead>
<tr>
<th>TABLE 4</th>
<th>Incidence of monochorionic multiples within higher-order multiple gestations within group 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of multiple pregnancy</td>
<td>Group 2</td>
</tr>
<tr>
<td></td>
<td>1000-1200</td>
</tr>
<tr>
<td>Monochorionic twins (n)</td>
<td>5</td>
</tr>
<tr>
<td>Monochorionic triplets (n)</td>
<td>0</td>
</tr>
<tr>
<td>Total (n)</td>
<td>5 (2.5%)</td>
</tr>
</tbody>
</table>
A prospective, randomized, multicenter trial of amnioreduction vs selective fetoscopic laser photocoagulation for the treatment of severe twin-twin transfusion syndrome

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OBJECTIVE: The objective of the study was to examine the effect of selective fetoscopic laser photocoagulation (SFLP) vs serial amnioreduction (AR) on perinatal mortality in severe twin-twin transfusion syndrome (TTTS).

STUDY DESIGN: This was a 5 year multicenter, prospective, randomized controlled trial. The primary outcome variable was 30 day postnatal survival of donors and recipients.

RESULTS: There was no statistically significant difference in 30 day postnatal survival between SFLP or AR treatment for donors at 55% (11 of 20) vs 55% (11 of 20) (P = 1.0, odds ratio [OR] 1.95 confidence interval [CI] 0.242 to 4.14) or recipients at 30% (6 of 20) vs 45% (9 of 20) (P = .51, OR 1.88, 95% CI 0.44 to 8.64). There was no difference in 30 day survival of 1 or both twins on a per-pregnancy basis between AR at 75% (15 of 20) and SFLP at 65% (13 of 20) (P = .73, OR 1.62, 95% CI 0.34 to 8.09). Overall survival (newborns divided by the number of fetuses treated) was not statistically significant for AR at 60% (24 of 40) vs SFLP 45% (18 of 40) (P = .18, OR 2.01, 95% CI 0.76 to 5.44). There was a statistically significant increase in fetal recipient mortality in the SFLP arm at 70% (14 of 20) vs the AR arm at 35% (7 of 20) (P = .25, OR 5.31, 95% CI 1.19 to 27.6). This was offset by increased recipient neonatal mortality of 30% (6 of 20) in the AR arm. Echocardiographic abnormality in recipient twin Cardiovascular Profile Score is the most significant predictor of recipient mortality (P = .055, OR 3.025/point) by logistic regression analysis.

CONCLUSION: The outcome of the trial did not conclusively determine whether AR or SFLP is a superior treatment modality. TTTS cardiomyopathy appears to be an important factor in recipient survival in TTTS.

Key words: amnioreduction, selective fetoscopic laser photocoagulation, twin-twin transfusion syndrome

Twin-twin transfusion syndrome (TTTS) is the single most common serious complication of monochorionic twin gestations accounting for 10-17% of all perinatal mortality. The natural history of untreated TTTS is well established, with mortality rates up to 90%. The incidence of this serious complication has been increasing in parallel with the increasing incidence of multiple gestations.

Numerous treatment options for TTTS have been reported, including amnioreduction (AR), intertwin septostomy, nonselective fetoscopic laser coagulation (SFLP), and cord coagulation, all of which have improved survival rates, compared with the untreated natural history of TTTS. Which of these treatment options would provide the best outcome in specific cases of TTTS has been controversial.

AR has been associated with an average survival of 50%, with large international registries reporting 60-65% survival. Saade et al suggested the reason AR works is because it inadvertently creates an intertwin septostomy. This inadvertent septostomy has been suggested to account for the single amnioreduction paradox in which TTTS appears to stop in up to 20% of patients after only a single AR. Consistent with this view, a prospective, randomized trial comparing AR with septostomy found the same 65% survival in each arm of the study. The problem with septostomy has been the risk of creating a monoamniotic gestation and the attendant risk of cord entanglement with double fetal demise. Cord coagulation, either by fetoscopic bipolar cautery or radiofrequency ablation, at best can achieve only a 50% survival and is unacceptable to many parents because it necessitates the sacrifice of 1 fetus.

Fetoscopic laser photocoagulation for TTTS was first described by De Lia et al. as a means of functionally converting a monochorionic placenta to a dichorionic one by occluding vascular connections between the twins normally present in monochorionic placentas. Although these vascular connections are not the cause of TTTS, they are a necessary prerequisite for the syndrome. This technique as originally described, and still in use in some centers, is nonselective in nature, meaning all vessels found to cross the intertwin membrane were photocoagulated. Survival with this approach is reported to be 53-56%, comparable with survival rates observed with AR. An intriguing finding that stimulated interest in this approach was the observation that newborn survivors treated by fetoscopic laser appeared to have a lower incidence of head ultrasound or magnetic resonance imaging (MRI) abnormalities (5-7% vs 14-25%). Subsequently Quintero et al recognizing there is no anatomic relationship between the position of the intertwin membrane and the vascular equator, described a selective technique of fetoscopic laser photocoagulation in which only vessels demonstrated to be communicating between the 2 twins are treated. This selective fetoscopic laser technique is thought to preserve noncommunicating vessels and has been associated with survival rates of 62-77%.

To eliminate the up to 20% of patients with TTTS who may respond to a single AR, the so-called single amnioreduction paradox, and to compare the impact on 30-day neonatal survival in patients at the most severe end of the spectrum of TTTS, we conducted a prospective, randomized clinical trial on subjects with TTTS presenting prior to 22 weeks’ gestation, stages II, III or IV, who failed to respond to AR, and randomized to either SFLP or serial AR.

MATERIALS AND METHODS

Study design
This trial was a 5 year multicenter, prospective, randomized, controlled trial to examine the effect of SFLP, compared with serial AR, on perinatal mortality in severe TTTS. Patients meeting entry criteria (see Table) and consenting to enter the trial were randomized to 1 of the 2 treatment arms with equal likelihood.

Primary and secondary outcomes
The primary outcome variable to be assessed in this trial comparing AR with SFLP was the 30 day neonatal survival of donors and recipients. In addition, secondary outcome variables to be assessed included the number of pregnancies in each arm with 1 or more twins surviving; and the overall survival to 30 days (total number of twins surviving to 30 days divided by the total number of fetuses treated).

Demographics
Subjects were pregnant women 18 years old or older, diagnosed with TTTS at less than 22 weeks’ gestation, and randomized and treated prior to 24 weeks’ gestation. The subjects must have had no contraindication to general anesthesia or abdominal surgery, history of preterm labor, or uterine anomalies (see Table).

Sample size considerations
Subjects were assigned to AR or SFLP with equal probability (1:1 odds). The plan consisted of 3 interim analyses at accrual of 1 of 4, 2 of 4, and 3 of 4 of the planned sample size. The criteria for stopping were based on the O’Brien-Fleming stopping rules. The primary null hypothesis to be tested was the survival rates of donor/recipient twins at 30 days after birth and no treatment failure are the same between the 2 treatment groups. With a 2-sided type 1 error of 0.025 for each of the 2 primary outcomes and a power of 0.80, it would take 146 patients to detect an increase from a 50% survival rate with AR to a 75% survival rate with SFLP. These estimates were based on pooled reports of overall survival to delivery from published series of patients treated by AR or SFLP.

Study procedures
Prescreening criteria: Prior to randomization, each subject underwent a diagnostic evaluation, which included ultrasound to confirm monochorionic diamniotic gestation with like-sex twins, single placental mass, and a thin intertwin membrane. In addition, there had to be a “stuck” donor twin defined as a twin with a deepest vertical pocket of 2 cm or less and a recipient cotwin with polyhydramnios defined with a deepest vertical pocket of more than 8 cm, with or without Doppler or echocardio-
graphic changes, and presenting prior to 22 weeks’ gestation. The empty bladder in the donor twin should not be seen to fill during the ultrasound examination (Quintero stage II) unless there are Doppler velocimetry changes in umbilical artery, umbilical vein, or ductus venosus waveforms (Quintero stage III). In addition, structural anomalies and central nervous system abnormalities were specifically excluded. A transvaginal ultrasound was performed after the qualifying AR to exclude foreshortened cervix and length less than 2.0 cm excluded the mother from the trial. To ensure uniformity, determination of projected gestational age was based on the method used by the National Institute of Child Health and Human Development–sponsored Maternal-Fetal Medicine Units Network.

A single diagnostic and therapeutic qualifying amniocentesis was performed on the polyhydramniotic sac with sufficient fluid removed to reduce the deepest vertical pocket to less than 5 cm. This initial qualifying AR was performed by a participating investigator per a standard protocol. A follow-up ultrasound was performed within 1 hour of the procedure to detect evidence of inadvertent microseptostomy, which, if detected, excluded the patient from eligibility for randomization.

Final screening: If the follow-up ultrasound obtained 12-24 hours after the qualifying amniocentesis visualized a decompressed bladder in the donor twin, which is not seen to fill during the examination, and a deepest vertical pocket of less than 2 cm in the donor sac, the subject was considered a candidate for entry into the trial and offered randomization. If the donor deepest vertical pocket was greater than 2 cm or the bladder was not decompressed and/or seen to fill during the follow-up ultrasound examination 12-24 hours later, the subject was considered a responder and not a candidate for randomization (see Table).

Pretreatment studies: Prior to initiation of treatment, baseline studies including ultrasound, fetal echocardiogram, and MRI were obtained and recorded on blinded tapes/films and sent to the data coordinating center. In the SFLP arm, maternal screening for anesthesia by an anesthesiologist and preoperative CXR, electrocardiogram, and complete blood count were obtained. An ultrasound and echocardiogram were obtained on each fetus at least weekly. Each of these surveillance tests had a checklist of data to be obtained with the examination.

Randomization plan
Randomization was centralized via faxed case report forms and stratified by cluster and gestational age group. AR was performed at the local clinic. SFLP subjects were sent to either Cincinnati, Children’s Hospital of Philadelphia, or University of California, San Francisco based on geographical location.

SFLP
Mothers randomized to the SFLP arm of the study underwent the procedure under epidural anesthesia and intravenous sedation for comfort. Although most of the procedures could be performed by a single percutaneous port, some cases with an anterior placenta required a minilaparotomy to expose the surface of the uterus. A 4 mm incision was made in the skin to allow ultrasound-guided placement of a 3.3 mm 3-port fetoscope that has a lens port as well as 2 working ports; 1 for the 600 μ laser endostat and the other for the level I for rapid infusion of physiologic saline, if needed, to clear the amniotic cavity. The chorionic plate of the placenta was mapped 3 times: (1) trace every vessel that leaves the placental cord insertion of the recipient twin to its termination in a cotyledon or in an anastomosis to a vessel crossing to the donor twin’s umbilical cord insertion, (2) intraoperatively mark and record location of all connecting vessels for subsequent photoagulation, and (3) verify that no connecting vessels were missed. All procedures were digitally recorded and a permanent record of vascular anatomy was made for each procedure. All recorded SFLP procedures were independently externally reviewed to ensure quality control. The vessels were photoagulated using 60 W power with the endostat positioned at 1 cm from the surface of the vessel. The amniotic fluid volume was reduced such that the deepest vertical pocket was less than 5 cm. Antibiotics were instilled into the amniotic cavity prior to trocar removal.

During a 9 month period, the trial was placed on pause when the principal investigator moved from Philadelphia to Cincinnati. During this pause, the National Institutes of Health and Data and Safety Monitoring Board (DSMB) requested that we incorporate the use of the new Storz remote head fetoscope into the trial as standard equipment at all 3 laser centers. This required institutional review board approval at all centers because this device at the time was not Food and Drug Administration approved. Up to that time, all 3 centers were using the Storz 3-3 mm fetoscope with standard head for camera attachments.

AR
Mothers who were randomized to the aggressive serial AR arm of the study underwent ultrasound examination on a Monday, Wednesday, and Friday schedule. AR was performed whenever the recipient twin sac deepest vertical pocket was 8.0 cm or greater. The volume of fluid removed during these procedures was sufficient to reduce the deepest vertical pocket to 5 cm or greater. The AR procedure was standardized by protocol to require use of a 20-gauge needle and vacuum suction bottle. The patient was assessed for an inadvertent microseptostomy by checking the deepest vertical pocket around the donor within 1 hour of the procedure. All fetuses delivered as clinically indicated.

Definition of treatment failure
A failure of therapy was defined as the progression of nonimmune hydrops or cardiac failure with imminent fetal demise despite therapy in 1 of the 2 treatment arms. A fetus would have to meet at least 2 of the 4 following criteria to meet the definition of cardiac failure despite therapy: (1) development or progression of severe atrioventricular valve (mitral or tricuspid) regurgitation, (2) reversal of diastolic flow in the ductus venosus, (3) reversal of diastolic flow in the umbilical
One hundred ninety-six subjects were screened a total of 251 times with 138 subjects not found to be eligible. Of the 58 found to be eligible for the trial, 15 declined randomization (71.4% of 58 eligible; 21.9% of 196 total). Of the 58 eligible, 43 consented to randomization, but 1 withdrew. Twenty-one subjects were randomized to each arm of the study, and a single subject dropped out of each arm.


The distribution by stage using the Quintero staging system is equally distributed among stages II, III, and IV in both the AR and SFLP groups. There were no stage I patients in the trial. There is no statistically significant difference in stage distribution between AR and SFLP.


Statistical considerations

Interim analysis and early trial closure: The DSMB reviewed the trial for efficacy for the primary outcome at the interim analyses performed after 38 randomized patients were evaluable. Adverse events and the primary outcome of perinatal mortality were the only outcome variables considered when deciding to close the trial.

Primary analysis: The primary outcome variables, survival of the donor and recipient twins at 30 days after birth, were analyzed using a logistic regression model with treatment (AR, SFLP) as 1 of a list of predictor covariates. Other covariates included gestational age at diagnosis, Quintero stage (II, III, or IV), maternal status, cohort, transportation for SFLP, and an echocardiographic score based on the Cardiovascular Profile Score (CVPS) described by Huhta. Adjustments for pairing and clustering, as is done in studies of paired organs such as eyes and kidneys, were unnecessary because the donor and recipient results were analyzed separately.

The primary null hypothesis to be tested is that there is no difference in success rates between pregnancies randomized to AR or SFLP treatment. The primary alternative hypothesis is that SFLP is superior to serial AR. For power analysis we assumed values of 50% success rate with AR and a 75% success rate with SFLP, a difference considered significant in a clinical sense.

RESULTS

The study was stopped early, after 42 subjects were randomized, at the request of the investigators. Referring physicians were increasingly unwilling to refer eligible subjects for evaluation to participating centers in which SFLP was available only through randomization in the trial. At the same time that the investigators’ meeting was held to decide to stop the trial, the Trial Oversight Committee, charged with evaluating all adverse events and serious adverse events, detected a statistical trend in adverse outcome affecting the recipient twin in 1 treatment arm. The Trial Oversight Committee was blinded to the treatment received in each arm. A recommendation was made to the DSMB that the trial be stopped to allow biostatistical analysis of this adverse trend. The DSMB concurred with the decision to stop the trial.

Enrollment

One hundred ninety-six cases (in which each case consisted of 3 individuals: mother, donor, and recipient) were screened a total of 251 times or an average of 1.28 screens per case (see Figure 1). Patients in the early stages of TTTS were often screened on more than 1 occasion until they either met criteria or were ineligible. Of the 196 cases, 58 (29.6%) were deemed eligible by meeting all inclusion criteria (54 cases) or passing via protocol deviation (4 cases had a visible bladder [stage I] but had Doppler changes upstaging them to stage III). Twelve patients refused a qualifying AR. Of the 46 patients who consented to an AR, only 4 of 46 (8.7%) were ineligible because of a positive therapeutic response to the AR. Of those 4, 2 had evidence of amniotic fluid around the donor within 1 hour of the AR, consistent with septostomy. Of the 58 cases eligible, 43 (21.9% of screened, 74.1% of eligible) consented to enrollment. Only 42 cases
There is no statistically significant difference in survival of 1 or both twins to 30 days of life. Crombleholme. Amnioreduction vs selective fetoscopic laser photocoagulation for TTTS. Am J Obstet Gynecol 2007

TTTS Trial

Primary outcome by treatment

There was no significant difference in the primary outcome of 30 day postnatal survival between SFLP or AR treatment for donors in the AR arm (55%, 11 of 20) vs the SFLP arm (55%, 11 of 20) ($P = 1.0$, odds ratio [OR] 1.00, 95% confidence interval [CI] 0.24 to 4.14) or recipients in the AR arm (45%, 9 of 20) vs the SFLP arm (30%, 6 of 20) ($P = .51, OR 1.88, 95\% CI 0.44 to 8.64$) (see Figure 3).

There was no significant difference in 30 day survival of 1 or both twins on a per-pregnancy basis between AR 75% (15 of 20) and SFLP 65% (13 of 20) ($P = .73, OR 1.62, 95\% CI 0.34 to 8.09$) (see Figure 4). There was no significant difference in overall 30 day survival (newborns divided by fetuses treated) between AR and SFLP at 60% (24 of 40) vs 45% (18 of 40) ($P = .18, OR 2.01, 95\% CI 0.76 to 5.44$) (see Figure 5).

There was a statistically significantly increased fetal mortality among recipient twins treated by SFLP 70% (14 of 20) vs AR 35% (7 of 20) ($P = .03, OR 5.31, 95\% CI 1.19 to 27.6$) (see Figure 6). Within stages III and IV, there was a significant difference in 30 day survival for AR of 67% vs SFLP of 12.5% ($P = .03, OR 14.21$) (see Figure 7).

Despite the differences observed in recipient fetal mortality, there were no statistically significant differences in overall survival to 30 days of age. The reason was that although there were more fetal recipient deaths in the SFLP arm than the AR arm of the study, more recipients were declared treatment failures in the AR arm (7 of 20) vs the SFLP arm (0 of 20). Neonatal death occurred in 6 of the 7 cases declared treatment failures.


There is a statistically significantly increased fetal mortality in recipients treated by SFLP (70%) vs those treated by AR (35%, $P < .025$, OR 5.31, 95% CI 1.19 to 27.6). Crombleholme. Amnioreduction vs selective fetoscopic laser photocoagulation for TTTS. Am J Obstet Gynecol 2007

Efficacy adjusted for covariates

A straightforward logistic regression of recipient success based on treatment, after adjusting for all other covariates or progression of severe biventricular dysfunction despite AR treatment (3 of 7). Neonatal death occurred in 6 of the 7 recipients in the AR arm meeting criteria for treatment failure and offsetting the increased fetal recipient mortality in the SFLP arm.

The difference in fetal recipient survival is more pronounced among recipients in stages III and IV that were treated by AR (63%) vs SFLP (12.5%, $P < .03$). Despite these differences in fetal recipient survival, there is no difference in survival at 30 days because 7 of 20 recipients in the AR arm met criteria for treatment failure (in which 6 of the 7 were neonatal deaths), whereas 0 of 20 in the SFLP arm did so.

of interest (stage, marital status, echocardiographic score, long-distance transportation, gestational age, and cohort [pre/post pause for equipment changes]), shows a nonsignificant difference between SFLP and AR in the full model but with SFLP having a worse outcome that is lost during backward variable selection. For donors there were only negligible differences between treatment arms in the full model, which was also lost during backward variable selection.

The most predictive models for recipients used only their echocardiographic score (OR 3.025/point, P = .0552). The score was based on the 10-point CVPS described by Huhta using as many parameters as possible with the measures and observations available from ultrasound and echocardiograms. Missing values were replaced with means in computing the score. For donors, the most predictive models were stage (OR 0.446/stage, P = .1249) and gestational age (OR 1.052/day, P = .0987). In addition, fitting models including each covariate alone with treatment in the predictor list had similar results.

### Additional approaches

Several alternative analysis approaches were explored because of the small sample size and to further examine the roles of the covariates. The use of alternative analysis methods also helped to show whether results are robust to variations in approach. With conditional logistic regression, the results show that echocardiographic score is a useful predictor for recipients (OR 2.84, P = .0576) in the same direction as before. No other covariates or treatment are significant using this approach.

We also created new treatment categorizations to more accurately reflect the AR cases who experienced treatment failure and went on to receive other treatments. Similarly, we examined different definitions of outcome such as survival to 30 days, ignoring treatment failure. Once TTTS reaches this severity, the mortality among recipients will be considerable, but the losses may occur at different times, depending on treatment. The impact of TTTS severity on fetal survival is further supported by the significantly worse fetal survival among recipient twins in stages III and IV, compared with stage II. One of the strongest predictors of recipient demise is echocardiographic evidence of TTTS cardiomyopathy. The losses of fetal recipients treated by SFLP usually occur within 24 hours of maternal transfusion but did compromise visualization and necessitated the procedure being stopped. There was a single case of spinal headache (2.3%) resulting from placement of an epidural catheter, which responded to blood patch. Maternal hospitalization at any time during the remainder of the pregnancy required for preterm labor, short cervix, PPROM, or to monitor for fetal growth occurred in 9.5% of cases in both the AR and SFLP arms of the study. The specific incidence of PPROM occurring prior to 28 weeks’ gestation was 0% in the AR arm and 4.8% in the SFLP arm. The incidence of preterm labor requiring tocolysis was 4.8% in the AR arm and 0 in the SFLP arm. The incidence of delivery prior to 28 weeks’ gestation was 4.8% (n = 1) in the AR arm and 9.5% (n = 2) in the SFLP arm. These differences are not statistically significant.

### Safety analyses

In this study, adverse events and serious adverse events were common and often closely related to the primary outcome, which is death and/or treatment failure. Every adverse event, whether serious or not, related or not, anticipated or not, was reported to the Trial Oversight Committee and the institutional review boards. Because of the broad definition of adverse events, these were frequent in both arms of the study. There were no maternal deaths and no serious maternal adverse events related to the operative procedure, even in the 7 cases in which a minilaparotomy was used to expose the surface of the uterus.

No patient in the study had a chorioamniotic separation as a result of the qualifying AR. In 1 case there was uterine bleeding occurring during an SFLP procedure (4.7%), which did not require
TTTS cardiomyopathy, which specifically affects the recipient twin, appears to be one of the most important contributing factors in recipient mortality in advanced cases of TTTS. The results of this trial suggest that in advanced cases of TTTS, the recipient survival may be compromised, no matter what treatment the patient receives. Consistent with these findings in the trial, Shah et al.\(^{41}\) recently reported the use of the CVPS as an indicator of the severity of TTTS cardiomyopathy, demonstrating a negative impact on recipient survival.

Despite differences in patient selection (22 vs 26 weeks’ gestation) and response to qualifying AR vs primary therapy, the National Institutes of Health–sponsored trial has comparable overall survival of 1 or both twins to those reported in the Eurofoetus trial.\(^{20}\) The survival with AR is significantly better in the National Institutes of Health trial (60%) than in the Eurofoetus trial (41%) and consistent with single institution series,\(^{6-12}\) a prospective, randomized trial,\(^{15,16}\) and registries (60–65%).\(^{34,35}\) This may be due to the standardized aggressive protocol used for serial AR in the National Institutes of Health trial. Conversely, the survival in the National Institutes of Health trial for SFLP-treated TTTS is significantly less than reported previously using this technique in both single institution series and the Eurofoetus trial.\(^{19-28}\)

One possible explanation for the poorer survival observed in the SFLP arm could be a lack of proficiency of the surgeons performing the SFLP in the 3 laser centers in the trial. However, every SFLP procedure was recorded and independently reviewed by fetal surgeons not participating in the trial with significant experience with the SFLP technique. This review independently confirmed the adequacy of the procedures. Minor differences in technique were identified among the laser centers in which 1 center frequently used a minilaparotomy to access the uterus, whereas the other 2 centers used an entirely percutaneous approach. The placental mapping and the SFLP technique used were the same in all 3 centers. The results in these centers with SFLP, before and after the National Institutes of Health trial, support the view that problems with technical proficiency do not account for the differential fetal survival of recipients observed in this trial.\(^{42}\) The more likely explanation, as noted in earlier text, is the severity of TTTS cardiomyopathy.

The outcome of this trial does not conclusively answer the question of which treatment, AR or SFLP, is the superior treatment modality. The results do suggest, however, that no matter which treatment is used, survival is likely to be compromised if treatment is initiated later in the disease progression, particularly among recipients. TTTS cardiomyopathy appears to be an important factor in recipient survival in TTTS. The Cincinnati modification of the Quintero staging system to incorporate echocardiographic changes into stage III, as suggested by Harkness and Crombleholme,\(^{42}\) may be useful to more appropriately stratify recipient risk. Comparisons between this trial and the Eurofoetus trial must be drawn cautiously, given the significant differences in the subjects that were treated in each.

Additional trials in TTTS are needed to define the best treatment for a given case of TTTS. It is unfortunate that the state of community equipoise, which resulted in a decline of subjects screened for this trial, may also prevent randomized trials in TTTS in the future. An alternative strategy may be rigorously designed and controlled prospective cohort studies. The impact of TTTS on neuroimaging, echocardiographic assessment, and long-term neurodevelopmental outcome of the subjects in this trial awaits analysis of the follow-up assessments of survivors.

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Intrafetal radiofrequency ablation for twin reversed arterial perfusion (TRAP): a single-center experience

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OBJECTIVE: The objective of the study was to review perinatal outcomes in pregnancies treated with intrafetal radiofrequency ablation (RFA) for twin reversed arterial perfusion (TRAP) sequence.

STUDY DESIGN: Perinatal outcome data from a quaternary care referral center were abstracted from a chart review of pregnancies with TRAP sequence treated in the midtrimester with umbilical cord RFA of the perfused twin.

RESULTS: Twenty-one pregnancies with TRAP sequence were evaluated. Two women had a pump twin demise prior to therapy, 1 with trisomy 21 declined treatment. Four of 20 were treated successfully with RFA but remain undelivered, and 1 was treated with fetoscopic cord coagulation. Twelve of 13 pump twins treated with RFA (94%) survived to 30 days of life. Mean preoperative cardiac combined cardiac output was 588 mL/kg and pump/twin ratio was 0.7 (range 0.4 to 1.1). The effect of RFA on postoperative cardiac output was variable (6-85%). The average gestational age at birth was 37 weeks (range 26-39 weeks).

CONCLUSION: Primary therapy with RFA is a successful modality for pregnancies complicated by TRAP sequence.

Key words: radiofrequency ablation, twin reversed arterial perfusion, twin reversed arterial perfusion sequence


Twin reversed arterial perfusion (TRAP) sequence is an uncommon congenital anomaly unique to multifetal gestation. The pathophysiology of TRAP sequence is arterioarterial chorionic-angiopagus resulting in a “pump twin” and a “perfused twin.” Perfused twins have various, often bizarre anatomic features and function more like a mass than a cotwin. Regardless of whether the perfused twin is acardiac or has a rudimentary heart structure, the hallmark sign of TRAP sequence is reversed Doppler flow in the umbilical cord of the perfused twin. Several authors report a diagnosis of TRAP sequence as early as the first trimester. Adverse perinatal outcomes are commonly attributed to high output pump twin cardiomyopathy, polyhydramnios, and preterm birth. Poor prognostic indicators include aneuploidy, hydrops fetalis, polyhydramnios, acardius acephalic perfused twin, a perfused to pump twin weight ratio of greater than 70% and high cardiac output in the pump twin.

A wide variety of therapeutic modalities have been described. Treatment for TRAP sequence is based on the premise of interrupting vascular connections. Tan and Sepulveda, in a systematic review of treatment modalities, concluded that intrafetal ablation is superior to cord occlusion techniques for the treatment of TRAP sequence. The goal of this study was to report a single center experience with intrafetal radiofrequency ablation in pregnancies complicated by TRAP sequence.

MATERIALS AND METHODS
Beginning with the opening of the Fetal Care Center of Cincinnati (Cincinnati, OH) in January 4, 2004, pregnancies complicated by TRAP sequence were evaluated by a standard protocol of detailed fetal anatomic survey by ultrasound, ultrafast magnetic resonance imaging, and echocardiography. Echocardiographic assessment was used to calculate combined ventricular output in the pump twin in a standard fashion. The weight of the pump twin was determined by the Hadlock formula. The weight of the perfused twin was calculated as previously described (weight [grams] = (−1.66 × length) + (1.21 × length²)). A perfused twin to pump twin ratio of greater than 70% was considered abnormal. All pregnancies had ultrasound-guided amniocentesis for fetal karyotype.

Candidates for intervention had any of the following: hydropic changes, elevated perfused twin to pump twin ratio, or evidence of high combined ventricular output in the pump twin. The arterial diameters of the ascending aorta, main pulmonary artery, and ductus arteriosus from each fetus were calculated. Blood flow in each artery was calculated as the
product of heart rate and flow-velocity integral and arterial cross-sectional area. Combined ventricular output is the sum of aortic and pulmonary artery flows and is elevated when values are greater than 2 SD above mean values.

Intrafetal radiofrequency ablation (RFA) was performed under local anesthesia and intravenous sedation with a 19-gauge LeVeen needle with 2 cm tines (Boston Scientific, Boston, MA). With ultrasound guidance, the tines deployed completely within the fetal abdomen at the umbilical cord insertion site. Radiofrequency ablation was started with 60 W of power for 60 seconds. Power was increased by 20 W in 60 second intervals to 120 W or when impedance dropped. Cessation of flow within the perfused twin was confirmed by Doppler ultrasound interrogation. Patients were observed overnight with toktodynamic monitoring. Women with regular uterine contractions were treated with intravenous magnesium sulfate until uterine quiescence was achieved. An ultrasound was performed on postoperative days 1 and 3. An echocardiogram was also performed on or before postoperative day 3. Those successfully treated by RFA were returned to the care of the referring physician. Therefore, care was subsequently not standardized. Survival was defined at hospital discharge and 30 days of life.

RESULTS
A total of 21 consecutive women referred to the Fetal Care Center of Cincinnati were evaluated. The average perfused to pump twin ratio was 0.7 (range 0.4 to 1.1). The average gestational age at treatment was 21 weeks (range 17-24 weeks). All but 1 pregnancy was diagnosed with TRAP prior to being evaluated at our center: a pregnancy referred for a monochorionic gestation with an anomalous twin actually had TRAP sequence with a 2-chamber heart in the perfused twin. One woman presented with a demise of the pump twin prior to the evaluation and 1 woman had a pump twin demise after the evaluation but before therapy. After 1 pregnancy was diagnosed with trisomy 21 in the pump twin, the patient refused therapy and an intrauterine demise occurred. One patient who had a monochorionic monoamniotic twin gestation with TRAP sequence and umbilical cord entanglement underwent an umbilical cord coagulation and release. The umbilical cords were untangled with fetoscopic guidance.

Pregnancies were managed with intrafetal RFA if the fetus was hydropic (n = 1), had an increased combined ventricular output (n = 10), or had an acardius to pump weight ratio exceeding 50% (n = 12). Seventeen women underwent RFA. Four pump twins survived the immediate postoperative recovery but remained undelivered at the time of this report. Twelve of the remaining 13 women treated with RFA (94%) delivered a pump twin survivor. The only postoperative pump twin demise occurred in a fetus that was hydropic at presentation.

There were no neonatal losses in the first 30 days of life. The average gestational age at birth was 37 weeks (range 26-39 weeks). The only pregnancy delivered at less than 32 weeks was complicated by premature rupture of membranes followed by preterm labor at 26 weeks. This very early preterm birth was the only significant neonatal morbidity. One ablation procedure was incomplete because an accessory vessel was not coagulated. This was discovered when the combined ventricular outflow declined by only 6% postoperatively. This patient was managed with close observation and delivered a viable fetus at term. The mean preoperative combined ventricular output was 588 mL/kg per minute in patients studied preoperatively. After RFA, the cardiac output was reduced between 6% and 85%. Besides exposure to tocolytics in 5 cases, there was no maternal morbidity.

COMMENT
Radiofrequency ablation is a highly successful therapy for pregnancies complicated by TRAP. In a recent review, Tan and Sepulveda9 concluded that intrafetal ablation is superior to minimally invasive umbilical cord coagulation modalities. Our experience is consistent with that of Tsao et al,14 who report an identical survival rate of 94% using an identical treatment modality with intrafetal RFA. Our high success rate compares favorably with the 80% survival in a large cohort (n = 60) treated with fetoscopic laser photocoagulation by Hecher et al.15 In that series, coagulation of blood flow was achieved in 49 of 60 patients (82%) by laser alone and in a further 9 of 60 patients (15%) by laser coagulation in combination with bipolar forceps. The overall survival rate of the pump twin was 48 of 60 (80%). Regardless of the treatment modality used, preterm birth is uncommon after therapy because most patients deliver at term.

In the seven patients who had measurements of the combined ventricular output, there was a wide-range initial reduction from as little as 6% to as much as 85% reduction by 3 days after RFA. Future work is needed to follow up the natural resolution of pump twin cardiomyopathy. A study of the prognostic value of the Tei myocardial performance index in TRAP is warranted. Like many single center reports of uncommon diseases, the number of patients in our study is small. All of our referred patients had 1 or more poor prognostic risk factors warranting intervention. This likely represents an ascertainment bias as those with favorable prognosis may not be referred to a center capable of invasive fetal therapy. Nevertheless, we conclude that intrafetal RFA is a highly efficacious treatment for TRAP sequence.

REFERENCES
Do vaginal birth after cesarean outcomes differ based on hospital setting?

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OBJECTIVE: The objective of the study was to test the null hypothesis that outcomes of vaginal birth after cesarean (VBAC) do not differ on the basis of the hospital setting.

STUDY DESIGN: The study was a retrospective cohort study of women who were offered VBAC in 17 hospitals from 1996 to 2000. VBAC attempts occurring in hospitals with and without obstetrics-gynecology residency programs were compared, as were outcomes from university and community hospitals. Bivariate and multivariate logistic regression analyses assessed the association between hospital setting and VBAC outcomes.

RESULTS: Of 25,065 women with 1 or more prior cesareans, the VBAC attempt rate was 56.1% at hospitals with obstetrics-gynecology residency programs, 51.3% at hospitals without obstetrics-gynecology residency programs, 61% at university hospitals, and 50.4% at community hospitals. The occurrence of failed VBAC, blood transfusion, or composite adverse outcome did not differ by hospital setting. There was a significant increase in the uterine rupture rate at community (1.2%) vs university hospitals (0.6%), but the absolute risk remained low.

CONCLUSION: The rate of VBAC-associated complications is low, independent of hospital setting.

Key words: cesarean delivery, community hospital, residency program, university hospital, vaginal birth after cesarean

As the rate of cesarean delivery has continued to increase over the past decade, the rate of vaginal birth after cesarean (VBAC) has progressively decreased. The concomitant increase in primary cesarean deliveries and decrease in VBAC attempts has driven the total cesarean rate to an all-time high of 30.2% in the United States in 2005. Despite efforts of obstetrical care providers to practice in a manner that optimizes the likelihood of vaginal delivery and limits the cesarean delivery rate, the rate has risen 46% since 1996.1,2 We continue to be acutely cognizant of the rare but ever-present risks associated with an attempted trial of labor after cesarean. These risks have led numerous birth centers, especially in the community hospital setting, to discontinue the practice of offering planned VBAC altogether.

Little is known about the differences in VBAC outcomes of women delivering in various hospital settings. One could hypothesize that VBACs are attempted more commonly in university and teaching hospitals because of more timely accessibility of obstetric and anesthesia support services in the event of a complication. It is possible that women choosing to attempt VBAC in academic centers are inherently at higher risk of complications because of the high-risk population of patients who deliver in these institutions. Likewise, it is also possible that women choosing to attempt VBAC in community birth centers are more highly selected to be at the lowest risk of complications (ie, only 1 prior cesarean) and fewer medical comorbidities. Despite these assumptions, it is unknown whether the rate of VBAC failure or VBAC complications (uterine rupture, hemorrhage, and damage to surrounding organs) differs between these types of hospitals. This study was undertaken to assess the risks associated with VBAC in several hospital settings, university and community hospitals, and hospitals with and without obstetrics-gynecology residency programs.

MATERIALS AND METHODS
This is a secondary analysis from a large retrospective cohort of women with 1 or more prior cesarean delivery to assess maternal outcomes associated with vaginal birth after cesarean.3 Data were collected from the medical records of women who delivered in 17 hospitals from 1996 to 2000. Sixteen of these hospitals were located in the Delaware Valley (southeastern Pennsylvania and Delaware), and the other was located in Rhode Island. In an effort to gain better insight into VBAC outcomes across populations, a variety of hospital types (university, community, and hospitals with and without obstetrics-gynecology residency programs) was included in the study. Among the 17 participating centers, 6 were university hospitals, 5 were community hospitals with obstetrics-gy-
A team of trained nurse data extractors reviewed the medical records of the women identified by the ICD code-based search. Data were collected from the medical records using standardized, closed-ended data collection forms. Data abstracted from the medical records included demographics, social history, preexisting medical conditions, medical complications of pregnancy, and intrapartum and postpartum events. Approximately 3% of the medical records were reabstracted at a later date for quality assurance purposes. All medical records with identified cases of uterine rupture were reviewed and confirmed by the principal investigator (G.A.M.).

VBAC attempts were defined as women with a prior cesarean delivery who underwent a trial of labor, regardless of the mode of delivery of the pregnancy being analyzed. Failed VBAC was defined as cases in which a trial of labor occurred, but the mode of delivery of the index pregnancy was a cesarean section. Uterine rupture was defined as complete separation of the uterine scar confirmed at laparotomy that occurred in the presence of a preceding nonreassuring fetal heart rate pattern, maternal signs/symptoms of acute hemorrhage (hypotension, tachycardia), or the visualization of blood in the maternal peritoneal cavity. The composite adverse outcome variable included cases of uterine rupture; uterine artery laceration; and bladder, ureter, or bowel injury.

For this secondary analysis, we stratified the study population by planned delivery mode (VBAC vs repeat cesarean) and initially subdivided the cohort into 2 groups: women who attempted VBAC in a university hospital and those who attempted VBAC in a community hospital. Demographic characteristics of the 2 groups were analyzed using unpaired Student t tests for continuous variables (maternal age, gestational age at birth, and birthweight) and χ² or Fisher exact tests for categorical variables. Bivariate techniques were then utilized to assess the association between maternal outcomes (VBAC attempt, failed VBAC, uterine rupture, maternal blood transfusion, and composite adverse outcome) and the hospital setting in which the VBAC attempt occurred (university vs community hospitals).
community hospital). Maternal outcomes were expressed as unadjusted relative risks with 95% confidence intervals.

Logistic regression models were constructed to assess the association of hospital type with maternal VBAC outcomes. First, bivariate techniques were used to identify factors significantly associated with the outcome of interest. These potential confounding factors were then included in the logistic regression model for each maternal outcome. Final regression models were established by adding or removing covariates and testing for differences between hierarchical models using the likelihood ratio test or Wald test. The final explanatory models for the study outcomes were those that included the most significant and parsimonious variables. Adjusted odds ratios (adjOR) and 95% confidence intervals were then reported for each of the maternal outcomes.

The analysis of VBAC outcomes based on delivery in hospitals with and without obstetrics-gynecology residency programs was then performed in the same manner. Statistical analysis was performed using STATA software (version 8, special edition, Stata Corp, College Station, TX).

**RESULTS**

The individual characteristics of the 17 hospitals included in this study are demonstrated in Table 1. The majority of university hospitals had 24 hour in-house anesthesia support services, 24 hour blood bank support, neonatal intensive care facilities, availability of a maternal-fetal medicine specialist on staff, and 24-hour in-house coverage by an obstetrics-gynecology physician. The community hospitals with obstetrics-gynecology residency programs had similar access to support services, but those without obstetrics-gynecology residency programs were more variable with respect to support service access. Tables 2 and 3 outline the demographic characteristics of the study population. The cohort analyzed included 25,065 women with 1 or more prior cesarean delivery. Of those, 10,229 women (40.8%) delivered in university hospitals, 14,836 (59.2%) in community hospitals, 18,240 (72.8%) in hospitals with an obstetrics-gynecology residency program, and 6825 (27.2%) in hospitals without. Women who delivered in university hospitals and hospitals with an obstetrics-gynecology residency program had a higher gravidity, but lower maternal age, gestational age at birth, and birthweight, compared with community hospitals and hospitals without obstetrics-gynecology residency programs, respectively. Women delivering in university hospitals and hospitals with an obstetrics-gynecology residency program were also more likely to be cigarette smokers; have gestational hypertension, preeclampsia, and gestational diabetes; and were less likely to be of white race and undergo an induction of labor. There were more women with chronic hypertension who delivered at university hospitals than community hospitals but fewer at hospitals with an obstetrics-gynecology residency program than without. More preeclampsia deliveries occurred in university hospitals and hospitals with obstetrics-gynecology residency programs. There were no significant differences in the number of women with more than 1 prior cesarean based on the hospital setting at which they delivered.

The cohort of 25,065 women with 1 or more prior cesarean in this study included 13,698 who underwent a VBAC attempt and 11,367 who underwent an elective repeat cesarean. Of those, there were 6234 VBAC attempts at university hospitals, 7464 at community hospitals, 10,206 at hospitals with an obstetrics-gynecology residency program, and 3492 at hospitals without (Tables 4 and 5). Of the 17 medical centers included in this study, 6 were university hospitals, 11 were community hospitals, 11 hospitals had an obstetrics-gynecology residency program, and 6 did not.

Table 4 demonstrates the VBAC outcomes of women who delivered in university vs community hospitals during the study period. VBACs were attempted 32% more often in university than community hospitals. Women attempting VBAC were equally likely to have a failed attempt in university and
TABLE 3
Demographic characteristics by hospital type: teaching vs nonteaching facility

<table>
<thead>
<tr>
<th>Hospital type</th>
<th>Ob/Gyn residency (n = 18,240)</th>
<th>No residency (n = 6825)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>30.6 ± 5.6</td>
<td>31.8 ± 4.6</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Gravity*</td>
<td>3.6 ± 1.9</td>
<td>3.2 ± 1.4</td>
<td>&lt; .001</td>
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<tr>
<td>Gestational age at birth (wks)*</td>
<td>38.2 ± 2.7</td>
<td>38.8 ± 2.7</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Birthweight (g)*</td>
<td>3295 ± 705</td>
<td>3525 ± 590</td>
<td>&lt; .001</td>
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<tr>
<td>Maternal white race (%)</td>
<td>53</td>
<td>83.5</td>
<td>&lt; .001</td>
</tr>
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<td>Cigarette smoking (%)</td>
<td>18.9</td>
<td>15.7</td>
<td>&lt; .001</td>
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<td>More than 1 prior uterine scar (%)</td>
<td>19.3</td>
<td>19.4</td>
<td>.194</td>
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<tr>
<td>Preterm delivery less than 37 wks (%)</td>
<td>12.2</td>
<td>4.0</td>
<td>&lt; .001</td>
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<tr>
<td>Induction of labor (%)</td>
<td>13.4</td>
<td>17.1</td>
<td>.004</td>
</tr>
<tr>
<td>Pitocin therapy (%)</td>
<td>21.4</td>
<td>23.8</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Chronic hypertension (%)</td>
<td>3.7</td>
<td>23.8</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Gestational hypertension (%)</td>
<td>4.6</td>
<td>3.1</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Preeclampsia (%)</td>
<td>3.4</td>
<td>1.4</td>
<td>&lt; .001</td>
</tr>
<tr>
<td>Gestational diabetes (%)</td>
<td>5.9</td>
<td>5.9</td>
<td>.890</td>
</tr>
<tr>
<td>Pregestational diabetes (%)</td>
<td>1.8</td>
<td>0.8</td>
<td>&lt; .001</td>
</tr>
</tbody>
</table>

Ob/Gyn, obstetrics-gynecology.
* Continuous variables expressed as mean ± SD.

TABLE 4
Maternal outcomes for VBAC attempts in university vs community hospitals

<table>
<thead>
<tr>
<th>Outcome</th>
<th>University hospital</th>
<th>Community hospital</th>
<th>Unadjusted RR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or more prior cesarean, no. total</td>
<td>n = 10,229</td>
<td>n = 14,836</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VBAC attempt</td>
<td>61%</td>
<td>50.3%</td>
<td>0.83 (0.81 to 0.85)</td>
<td>0.68 (0.64 to 0.72)*</td>
</tr>
<tr>
<td>VBAC attempt outcomes</td>
<td>n = 6234</td>
<td>n = 7464</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failed VBAC</td>
<td>1,503 (24.1%)</td>
<td>1,862 (24.9%)</td>
<td>1.03 (0.98 to 1.10)</td>
<td>0.99 (0.90 to 1.08)†</td>
</tr>
<tr>
<td>Uterine rupture</td>
<td>40 (0.6%)</td>
<td>88 (1.2%)</td>
<td>1.84 (1.27 to 2.67)</td>
<td>1.60 (1.09 to 2.33)‡</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>46 (0.7%)</td>
<td>51 (0.7%)</td>
<td>0.93 (0.62 to 1.38)</td>
<td>0.96 (0.64 to 1.43)§</td>
</tr>
<tr>
<td>Composite adverse outcome**</td>
<td>136 (2.2%)</td>
<td>164 (2.2%)</td>
<td>1.01 (0.80 to 1.26)</td>
<td>1.00 (0.78 to 1.29)‖</td>
</tr>
</tbody>
</table>

CI, confidence interval; OR, odds ratio; RR, relative risk.
* The logistic regression models contain the following covariates: maternal age, race, twin gestation, >1 prior cesarean delivery, birthweight, gestational age at birth, chronic hypertension, and gestational diabetes.
† The logistic regression models contain the following covariates: placental abruption, birthweight, chronic hypertension, gestational diabetes, preeclampsia, induction of labor, and oxytocin therapy in labor.
‡ The logistic regression models contain the following covariates: maternal age, placental abruption, >1 prior cesarean delivery, and oxytocin therapy in labor.
§ The logistic regression models contain the following covariates: maternal age, placental abruption, induction of labor, and birthweight.
‖ The logistic regression models contain the following covariates: maternal age, placental abruption, induction of labor, and birthweight.
* The logistic regression models contain the following covariates: composite adverse outcome includes uterine rupture, uterine artery laceration, and bladder, ureter, or bowel injuries.
Concern borne from many case reports of VBAC-associated uterine rupture has led the obstetrical community to become increasingly concerned regarding the safety of VBAC in community hospitals over the past decade. The American College of Obstetricians and Gynecologists’ most recent recommendation regarding vaginal birth after previous cesarean delivery states that “because uterine rupture may be catastrophic, VBAC should be attempted in institutions equipped to respond to emergencies with physicians immediately available to provide emergency care.” This concern has caused many birth centers to discontinue the practice of planned trials of labor after cesarean. The changing practice pattern is certainly a contributor to the rising cesarean delivery rate in the United States.

The increasing rate of cesarean delivery, declining rate of VBAC, and concern with regard to the potentially catastrophic complications associated with a VBAC attempt cause obstetrical care providers to closely scrutinize which candidates and environments are optimal for a safe trial of labor in women with a prior cesarean scar. Recent studies from this large cohort of women and others have demonstrated that some subgroups of women with a prior cesarean are at lower risk of uterine rupture and other serious complications of VBAC than previously recognized. The large size of the cohort of women with 1 or more prior cesareans in this study, as well as the heterogeneity of hospital types in which they delivered, makes it an optimal group in which to analyze the effect of hospital setting on VBAC outcomes.

Although it is clearly prudent to select VBAC candidates cautiously, our study suggests that the rate of complications with VBAC largely do not differ by hospital setting. We find that women attempting VBAC have low complication rates, regardless of the hospital setting in which they deliver. The risks of VBAC failure, maternal blood transfusion, and composite adverse outcome were similar whether the VBAC attempt took place in a community hospital or a hospital without an obstetrics-gynecology residency program. The risk of uterine rupture associated with a VBAC attempt was also not influenced by whether the delivering hospital had an obstetrics-gynecology residency program.

Women undergoing a VBAC attempt in university vs community hospitals were more likely to be high-risk (ie, have higher rates of chronic hypertension, preeclampsia, diabetes, tobacco use, and preterm birth). Despite these distinct population differences, we did find that the rate of uterine rupture was significantly lower in university hospitals, compared with community hospitals. The overall risk remained low, although with an absolute risk difference of only 0.6%. This may be important information for hospitals to consider as they plan their scope of obstetrical care.

One limitation is that our study did not analyze the effect of hospital setting on women attempting VBAC who are at especially low risk of uterine rupture, such as those with a prior vaginal delivery or with a single prior low-transverse cesarean. It is likely that these women who are at especially low risk will have optimal outcomes, regardless of the hospital setting in which they deliver. Likewise, women who are at higher risk of maternal complications associated with a VBAC attempt, such as those with multiple prior low transverse hysterotomies or women undergoing induction of labor with multiple labor-stimulating agents are likely to be at higher risk of complications in any hospital setting. These women with risk factors for VBAC complications may be better served to plan delivery in hospitals with the capabilities to rapidly respond in the event of a catastrophic outcome. Also, it is possi-

### TABLE 5

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Residency</th>
<th>No residency</th>
<th>Unadjusted RR (95% CI)</th>
<th>Adjusted OR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or more prior cesareans, no. total</td>
<td>n = 18,240</td>
<td>n = 6,825</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VBAC attempt</td>
<td>56%</td>
<td>51.2%</td>
<td>0.91 (0.89, 0.93)</td>
<td>0.88 (0.82, 0.95)*</td>
</tr>
<tr>
<td>VBAC attempt outcomes</td>
<td>n = 10,206</td>
<td>n = 3,492</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Failed VBAC</td>
<td>2,481 (24.3%)</td>
<td>884 (25.3%)</td>
<td>0.96 (0.90 to 1.02)</td>
<td>1.04 (0.93 to 1.16)*</td>
</tr>
<tr>
<td>Uterine rupture</td>
<td>96 (0.9%)</td>
<td>32 (0.9%)</td>
<td>0.97 (0.65 to 1.45)</td>
<td>0.86 (0.57 to 1.29)*</td>
</tr>
<tr>
<td>Blood transfusion</td>
<td>70 (0.7%)</td>
<td>27 (0.8%)</td>
<td>1.13 (0.72 to 1.75)</td>
<td>1.17 (0.75 to 1.83)*</td>
</tr>
<tr>
<td>Composite adverse outcome†</td>
<td>226 (2.2%)</td>
<td>74 (2.1%)</td>
<td>0.96 (0.74 to 1.24)</td>
<td>0.91 (0.67 to 1.23)*</td>
</tr>
</tbody>
</table>

CI, confidence interval; Ob/Gyn, obstetrics-gynecology; OR, odds ratio; RR, relative risk.
* The logistic regression model contains the following covariates: maternal age, race, twin gestation, more than 1 prior cesarean delivery, birthweight, gestational age at birth, chronic hypertension, and gestational diabetes.
† The logistic regression model contains the following covariates: placental abruption, birthweight, chronic hypertension, gestational diabetes, preeclampsia, induction of labor, and oxytocin therapy in labor.
‡ The logistic regression model contains the following covariates: maternal age, placental abruption, more than 1 prior cesarean delivery, and oxytocin therapy in labor.
§ The logistic regression model contains the following covariates: twin gestation, placental abruption, and preeclampsia.
¶ The logistic regression model contains the following covariates: maternal age, placental abruption, induction of labor, and birthweight.
* Composite adverse outcome includes uterine rupture, uterine artery laceration; and bladder, ureter, or bowel injuries.
ble that the community and nonteaching hospitals in our analysis may not be repre-
sentative of other hospitals of the same type across the country, many of which
may not have similar availability of phys-
cians and support services to respond
in case of emergencies.

Another limitation of our study is the
effect of selection bias on maternal out-
comes. This analysis could not account
for the inherent differences that exist be-
tween women who choose a VBAC at-
tempt vs elective repeat cesarean delivery
or those who choose to deliver in various
hospital types.

We conclude the rate of VBAC-asso-
ciated complications is low, regardless
of hospital setting. Significant differ-
ences do exist among hospitals of the
same type with regard to their individ-
ual capabilities to respond to the emer-
gent complications that can occur with
VBAC. Planning VBAC in specific in-
stitutions should be considered indi-
vidually, based on patient risk factors
and the individual hospital’s ability to
respond expeditiously in the event of a
complication.

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a viable option? Am J Obstet Gynecol
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repeat cesarean safer in women with a prior
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with multiple and single prior cesarean delivery.
Case-control comparison of cesarean hysterectomy with and without prophylactic placement of intravascular balloon catheters for placenta accreta

Vineet Shrivastava, MD; Michael Nageotte, MD; Carol Major, MD; Michael Haydon, MD; Deborah Wing, MD

OBJECTIVE: The objective of the study was to compare outcomes of women with placenta accreta who underwent cesarean hysterectomy with and without prophylactic intravascular balloon catheters.

STUDY DESIGN: Case-control study of women at risk for placenta accreta identified using hospital databases and billing records from January 1995 to January 2006. Subjects with preoperative intravascular balloon catheter (BC) placement plus hysterectomy were compared with those that had hysterectomy alone.

RESULTS: Sixty-nine subjects had cesarean hysterectomy performed for placenta accreta; 19 subjects had balloon catheters plus hysterectomy and 50 subjects had hysterectomy alone. No significant differences were noted in estimated blood loss ($P = .79$), transfused blood products ($P = .60$), operative time ($P = .85$), and postoperative hospital days ($P = .85$). There were no significant differences in secondary outcomes between groups. Three of the 19 BC subjects (15.8%) had complications from catheter placement; 2 required stent placement and/or arterial bypass.

CONCLUSION: Prophylactic intravascular balloon catheters did not benefit women with placenta accreta undergoing cesarean hysterectomy.

Key words: cesarean hysterectomy, intravascular balloon catheter, placenta accreta


Placenta accreta and its variants, increta or percreta, represent 1 of the most challenging situations confronted by obstetricians. The rising rate of cesarean delivery appears to be directly linked to the increasing placenta previa incidence. In turn, there has been an increase in the incidence of placenta accreta. Miller et al reported a 10-fold increase in placenta accreta over the past 50 years with a relative frequency of 1 per 2500 deliveries. More recently Wu et al observed an incidence of 1 in 533 at their institution between the years of 1982 and 2000. Risk factors include not only previous cesarean deliveries and placenta previa but also multiparity, advanced maternal age, prior myomectomy, and submucosal leiomyomata. Improvements in ultrasound technology and the implementation of other imaging modalities such as magnetic resonance imaging have allowed for antenatal diagnosis and the use of strategies to attempt to minimize blood loss and complications at time of delivery. The standard management of placenta accreta is cesarean delivery followed by hysterectomy.

The most common complication encountered in these cases is hemorrhage, attributed to the extensive collateral lateral circulation in the gravid uterus. Hudon et al reported in a recent case series of placenta accreta an average blood loss of 3000-5500 mL. Intraoperative blood loss may necessitate significant blood transfusion with the associated complications of disseminated intravascular coagulation (DIC), fluid overload, acute respiratory distress syndrome (ARDS) and infection. Other significant surgical morbidities include ureteral injury, bowel trauma, and bladder lacerations. In an effort to minimize blood loss and facilitate surgery, novel approaches have been described. One such approach is the use of balloon catheters for occlusion and/or embolization of the pelvic vasculature (Figure). The theory is that reduced uterine perfusion allows for a more controlled hysterectomy with decreased hemorrhage and surgical complications. To date however, the literature regarding this modality has been limited to descriptive reports and small case-control comparisons.

The purpose of this study was to compare outcomes of women who underwent cesarean hysterectomy for placenta accreta with and without the use of prophylactic occlusive balloon catheters.

MATERIALS AND METHODS
This was a retrospective, case-controlled study performed at the University of California Irvine Medical Center and the Women’s Pavilion at Long Beach Memorial Medical Center/Miller Children’s Hospital from January 1995 to January 2006.

2006. Investigational review board approval at each institution was obtained. Subjects were identified through billing records and clinical databases using the following diagnoses and procedures codes: cesarean hysterectomy, hysterectomy at cesarean delivery, placenta previa, placenta accreta, placenta percreta, and placenta increta. Inclusion criteria for the study included subjects that had cesarean hysterectomy performed for a presumed placenta accreta or 1 of its variants. Subjects were excluded if they had cesarean hysterectomy performed for other indications such as uterine atony, cervical dysplasia, cervical cancer, or uterine rupture.

The subjects were subdivided into categories based on the preoperative placement of occlusive balloon catheters into the anterior division of the internal iliac arteries. Subjects were identified either sonographically or by obstetrical history with risk factors for placenta accreta such as placenta previa and/or previous cesarean delivery. Control subjects were those who had cesarean hysterectomy alone (HA) for presumed placenta accreta. Women who required emergency hysterectomy at time of cesarean delivery for newly diagnosed placenta accreta were included in the control group.

Study subjects were those who had predelivery placement of occlusive balloon catheters (BC). On the day of delivery, the patient was taken to the angiography suite by interventional radiology, in which under fluoroscopic guidance, catheterization of the common femoral artery was performed. A bilateral contralateral approach was used to guide placement of the occlusive balloon catheters (Boston Scientific, Watertown, MA) into the internal iliac arteries and their anterior divisions. The catheters were secured once proper confirmation of placement was made by fluoroscopy, and the patient was subsequently taken directly to the operating room for cesarean delivery. After delivery of the infant, the balloon catheters were inflated in all subjects and a supracervical or total hysterectomy was performed. The balloons were usually deflated intraoperatively to assure that hemostasis was achieved after the uterus had been removed. However, catheter removal was reserved until postoperative day 1 and was dependent on the patient’s hemodynamic status and coagulation profile.

Primary outcomes for this study included estimated blood loss (milliliters), number of units of total blood products received (packed red blood cells and fresh frozen plasma), total operative time in minutes, and number of postoperative hospital days. Secondary outcomes included the development of DIC, febrile morbidity, postoperative ileus, wound complications, and need for reoperation. Statistical analysis for the primary outcomes was performed using the Mann-Whitney U test. The association between interventions and discrete variables in the secondary outcomes were analyzed using the Fisher exact test. Statistical analysis was performed with SPSS software (version 12.0; SPSS Inc, Chicago, IL), and significance was considered at a probability value of less than .05.

**RESULTS**

From the billing records and hospital databases, 156 subjects were identified as having cesarean hysterectomy from January 1995 to January 2006. Eighty-seven subjects were excluded for having hysterectomies performed for uterine atony, uterine rupture, or cervical dysplasia. Sixty-nine subjects were identified as having hysterectomies at time of cesarean delivery for abnormal placentation. Of these, 19 subjects had preoperative balloon catheter placement, whereas 50 subjects had HA.

Maternal and fetal demographic characteristics for both groups of subjects are listed in **Table 1**. Although there were essentially no differences noted in maternal age between the groups, there were significant differences noted in ethnicity and the number of prior cesarean deliveries performed between the groups. The HA group had higher incidence of women with no prior cesarean delivery; however, the BC group had a greater proportion of subjects with 4 or more prior cesarean deliveries.

Not unexpectedly, the method of diagnosis of placenta accreta or its subtypes varied considerably between the groups. In the HA group, placenta accreta diagnosis was made by ultrasound (16%), obstetrical history (46%), and intraoperative findings (38%). The BC group was identified solely by ultrasound (52%) or clinical history (48%). There were no differences in the final pathology results between the groups.

In terms of the primary surgical outcomes of median estimated blood loss, numbers of units of blood products transfused, duration of surgery, or median number of postoperative hospital days, no statistical differences were observed between the 2 groups (**Table 2**). Similarly, no significant differences were demonstrated in any of the secondary outcomes evaluated. The development of DIC in the BC group (26% or 5 of 19) was statistically similar to that of the HA group (20% or 10 of 50) \( P = .40 \). The incidence of postoperative ileus was just as likely in the BC subjects 26% (5 of 19) as they were in the HA subjects 20% (10 of 50) \( P = .32 \). Febrile morbidity likewise was similar between the groups (BC 42% [8 of 19] vs HA 26% [13 of 50], \( P = .15 \)). The manifestation of wound complications in the BC group was 11% (2 of 19) as compared with HA group of 6% (3 of 50) \( P = .42 \). The need for reoperation was observed to be statistically similar between the groups.
### TABLE 1
Demographic characteristics

<table>
<thead>
<tr>
<th></th>
<th>BC plus hysterectomy (n = 19)</th>
<th>HA (n = 50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>33 ± 4.7</td>
<td>34 ± 6.4</td>
<td>.71</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Caucasian</td>
<td>7 (37%)</td>
<td>13 (26%)</td>
<td>.01*</td>
</tr>
<tr>
<td>Hispanic</td>
<td>9 (47%)</td>
<td>27 (54%)</td>
<td>.79</td>
</tr>
<tr>
<td>African American</td>
<td>1 (5%)</td>
<td>6 (12%)</td>
<td>.66</td>
</tr>
<tr>
<td>Asian</td>
<td>2 (11%)</td>
<td>4 (8%)</td>
<td>.30</td>
</tr>
<tr>
<td>Gravidity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 or fewer</td>
<td>8 (42%)</td>
<td>28 (56%)</td>
<td>.41</td>
</tr>
<tr>
<td>5 or more</td>
<td>11 (58%)</td>
<td>22 (44%)</td>
<td>.41</td>
</tr>
<tr>
<td>Parity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 or fewer</td>
<td>17 (89.5%)</td>
<td>44 (88%)</td>
<td>1.00</td>
</tr>
<tr>
<td>5 or more</td>
<td>2 (10.5%)</td>
<td>6 (12%)</td>
<td>.13</td>
</tr>
<tr>
<td>Type of gestation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Singleton</td>
<td>17 (96%)</td>
<td>48 (90%)</td>
<td>.30</td>
</tr>
<tr>
<td>Twin</td>
<td>2 (4%)</td>
<td>2 (10%)</td>
<td>.13</td>
</tr>
<tr>
<td>Number of previous cesareans</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td>1 (5.3%)</td>
<td>9 (18%)</td>
<td>.26</td>
</tr>
<tr>
<td>1</td>
<td>5 (26.3%)</td>
<td>15 (30%)</td>
<td>1.00</td>
</tr>
<tr>
<td>2-3</td>
<td>8 (42%)</td>
<td>23 (46%)</td>
<td>.79</td>
</tr>
<tr>
<td>4 or more</td>
<td>5 (26.3%)</td>
<td>3 (6%)</td>
<td>.03*</td>
</tr>
<tr>
<td>Foundation for diagnosis</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ultrasound examination</td>
<td>10 (52%)</td>
<td>8 (16%)</td>
<td>.02*</td>
</tr>
<tr>
<td>Clinical suspicion</td>
<td>9 (48%)</td>
<td>23 (46%)</td>
<td>.29</td>
</tr>
<tr>
<td>Intraoperative findings</td>
<td>N/A</td>
<td>19 (38%)</td>
<td>N/A</td>
</tr>
<tr>
<td>Placenta previa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Previa present</td>
<td>16 (84%)</td>
<td>37 (74%)</td>
<td>.52</td>
</tr>
<tr>
<td>No placenta previa</td>
<td>3 (16%)</td>
<td>13 (26%)</td>
<td></td>
</tr>
<tr>
<td>Placental pathology</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Accreta</td>
<td>13 (68%)</td>
<td>36 (72%)</td>
<td>.77</td>
</tr>
<tr>
<td>Increta</td>
<td>4 (21%)</td>
<td>8 (16%)</td>
<td>.72</td>
</tr>
<tr>
<td>Percreta</td>
<td>2 (11%)</td>
<td>6 (12%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Type of hysterectomy performed</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total hysterectomy</td>
<td>15 (79%)</td>
<td>41 (82%)</td>
<td>.74</td>
</tr>
<tr>
<td>Supracervical hysterectomy</td>
<td>4 (21%)</td>
<td>9 (18%)</td>
<td>.74</td>
</tr>
<tr>
<td>Mean gestational age (wks)</td>
<td>35.3 ± 1.8</td>
<td>33.6 ± 4.8</td>
<td>.12</td>
</tr>
<tr>
<td>Apgar scores</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 min less than 7</td>
<td>2 (10.6%)</td>
<td>13 (26%)</td>
<td>.20</td>
</tr>
<tr>
<td>5 min less than 7</td>
<td>1 (5.3%)</td>
<td>7 (14%)</td>
<td>.43</td>
</tr>
<tr>
<td>Mean birthweight (g)</td>
<td>2872 ± 629</td>
<td>2127 ± 917</td>
<td>.02</td>
</tr>
</tbody>
</table>

Data presented as mean ± SD or n (%).
* Indicates significant values.

---

with the BC group at 21% (4 of 19) vs the HA group at 12% (6 of 50).

It can be inferred that women who have emergency cesarean hysterectomy for intraoperatively diagnosed placenta accreta have the potential for significantly more blood loss when compared with those who were sonographically identified or clinically suspected prior to delivery. This could be attributed to delay in diagnosis and initiation of definitive surgery. It could therefore be hypothesized that intraoperatively discovered placenta accetas would skew HA group toward more blood loss. However, excluding intraoperatively diagnosed subjects revealed no differences in the primary outcomes (Table 3) or secondary measures.

In the 19 subjects who had prophylactic balloon catheter placement, 3 subjects (15.8%) had severe complications directly related to the intervention. One patient was noted to have an internal iliac artery thrombosis and groin hematoma that resolved with expectant management. She remained hospitalized for 11 days. A second patient had an internal iliac artery dissection with 80–90% occlusion. A third patient had a femoral artery thrombosis. Both of these patients required iliofemoral bypass surgery for persistence of symptoms.

### Comment

In this case control study, we present data on 19 subjects who had intravascular balloon catheter placement and deployment, which represents the largest cohort of such cases reported. We failed to demonstrate statistically significant differences in either the primary or the secondary surgical outcomes measured. This comparison was with a cohort of patients undergoing cesarean hysterectomy for placenta accreta. No differences were discovered when comparing these 19 women with the total group without balloon catheters or when removing those cases in which hysterectomy was performed emergently for intraoperatively diagnosed placenta accreta.

Failure of occlusive balloons to reduce blood loss from hysterectomy may be explained by the degree of uterine blood...
flow with pregnancy and the extensive vascular anastomoses present in the gravid pelvis. Whereas reduction of blood flow to the uterine arteries likely occurs following balloon inflation in the hypogastric arteries, collateral circulation from cervical, ovarian, rectal, femoral, lumbar, and sacral arteries likely contributes to the overall blood loss.\(^7,8\)

Indeed, routine inflation of the balloons immediately following delivery of the infant may actually exacerbate collateral blood flow. Kidney et al\(^9\) theorized that despite the presence of pelvic vessel anastomoses around the site of catheter placement, the presence of distended balloons will reduce arterial pressure to that of venous pressure. It is by this means that the technique would be effective in controlling hemorrhage and promoting clot formation.\(^9\) Our data do not support this hypothesis.

In addition to lack of benefit of occlusive balloon catheters, we found that there may also be harm because 3 of 19 subjects (15.8\%) had complications directly associated with the placement, deployment, or removal of balloon catheters. There are other reports by Ojala et al\(^10\) of 3 of 22 cases with complications following catheterization for obstetric hemorrhage and Sewell et al\(^11\) of a case of thrombus formation in the common iliac artery resulting from prophylactic use of endovascular catheters. It is difficult to ascertain the exact cause for the complications seen in our investigation. Possible contributing factors included those related to technique, operator experience, duration of catheter placement procedure, number of attempts, and inadvertent luminal trauma, among others and those inherent to the patients, including hemodynamic stability and presence of coagulopathy or its aggressive correction.

Other surgical complications observed included bowel injury (1 subject in each group), incidental ureteral injury (1 subject in the HA group with placenta percreta), and bladder injury (3 subjects in the BC group, 2 in the HA group).

This study highlights the importance of evaluating novel strategies for optimal management for cesarean hysterectomies. The National Center for Health Statistics reports that the cesarean rate in the United States for 2005 reached a new peak of 30.2\%.\(^12\) With this rise, an inevitable consequence has been an increase in the incidence of placenta accreta in all of its forms as well as an increase in cesarean hysterectomies. At our institutions between the years of 1995 and 2006, there were 76,087 deliveries. In these patients, we observed 69 cesarean hysterectomies performed for the cases that were clinically suspicious for placenta accreta or 1 of its variants. This represents an incidence of 1 in 1100. Cesarean hysterectomy is 1 of the most morbid procedures in obstetrics. Miller et al\(^2\) in a study of 62 placenta accretas in a 10 year time period reported 24.5\% of subjects with accretas had reported blood loss exceeding 5 L. Other complications of placenta accreta and its treatment include coagulopathy, infection, multiple transfusions of blood products, allergic reactions, infection, and ARDS.

With the routine use of ultrasound, antenatal diagnosis of placenta accreta is possible, which allows for planning to attempt reduction of the morbidity associated with this diagnosis. Specifically, radiological interventions have been used in an effort to reduce blood loss. Two different techniques have been described. The first involves placement of intravascular catheters with occlusive balloons into the pelvic vasculature to limit blood flow to the uterus. The second technique involves the placement of catheters with or without occlusive balloons and embolization of the uterine vasculature with microparticles, powder, pledgets, or coils following delivery of the infant and prior to proceeding with a hysterectomy.\(^13-16\)

The successful use of preoperative occlusive balloon catheters for cesarean hysterectomy has been described in a few case reports\(^17,18\) and a small prospective cohort study.\(^19\) The existing literature is limited by small sample sizes, retrospective analyses, and lack of randomized, controlled trials. In 1 series of 5 subjects undergoing prophylactic placement of occlusive balloon catheters in anticipation of cesarean hysterectomy, the mean estimated blood loss observed was approximately 2000 mL [range 1100–4000 mL]. Three of the 5 subjects required blood transfusions.\(^18\) Levine et al\(^19\) authored the only prospective cohort study of 5 subjects with occlusive balloons, compared with 4 subjects who un-

### TABLE 2

<table>
<thead>
<tr>
<th>Primary surgical outcomes</th>
<th>BC with hysterectomy (n = 19)</th>
<th>HA (n = 50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery duration (min)</td>
<td>182 (110-360)</td>
<td>180 (60-420)</td>
<td>.85</td>
</tr>
<tr>
<td>Estimated blood loss (mL)</td>
<td>2700 (800-8000)</td>
<td>3000 (600-7000)</td>
<td>.79</td>
</tr>
<tr>
<td>Blood products (U)</td>
<td>10 (0-43)</td>
<td>6.5 (0-50)</td>
<td>.60</td>
</tr>
<tr>
<td>Postoperative days</td>
<td>5 (4-11)</td>
<td>4 (3-44)</td>
<td>.85</td>
</tr>
</tbody>
</table>

Data presented as median with range.

### TABLE 3

<table>
<thead>
<tr>
<th>Primary surgical outcomes excluding intraoperatively diagnosed cases</th>
<th>BC with hysterectomy (n = 19)</th>
<th>HA (n = 31)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Surgery duration (min)</td>
<td>182 (110-360)</td>
<td>180 (75-405)</td>
<td>.95</td>
</tr>
<tr>
<td>Estimated blood loss (mL)</td>
<td>2700 (800-8000)</td>
<td>3000 (600-6000)</td>
<td>.90</td>
</tr>
<tr>
<td>Blood products (U)</td>
<td>10 (0-43)</td>
<td>8 (0-54)</td>
<td>.81</td>
</tr>
<tr>
<td>Postoperative days</td>
<td>5 (4-11)</td>
<td>5 (3-41)</td>
<td>.73</td>
</tr>
</tbody>
</table>

Data presented as median with range.
derwent hysterectomy alone. There was a lack of consistency in the placement of the occlusive balloons in the pelvic vasculature in these subjects. Additionally, no significant differences were noted in estimated blood loss, transfusion requirements, or length of hospitalization. 19

As an alternative to occlusive balloons, the preoperatively placed intravascular catheters may also be used for embolization following delivery of the fetus. The use of this technique for obstetrical purposes was first described by Alvarez et al 20 in a report of 9 subjects. They reported decreased mean blood loss, fewer transfusion requirements, and shortened postoperative hospital stay in women who underwent embolization, compared with a group of subjects who received emergent postoperative catheter placement alone. 20 However, they did not make comparisons with a control group that had cesarean hysterectomy alone without catheters. Dubois et al 21 described the use of occlusive balloon catheters plus embolization in 2 subjects with pathologically confirmed placenta accreta and reported blood loss ranging from 1500 to 2000 mL. 21 Bodner et al 22 presented a cohort study of 28 consecutive subjects with placenta accreta. Six had preoperative occlusive balloon catheter placement and embolization prior to initiating hysterectomy, and their outcomes were compared with 22 subjects who had hysterectomy alone. No differences were found in estimated blood loss, transfusion of blood products, operative time, or postoperative recovery. 22

One strength of our study is the number of patients with 4 or more cesarean sections being greater in women receiving balloon catheters. However, there were only 8 such patients, 5 in the BC group and 3 in the HA group. Lastly, this study did not assess the role of embolization in the setting of placenta accreta and its management

In summary, we were unable to demonstrate any difference in primary surgical outcomes or associated complications in patients receiving prophylactic intravascular balloon catheters. Our data are congruent with previous reports in which comparisons are made between endovascular intervention and control groups. Importantly 16% of subjects had severe complications directly related to the intravascular catheters. Given these facts and the absence of prospective randomized trials, the use preoperative balloon catheter for placenta accreta is not recommended.

ACKNOWLEDGMENTS

We acknowledge the contributions of Christine Preslicka, RN, and Pamela J. Rumney, RNC.

REFERENCES

Level of glycemic control and pregnancy outcome in type 1 diabetes: a comparison between multiple daily insulin injections and continuous subcutaneous insulin infusions

Rony Chen, MD; Avi Ben-Haroush, MD; Alina Weissman-Brenner, MD; Nir Melamed, MD; Moshe Hod, MD; Yariv Yogev, MD

OBJECTIVE: We aimed to compare glycemic control and pregnancy outcome in type 1 diabetic patients treated by 2 modes of treatment: multiple daily injections of insulin (MDI) and continuous subcutaneous insulin infusions (CSII).

STUDY DESIGN: In a retrospective, matched-control study, patients treated by MDI were compared with patients treated by CSII in a ratio of 2:1. Level of glycemic control and pregnancy outcome was compared.

RESULTS: Overall, 90 women were evaluated; of them 30 were treated by CSII and 60 by MDI. No between-group differences were found in maternal age, nulliparity rate, severity and duration of diabetes, prepregnancy body mass index, and weight gain during pregnancy.

CONCLUSION: In type 1 diabetes, glycemic control and pregnancy outcome are compromised, regardless of treatment modality. CSII may be associated with higher rate of both maternal DKA and neonatal hypoglycemic events.

Key words: glycemic control, insulin pump, pregnancy, type 1 diabetes

Achievement of the desired level of glycemic control at conception and during pregnancy is essential for attaining optimal pregnancy outcome in patients with type 1 diabetes. Although major progress took place in recent years in the treatment of women with type 1 diabetes during pregnancy, in most national audits, pregnancy outcome is associated with an increased risk of congenital malformations, perinatal mortality, obstetric complications, and neonatal morbidity.1-7

Recently, subcutaneous insulin infusion (CSII) or insulin pump therapy has been used as an alternative to multiple-dose insulin injection (MDI). It is recognized that both CSII and MDI offer the advantage of frequent dose adjustment, which should lead to optimum blood sugar level attainment. However, insufficient data exist concerning the use of these different methods of insulin administration during pregnancy8-13 in terms of normalizing blood sugar level, reducing hypo/hyperglycemia, and pregnancy outcome. Thus, we aimed to compare glycemic control and pregnancy outcome in women with type 1 diabetes.

MATERIALS AND METHODS Subjects
We conducted a retrospective, matched controlled study of 90 patients with type 1 diabetes who were followed up and treated during pregnancy between 2003 and 2006. For each patient treated with CSII (n = 30), 2 patients treated by MDI were matched (n = 60). Matching criteria included prepregnancy maternal body mass index (BMI; weight in kilograms divided by the square of the height in meters), duration and severity of type 1 diabetes (the presence of microvascular complications), preconception or early pregnancy (prior to 6 weeks of gestation) hemoglobin (Hgb) A1c levels, gravidity, and parity.

For all patients, diagnosis of type 1 diabetes was established at least 1 year prior to the studied pregnancy and allocation for treatment modality (MDI or CSII) was based on patient preference. All women treated with CSII initiated this mode of treatment prior to the studied pregnancy. Women who were transferred from MDI to CSII or vice versa during pregnancy as well as all multiple pregnancies were excluded. The study was approved by the local institutional review board.
Management approach and level of glycemic control assessment

Our department offers patients with diabetes an intensive prepregnancy treatment protocol for a minimum of 3 months, which includes folic acid supplementation (5 mg daily), instruction in self-administration of multiple insulin injections (4-6 daily), or use of the continuous subcutaneous insulin pump. In both treated groups, both recombinant insulin and analogs were used as needed. All women were instructed in the measurement of blood glucose with a memory reflectance meter to ascertain reliable glucose data 7 times daily: after an overnight fast, before meals, 2 hours after meals, and at bedtime. Hgb A1c levels are taken prior to pregnancy or during the first trimester and during the third trimester prior to delivery.

Subjects with proliferative retinopathy are referred for laser treatment when necessary, and those with proteinuria are prescribed angiotensin converting enzyme (ACE) inhibitors. ACE inhibitor treatment is discontinued immediately after a missed period and a positive pregnancy test and was not used during pregnancy. Preexisting hypertension is defined as a systolic blood pressure greater than 140 and/or diastolic blood pressure greater than 90 mm Hg or the need for antihypertensive drugs before pregnancy. Mean blood pressure, maternal weight, and the presence of proteinuria were assessed. Fetal testing included non–stress testing starting at 32 weeks of gestation, fetal movement, and velocity (Doppler) studies; fetal growth assessment; and biophysical profile when medically indicated.

Pre-eclampsia was defined as systolic blood pressure 140 mm Hg or greater and diastolic blood pressure 90 mm Hg or greater and proteinuria on at least 2 occasions, at least 6 hours apart after the 20th week of gestation. Gestational hypertension was defined as hypertension in the absence of proteinuria.15,16 Preterm labor was defined as delivery prior to 37 weeks of pregnancy.

Hypoglycemic events were defined as: (1) symptomatic hypoglycemic episode when blood glucose was less than 40 mg/dL; (2) significant hypoglycemic episode when blood glucose was less than 40 mg/dL in conjunction with an inability of patients to treat their own symptoms.

Pregnancy outcome and adverse events

All patients were evaluated on a weekly basis. In each prenatal visit, level of glycemic control was assessed and insulin therapy was modified when needed. Moreover, blood pressure, maternal weight, and the presence of proteinuria were assessed. Fetal testing included non–stress testing starting at 32 weeks of gestation, fetal movement, and velocity (Doppler) studies; fetal growth assessment; and biophysical profile when medically indicated.

Hypoglycemic events were defined as: (1) symptomatic hypoglycemic episode when blood glucose was less than 40 mg/dL. Combined with symptoms, such as confusion, poor coordination, double vision, headache, or combativeness and (2) significant hypoglycemic episode when blood glucose was less than 40 mg/dL. In conjunction with an inability of patients to treat their own symptoms.

Infants were considered large for gestational age (LGA) when birthweight was greater than the 90th percentile and small for gestational age (SGA) when birthweight was less than the 10th percentile based on growth standards developed for our population.17 Macrosomia was defined as delivery weight greater than 4000 g. Neonatal respiratory outcomes included the presence or absence of hyaline membrane disease, transient tachypnea (respiratory distress in infants born near term that lasts for about 3 days), and respiratory support. Metabolic complications were defined by the presence of 1 or more of the following: neonatal prefixed hypoglycemia (less than 40 mg/dL), polycythemia (hematocrit greater than 60%), hyperbilirubinemia (greater than 12 mg/dL), or hypocalcemia (less than 8 mg/dL).

Statistical analysis

Data analysis was performed with SPSS software (version 13.0, SPSS Inc., Chicago, IL). A Student t test was used to compare continuous variables between the groups, and a χ² test was used for categorical variables. A P value less than .05 was considered significant.

Results

Selected patient characteristics are presented in Table 1. Both studied groups were characterized by similar maternal age, nulliparity rate, severity and duration of diabetes, prepregnancy BMI, Hgb A1c level, and weight gain during pregnancy (Table 1).

Gestational age at delivery, rate of preterm delivery, mean birthweight, and cesarean delivery rate were similar in both groups (Table 2). No differences were found in fetal metabolic complications, but the rate of neonatal hypoglycemia was found to be significantly higher in the CSII group (36% vs 13%, P = .016) (Table 2).

No differences were found in the rate and severity level of maternal hypoglycemic events, hypertensive complications, or level of glycemic control as reflected by mean blood glucose and Hgb A1c taken prior to delivery (Table 3). Type 1 patients treated with CSII had a signifi-
cantly higher rate of diabetic ketoacidosis episodes (13% vs 1.6%, P = .04) (Table 3).

**COMMENT**

Pregnancy in women with type 1 diabetes mellitus is associated with an increased risk of congenital malformations, perinatal mortality, obstetric complications, and neonatal morbidity. Almost 17 years ago, the St. Vincent's declaration was established, which aimed to approximate pregnancy outcome of women with type 1 diabetes to that of the nondiabetic population, a goal fated to be achieved in 5 years. Nevertheless, emerging and growing data imply, though, more than 17 years afterward, that poor pregnancy outcome is obtained among women with type 1 diabetes.

The goal of management in pregnancy complicated by diabetes is to maintain blood glucose as near to normal as possible. Intensive therapy involves using memory-based self-monitoring blood glucose, multiple injections of insulin or CSII, diet, and an interdisciplinary team effort.

Regardless of the treatment strategy, the purpose of intensified therapy is to achieve the targeted level of glycemic control that diminishes the rate of hypoglycemia and ketosis and maximizes perinatal outcome. Although there is ample evidence associating glycemic control and the occurrence of maternal-fetal complications, association does not prove cause and effect. It does provide the rationale for glucose control, however.

It is recognized that both CSII and MDI offer the advantage of frequent insulin dose adjustment, which should lead to optimum blood sugar level attainment. It is less clear, however, because of the lack of any systematic meta-analysis or randomized studies during pregnancy, which method achieves the best outcome in terms of normalizing blood sugar level and reducing hyperglycemia to prevent associated complications for both mother and child.

Our study aimed to compare pregnancy outcome and level of glycemic control in patients with type 1 diabetes treated by CSII or MDI. Our main findings included the following: (1) glycemic control is compromised, regardless of treatment modality; (2) maternal and fetal outcomes of pregnant women with type 1 diabetes treated with MDI therapy are compared with those of women treated with CSII; (3) the rate of maternal hypoglycemia events (mild and severe) is similar with MDI and CSII therapy; and (4) CSII may be associated with a higher rate of both maternal diabetic ketoacidosis and neonatal hypoglycemic events.

### Table 1
Clinical characteristics of type 1 diabetic women, by treatment modality

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CSII (n = 30)</th>
<th>MDI (n = 60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)</td>
<td>29.6 ± 5.0</td>
<td>29.3 ± 4.9</td>
<td>.62</td>
</tr>
<tr>
<td>Duration of diabetes (y)</td>
<td>12.3 ± 5.2</td>
<td>13.4 ± 4.7</td>
<td>.32</td>
</tr>
<tr>
<td>Prepregnancy BMI (kg/m²)</td>
<td>24.7 ± 4.1</td>
<td>24 ± 4.2</td>
<td>.45</td>
</tr>
<tr>
<td>Obesity (%)</td>
<td>7 (23%)</td>
<td>13 (22%)</td>
<td>.32</td>
</tr>
<tr>
<td>Prepregnancy consultation (%)</td>
<td>16 (53%)</td>
<td>34 (56%)</td>
<td>.41</td>
</tr>
<tr>
<td>Gravidity</td>
<td>1.4 ± 0.8</td>
<td>1.7 ± 1.0</td>
<td>.25</td>
</tr>
<tr>
<td>Nulliparity rate (%)</td>
<td>43%</td>
<td>45%</td>
<td>.45</td>
</tr>
<tr>
<td>Weight gain (kg)</td>
<td>14.0 ± 5.2</td>
<td>11.7 ± 5.1</td>
<td>.11</td>
</tr>
<tr>
<td>Hgb A1c (%)</td>
<td>6.9 ± 0.6</td>
<td>7.1 ± 1.7</td>
<td>.28</td>
</tr>
<tr>
<td>Number of pregestation DKA episodes (%)</td>
<td>2 (6.6%)</td>
<td>3 (5.0%)</td>
<td>.31</td>
</tr>
<tr>
<td>Prepregnancy retinopathy (%)</td>
<td>7 (22%)</td>
<td>12 (20%)</td>
<td>.48</td>
</tr>
<tr>
<td>Prepregnancy proteinuria (%)</td>
<td>1 (3.2%)</td>
<td>—</td>
<td>.34</td>
</tr>
</tbody>
</table>

DKA, diabetic ketoacidosis.

* DKA event in the year prior to the current pregnancy.

### Table 2
A comparison in fetal outcome for women with type 1 diabetes treated by CSII or MDI

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>CSII (n = 30)</th>
<th>MDI (n = 60)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gestational age at delivery (wks)</td>
<td>36.2 ± 5.1</td>
<td>36.1 ± 6.5</td>
<td>.40</td>
</tr>
<tr>
<td>Preterm delivery (less than 37 wks)</td>
<td>9 (30%)</td>
<td>21 (35%)</td>
<td>.33</td>
</tr>
<tr>
<td>Birth weight (kg)</td>
<td>3454 ± 633</td>
<td>3488 ± 662</td>
<td>.48</td>
</tr>
<tr>
<td>Macrosomia</td>
<td>8 (26%)</td>
<td>17 (28%)</td>
<td>.55</td>
</tr>
<tr>
<td>Cesarean delivery rate (%)</td>
<td>19 (63%)</td>
<td>36 (60%)</td>
<td>.88</td>
</tr>
<tr>
<td>LGA (%)</td>
<td>16 (53%)</td>
<td>24 (40%)</td>
<td>.43</td>
</tr>
<tr>
<td>SGA (%)</td>
<td>0</td>
<td>1 (1.6%)</td>
<td>—</td>
</tr>
<tr>
<td>Fetal metabolic complications</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hypocalcemia (%)</td>
<td>0</td>
<td>2 (3.3%)</td>
<td>.43</td>
</tr>
<tr>
<td>Hyperbilirubinemia (%)</td>
<td>8 (26.6%)</td>
<td>9 (15%)</td>
<td>.11</td>
</tr>
<tr>
<td>Polycythemia (%)</td>
<td>0</td>
<td>3 (5%)</td>
<td>.26</td>
</tr>
<tr>
<td>Hypoglycemia (%)</td>
<td>11 (36%)</td>
<td>8 (13%)</td>
<td>.016</td>
</tr>
<tr>
<td>Convulsions (%)</td>
<td>0</td>
<td>2 (3.3%)</td>
<td>.43</td>
</tr>
<tr>
<td>Respiratory complications (%)</td>
<td>4 (13%)</td>
<td>7 (11%)</td>
<td>.19</td>
</tr>
</tbody>
</table>
Suboptimal pregnancy outcome in patients with type 1 diabetes is usually elucidated by the combination of both the low rate of preconception care and the low proportion of patients achieving the desired level of glycemic control. In our cohort, only half of the pregnancies were planned, and only 50% of the women underwent prepregnancy medical consultation. Moreover, approximately half of the women (the same rate as in CSII or MDI therapy) achieved the desired level of glycemic control. Although in both treated groups Hgb A1c levels decreased during pregnancy, the mean blood glucose levels were higher than the levels expected for both groups (Table 3).

These findings may explain the high rate of LGA and macrosomia, fetal metabolic complications, and high cesarean section rate found in our study (Table 2). We speculate that achievement of desired level of glycemic control by higher proportion of our patients (in both treatment modalities) would have been associated with enhanced pregnancy outcome and especially the lower rate of both LGA and macrosomia. The increased rate of neonatal hypoglycemia in the CSII-treated group may be explained by transient maternal hyperglycemia during delivery.

Our findings are in agreement with other studies that found similar pregnancy outcome and the level of glycemic control in both MDI- and CSII-treated patients, however, other studies have shown that CSII is superior to conventional therapy. Notably, these studies were limited by different definitions for pregestational diabetes mellitus, and only a few evaluated the level of glycemic control or firmly defined pregnancy outcome, and the studies usually assessed only 1 aspect of pregnancy outcome (the rate of congenital anomalies). Nevertheless, a recent study has noted that women who initiate insulin pump therapy during pregnancy are highly likely to continue with the pump after they deliver. Moreover, they maintain better glucose control than do patients remaining on multiple insulin injections.

Maternal hypoglycemia is a common finding in pregnancies complicated by type 1 diabetes. Clinically significant hypoglycemia requiring assistance from another person was reported in approximately 40-70% of pregnant patients with type 1 diabetes. With the advantages of CSII in decreasing hypoglycemia and improving glycemic variability, it is logical to assume that CSII would be beneficial for pregnant women with diabetes, especially during the first trimester. In our study, most hypoglycemic events although symptomatic, were mild, and we did not find any difference in the rate of overall maternal hypoglycemic events both symptomatic and severe between CSII- and MDI-treated patients.

Diabetic ketoacidosis has been recognized as a potential complication of insulin pump failure during pregnancy. In our study, 4 patients in the CSII group did require hospitalization for mild ketoacidosis (all because of mechanical pump failure), but neither episode adversely affected pregnancy outcome. Thus, the overall risks for ketoacidosis associated with CSII (although increased in comparison with MDI therapy) during pregnancy appear to be small.

Our results represent a single-center experience, in which all patients were treated by the same diabetic protocol and by the same care providers. However, it is a retrospective analysis with a limited number of patients who were not randomly assigned to either pump therapy or conventional treatment. Importantly, although the overall costs of CSII are much higher in comparison with MDI because of the fact that national medical insurance covers these costs, the allocation to treatment modality was performed by patient’s preference. Thus, it is likely that the 2 groups were similar in most socioeconomic and demographic aspects, a fact that reduces potential biases for comparison between the studied groups. Nevertheless, it may be that patients who were treated by CSII are those who had difficulty controlling glucose using MDI in the past.

In conclusion, our study found overall both similar maternal and neonatal outcome in both the CSII- and MDI-treated patients. It is understandable that patient satisfaction and lifestyle flexibility is increased under CSII treatment in comparison with MDI treatment. CSII allows the patients to modify insulin availability hour by hour and avoid multiple injections during the day.

This increased flexibility, especially during pregnancy, may be fueling the upsurge in patient demand for CSII more than any other factor. However, it

| TABLE 3 | A comparison in maternal outcome for women with type 1 diabetes treated by CSII or MDI |
|---|---|---|
| | CSII (n = 30) | MDI (n = 60) | P value |
| Maternal hypoglycemia | | | |
| Overall hypoglycemic events (%) | 18 (60%) | 37 (62%) | .51 |
| Symptomatic hypoglycemic episode | 13/18 (72%) | 28/37 (75%) | — |
| Severe hypoglycemic episode | 5/18 (28%) | 9/28 (25%) | — |
| Deterioration in retinopathy (%) | 4 (13%) | 9 (15%) | .55 |
| Deterioration in nephropathy (%) | 7 (23%) | 7 (12%) | .14 |
| Hypertensive complications (PET and gestational HTN) | 6 (25%) | 13 (22%) | .17 |
| Number of diabetic ketoacidosis episodes (%) | 4 (13%) | 1 (1.6%) | .044 |
| Third-trimester Hgb A1c (%) | 6.2 ± 0.4 | 6.3 ± 0.8 | .36 |
| Mean blood glucose (mg/dL) | 123 ± 23 | 128 ± 31 | .17 |
| Well controlled | 52% | 53% | .55 |

HTN, hypertension.
should be remembered that the risk of diabetic ketoacidosis (a potentially life-threatening outcome for both mother and fetus) is increased in CSII-treated women. Because more women are choosing the CSII for lifestyle issues, there is a potential for more adverse outcomes.

Nevertheless, to establish clear benefit of CSII (other than improvement in lifestyle) with regard to pregnancy outcomes, a large prospective, randomized study is needed.

REFERENCES

Neonatal outcomes in pregnancies with preeclampsia or gestational hypertension and in normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation

Mounira Habli, MD; Richard J. Levine, MD; Cong Qian, MS; Baha Sibai, MD

OBJECTIVE: The purpose of this study was to compare neonatal outcomes of pregnancies with preeclampsia or gestational hypertension with those of normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation separately.

STUDY DESIGN: Secondary analysis of neonatal outcomes by week of delivery between 35 and 37 weeks 6 days of gestation to 4293 nulliparous women who were enrolled in a multicenter National Institute for Child Health and Human Development study. Outcomes included the percentage of neonatal intensive care unit admission, duration of neonatal hospitalization, and neonatal complications.

RESULTS: As compared with normotensive pregnancies, hypertensive pregnancies that delivered at 35 and 36 weeks of gestation had higher rates of small for gestational age births (17.9% vs 1.7% \( P < .05 \)) and 33.3% vs 12.2% \( P < .01 \), respectively) and neonatal intensive care unit admission (57.1% vs 34.5% \( P < .05 \) and 33.3% vs 10.7% \( P < .001 \)). The rate of neonatal intensive care unit admission (25.6% vs 8.7%; \( P < .001 \)) and duration of neonatal stay (3.9 vs 2.0 days; \( P < .001 \)) were greater in hypertensive pregnancies that delivered at 37 weeks of gestation. These differences were observed largely in women whose condition required labor induction, regardless of the severity of the hypertensive disease.

CONCLUSION: Pregnancies with preeclampsia or gestational hypertension that delivered between 35 and 37 weeks of gestation had higher rates of neonatal intensive care unit admission, small for gestational age, and longer neonatal stay than normotensive pregnancies, regardless of the severity of the hypertensive disease.

Key words: labor induction, late preterm birth, neonatal outcome, preeclampsia

Cite this article as: Habli M, Levine RJ, Qian C, Sibai B. Neonatal outcomes in pregnancies with preeclampsia or gestational hypertension and in normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation. Am J Obstet Gynecol 2007;197:406.e1-406.e7.

Preeclampsia affects 3%-7% of pregnancies\(^1,2\); its cause remains unknown. The main treatment is delivery of the placenta.

Hypertension in pregnancy, especially preeclampsia, is a major cause of maternal and perinatal morbidity and death.\(^3,4\) Hypertensive disorders account for 16% of maternal deaths in developed countries, for 25% of maternal deaths in Latin America, and for 9% of maternal deaths each in Africa and Asia.\(^5\)

The current literature emphasizes the increased risk of adverse perinatal outcomes in preeclampsia and severe gestational hypertension (GH) at <34 weeks of gestation and at term.\(^6\) Several studies have reported an increased incidence of late preterm birth among women with GH or preeclampsia.\(^7,8\) The rate of late preterm birth was reported to range from 4%-6% among women with GH and from 10%-11% among women with preeclampsia.\(^8\) Numerous studies have shown increased rates of morbidity and mortality in late preterm infants of women with GH or preeclampsia, including more neonatal intensive care unit (NICU) admissions, hypoglycemia, need for respiratory support, and rehospitalization.\(^9\) In 2002 in the United States, 41% of deliveries that included those pregnancies that were complicated by hypertension resulted from interventions for medical indications of which 7.4% occurred between 34 and 36 weeks of gestation.\(^10\)

Induction of labor is 1 of the most common medical interventions, especially in pregnancies that are complicated with hypertension. However, it is still unclear whether the increase in adverse perinatal outcomes in late preterm infants who are born to...
women with hypertension in pregnancy is due to shorter gestation, the hypertensive disease, and/or the mode of delivery or induction of labor.

Most studies have attempted to address the relative importance of neonatal outcomes that are based on the severity of disease in preterm infants who are born at a wide range of gestational ages (eg, at <33 weeks of gestation). However, few studies, if any, have addressed neonatal outcomes in late preterm infants by gestational age at delivery.

The aim of our study was to compare neonatal outcomes by week of delivery between pregnancies with preeclampsia or GH and normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation separately.

**Materials and Methods**

We performed a secondary analysis of neonatal outcomes of infants who were born between 35 and 37 weeks 6 days of gestation to healthy nulliparous women with singleton pregnancies who were enrolled in a multicenter randomized trial in the United States (Calcium for Preeclampsia Prevention [CPEP]) conducted from 1992-1995 to compare supplementation with calcium or placebo for the prevention of preeclampsia. The CPEP trial was directed by the National Institute of Child Health and Human Development. All women with pregnancies that were delivered between 35 and 37 weeks 6 days of gestation that resulted in live births or stillbirths were eligible for inclusion in this study. All women were seen before 21 weeks of gestation, and gestational age was determined from the earliest obstetric ultrasound examination. Women who were enrolled in CPEP received prenatal care with standardized measurement of blood pressure and urine protein. The study procedures were in accordance with institutional review board policies at all participating institutions.

Only women with blood pressures of <135/85 mm Hg and absent dipstick proteinuria at screening or randomization clinic visits were included in CPEP. Women with medical or obstetric complications (eg, chronic hypertension, diabetes mellitus, and lupus) and women with known fetal complications at randomization (eg, hydatidiform mole, multiple gestation, chromosomal or major congenital anomalies) were excluded. Medical history and demographic and lifestyle data were collected before randomization. Study subjects were seen by trained research staff at clinic visits that were scheduled every 4 weeks through 29 weeks of gestation, every 2 weeks between 30 and 35 weeks of gestation, and weekly thereafter. Seated blood pressure was measured by certified research staff who used a standard mercury sphygmomanometer, according to a published protocol. Two measurements that were taken at least 1 minute apart were averaged. Diastolic blood pressure was determined with the fifth Korotkoff sound, unless 1 or both measurements were 0, in which case the fourth sound was used. Voided urine specimens were collected for measurement of protein by dipstick. Dipsticks that indicated proteinuria of at least 1+ (300 mg/L) were confirmed in clean-catch, midstream urine samples. A dipstick of zero or trace in the confirmatory sample was considered negative.

*GH* was defined as diastolic blood pressure of at least 90 mm Hg on 2 occasions 4-168 hours apart. *Proteinuria* was defined as ≥300 mg protein in a 24-hour urine collection, 2 random urine specimens 4-168 hours apart that contained at least 1+ protein by dipstick, a single urine sample with a protein/creatinine ratio at least 0.35, or a single random urine specimen that contained at least 2+ protein by dipstick. After rupture of the membranes or in the presence of vaginitis, urine specimens were collected by catheter. *Preeclampsia* was defined as GH plus proteinuria within 7 days of each other or as hemolysis, elevated liver enzymes, and low platelet count syndrome (HELLP) or eclampsia.

Comparative endpoints included severity of disease (GH, mild preeclampsia, severe preeclampsia), induction status (induced labor or cesarean section vs spontaneous labor), and selected neonatal outcomes that were documented by newborn infant chart review. Selected neonatal outcomes included admission to the NICU, total neonatal hospital stay, days in NICU, respiratory distress syndrome, respiratory support (oxygen, continuous positive airway pressure, or mechanical ventilation), and small for gestational age (SGA; below the 10th percentile birthweight for gestational age). These neonatal outcomes in normotensive and preeclamptic pregnancies were compared separately at each gestational week of delivery. Indications of delivery that included SGA, rupture of membranes, and worsening of maternal or fetal status were analyzed.

Statistical analysis was conducted after stratification of patients by gestational age, normotensive/severity of hypertensive pregnancies, and induction status (induced labor or cesarean vs spontaneous labor). Group comparisons were performed with the chi-square or Fisher exact test for categoric data and 2-sample *t*-test for continuous variables. Data are presented as mean ± SD or as N (%). A 2-sided probability value of <.05 was considered statistically significant.

**Results**

Among the 4589 women who were enrolled in the study, 296 women were excluded for the following reasons: 253 women were lost to follow-up; 21 women whose pregnancy terminated before 20 weeks of gestation; 13 women had missing maternal or perinatal outcome data; and 9 women had unverified hypertension. Among the remaining 4293 women, 36 women had stillbirth delivery, of whom 32 were normotensive, 2 had GH, and 2 had preeclampsia. The birthweight and SGA status of these 36 infants were treated as missing in the analysis. Among all 4293 study subjects, 3229 subjects remained normotensive during pregnancy, and 1064 subjects experienced preeclampsia or GH. Fifty-nine of 3229 normotensive subjects (1.8%) were delivered at 35 weeks of gestation vs 28 of 1064 hypertensive (preeclampsia/GH) subjects (2.6%); 124 normotensive subjects (3.8%) were delivered at 36 weeks of gestation vs 42 subjects (3.9%) who were hypertensive; and 196 normotensive subjects (6.1%) were delivered at 37 weeks vs 86 patients (8.1%) who were hypertensive. Overall, deliveries at
35-37 weeks of gestation accounted for 11.7% of deliveries to normotensive women and 14.7% of deliveries to women with GH or preeclampsia (P = .01).

The baseline characteristics of normotensive and hypertensive women according to week of delivery are shown in Table 1. Among women who delivered at 37 weeks of gestation, those women with hypertensive pregnancies were more likely to have higher blood pressure and body mass index at enrollment than those who were normotensive. There were no statistically significant differences between normotensive and hypertensive women who delivered at 35, 36, or 37 weeks of gestation with respect to race, gestational age at enrollment, or smoking during pregnancy.

Selected neonatal outcomes in normotensive, as compared with hypertensive (preeclampsia/GH), pregnancies by gestational week of delivery (35, 36, or 37 weeks) are shown in Table 2. A higher induction rate was noted in hypertensive pregnancies at 35 weeks (53.6% vs 25.4%; P < .001), 36 weeks (66.7% vs 26.6%; P < .001) and at 37 weeks (67.4% vs 25.5%; P < .001) of gestation. A greater proportion of hypertensive pregnancies was delivered at 37 weeks of gestation by cesarean section (25.6% vs 9.2%; P < .001). Among hypertensive pregnancies, there were significantly higher rates of SGA infants delivered at 35 and 36 weeks of gestation and NICU admissions among infants who were delivered at 35, 36, and 37 weeks of gestation. There were no significant differences between hypertensive and normotensive pregnancies that delivered at 35 or 36 weeks of gestation, with respect to neonatal respiratory support (Table 2). As compared with normotensive pregnancies (Table 2), a greater percentage of hypertensive pregnancies that were delivered at 36 weeks of gestation developed respiratory distress syndrome (9.5% vs 1.6%; P < .05), and infants delivered of hypertensive pregnancies at 36 and 37 weeks had longer total neonatal stay (5.5 vs 2.8 days [P < .01] and 3.9 vs 2.0 days [P < .001], respectively).

Table 3 compares neonatal outcomes among infants who were born at 35-37 weeks of gestation to normotensive or hypertensive women, according to whether labor was spontaneous or induced or delivered by cesarean. Hypertensive pregnancies that were delivered at 35-37 weeks of gestation that required labor induction had higher rates of SGA (24.8% vs 9.1%; P < .05, respectively) and longer total neonatal stay (5.1 days vs 3.4 days; P < .05, respectively) than hypertensive pregnancies with spontaneous labor (Table 3). Similarly, normotensive pregnancies that required labor induction had more adverse neonatal outcomes than normotensive pregnancies with spontaneous labor (Table 3).

Tables 4 and 5 compare neonatal outcomes by gestational week of delivery in normotensive and hypertensive pregnancies after stratification by spontaneous or induced labor. Hypertensive pregnancies that required labor induction had higher rates of NICU admission at 36 weeks of gestation and more prolonged NICU and total neonatal stay at 37 weeks of gestation, as compared with normotensive pregnancies (Table 3). The most common reported causes for induction in hypertensive pregnancies

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Gestational age at delivery (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>Age (y)*</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)*</td>
<td></td>
</tr>
<tr>
<td>Body mass index (kg/m²)*</td>
<td></td>
</tr>
<tr>
<td>Systolic blood pressure (mm Hg)*</td>
<td></td>
</tr>
<tr>
<td>Diastolic blood pressure (mm Hg)*</td>
<td></td>
</tr>
<tr>
<td>Gestational age at enrollment (d)*</td>
<td></td>
</tr>
<tr>
<td>Current smoker (n)</td>
<td></td>
</tr>
<tr>
<td>White, non-Hispanic (n)</td>
<td></td>
</tr>
<tr>
<td>White, Hispanic (n)</td>
<td></td>
</tr>
<tr>
<td>Black (n)</td>
<td></td>
</tr>
<tr>
<td>Unknown/other race (n)</td>
<td></td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD.
† P < .05.
‡ P < .001.
§ P < .01.
### TABLE 2
Delivery characteristics and neonatal outcomes for women who had experienced preeclampsia or GH and for women who had had normotensive pregnancies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gestational age (wk)</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>35</td>
<td>36</td>
<td>37</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Normotensive</td>
<td>Preeclampsia/GH</td>
<td>Normotensive</td>
<td>Preeclampsia/GH</td>
<td>Normotensive</td>
</tr>
<tr>
<td>N</td>
<td></td>
<td>59</td>
<td>28</td>
<td>124</td>
<td>42</td>
<td>196</td>
</tr>
<tr>
<td>SGA (n)*</td>
<td></td>
<td>1 (1.7%)</td>
<td>5 (17.9%)†</td>
<td>15 (12.2%)</td>
<td>14 (33.3%)§</td>
<td>21 (10.8%)</td>
</tr>
<tr>
<td>Admission to NICU (n)</td>
<td></td>
<td>20 (34.5%)</td>
<td>16 (57.1%)†</td>
<td>13 (10.7%)</td>
<td>14 (33.3%)§</td>
<td>17 (8.7%)</td>
</tr>
<tr>
<td>NICU stay (d)†</td>
<td></td>
<td>6.0 ± 6.4</td>
<td>5.3 ± 4.0</td>
<td>11.2 ± 10.3</td>
<td>10.3 ± 8.6</td>
<td>2.5 ± 2.7</td>
</tr>
<tr>
<td>Total neonatal stay (d)†</td>
<td></td>
<td>5.0 ± 5.3</td>
<td>4.9 ± 4.9</td>
<td>2.8 ± 4.2</td>
<td>5.5 ± 4.8‡</td>
<td>2.0 ± 2.1</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)</td>
<td></td>
<td>3 (5.2%)</td>
<td>1 (3.6%)</td>
<td>2 (1.6%)</td>
<td>4 (9.5%)†</td>
<td>4 (2.1%)</td>
</tr>
<tr>
<td>Respiratory support (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td></td>
<td>47 (81.0%)</td>
<td>20 (71.4%)</td>
<td>108 (89.3%)</td>
<td>34 (81.0%)</td>
<td>182 (93.8%)</td>
</tr>
<tr>
<td>Oxygen/continuous positive airway pressure/mechanical ventilation</td>
<td>11 (19.0%)</td>
<td>8 (28.6%)</td>
<td>13 (10.7%)</td>
<td>8 (19.1%)</td>
<td>12 (6.2%)</td>
<td>13 (15.1%)†</td>
</tr>
<tr>
<td>Induced labor/cesarean section (n)</td>
<td></td>
<td>15 (25.4%)</td>
<td>15 (53.6%)†</td>
<td>33 (26.6%)</td>
<td>28 (66.7%)§</td>
<td>50 (25.5%)</td>
</tr>
<tr>
<td>Cesarean delivery (n)</td>
<td></td>
<td>9 (15.3%)</td>
<td>4 (14.3%)</td>
<td>13 (10.5%)</td>
<td>8 (19.2%)</td>
<td>18 (9.2%)</td>
</tr>
</tbody>
</table>

* Ascertained among women with delivery of a live infant.
† P < .05.
‡ P < .01.
§ P < .001.
* Data are presented as mean ± SD.

### TABLE 3
Neonatal outcomes in infants born at 35-37 weeks of gestation to women with spontaneous labor or in whom labor was induced or delivery was by cesarean, according to maternal blood pressure status

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Normotensive Spontaneous labor</th>
<th>Induced labor/cesarean</th>
<th>Preeclampsia/GH Spontaneous labor</th>
<th>Induced labor/cesarean</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>281</td>
<td>98</td>
<td>55</td>
<td>101</td>
</tr>
<tr>
<td>Gestational age at delivery (wk)*</td>
<td>36.4 ± 0.7</td>
<td>36.4 ± 0.7</td>
<td>36.3 ± 0.8</td>
<td>36.4 ± 0.7</td>
</tr>
<tr>
<td>SGA (n)*</td>
<td>20 (7.2%)</td>
<td>17 (17.5%)†</td>
<td>5 (9.1%)</td>
<td>25 (24.8%)§</td>
</tr>
<tr>
<td>Admission to NICU (n)</td>
<td>31 (11.1%)</td>
<td>19 (19.8%)§</td>
<td>16 (29.1%)</td>
<td>36 (35.6%)</td>
</tr>
<tr>
<td>Neonatal intensive care stay (d)*</td>
<td>6.2 ± 7.6</td>
<td>6.2 ± 7.5</td>
<td>4.3 ± 5.6</td>
<td>7.9 ± 6.1</td>
</tr>
<tr>
<td>Total neonatal stay (d)*</td>
<td>2.4 ± 3.1</td>
<td>3.8 ± 4.7†</td>
<td>3.4 ± 3.4</td>
<td>5.1 ± 4.5§</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)</td>
<td>8 (2.9%)</td>
<td>1 (1.0%)</td>
<td>2 (3.6%)</td>
<td>9 (8.9%)</td>
</tr>
<tr>
<td>Respiratory support (n)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>None</td>
<td>258 (92.5%)</td>
<td>79 (84.0%)§</td>
<td>48 (87.3%)</td>
<td>79 (78.2%)</td>
</tr>
<tr>
<td>Oxygen/continuous positive airway pressure/mechanical ventilation</td>
<td>21 (7.5%)</td>
<td>15 (16.0%)§</td>
<td>7 (12.7%)</td>
<td>22 (21.8%)</td>
</tr>
</tbody>
</table>

* Data are presented as mean ± SD.
† Ascertained among women with delivery of a live infant.
‡ P < .01.
§ P < .05.
* P < .001.
### TABLE 4

Delivery characteristics and neonatal outcomes for women in whom labor was induced or delivery was by cesarean section, according to whether they had experienced preeclampsia or GH (GH) or had had normotensive pregnancies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gestational age at delivery (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 Normotensive</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>SGA (n)*</td>
<td>1</td>
</tr>
<tr>
<td>Admission to NICU (n)</td>
<td>6</td>
</tr>
<tr>
<td>NICU stay (d)†</td>
<td>4.5 ± 3.6</td>
</tr>
<tr>
<td>Total neonatal stay (d)‡</td>
<td>6.0 ± 2.4</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n [%])</td>
<td>0</td>
</tr>
</tbody>
</table>

Respiratory support (n)

| Oxygen/continuous positive airway pressure/ mechanical ventilation | 3 (21.4%) | 4 (26.7%) | 6 (19.4%) | 8 (28.6%) | 6 (12.2%) | 10 (17.2%) |

| Cesarean delivery (n) | 9 (60.0%) | 4 (26.7%) | 13 (39.4%) | 8 (28.6%) | 18 (36.0%) | 22 (37.9%) |

* Ascertained among women with delivery of a live infant.
† P < .05.
‡ Data are presented as mean ± SD.
§ P < .01.

### TABLE 5

Delivery characteristics and neonatal outcomes for women with spontaneous labor, according to whether they had experienced preeclampsia or GH or had had normotensive pregnancies

<table>
<thead>
<tr>
<th>Variable</th>
<th>Gestational age at delivery (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>35 Normotensive</td>
</tr>
<tr>
<td></td>
<td>N</td>
</tr>
<tr>
<td>SGA (wk)*</td>
<td>0</td>
</tr>
<tr>
<td>Admission to NICU (n)</td>
<td>14 (31.8%)</td>
</tr>
<tr>
<td>NICU stay (d)‡</td>
<td>6.5 ± 7.3</td>
</tr>
<tr>
<td>Total neonatal stay (d)‡</td>
<td>4.6 ± 5.9</td>
</tr>
<tr>
<td>Respiratory distress syndrome (n)</td>
<td>3 (6.8%)</td>
</tr>
</tbody>
</table>

Respiratory support (n)

| Oxygen/continuous positive airway pressure/ mechanical ventilation | 8 (18.2%) | 4 (30.8%) | 7 (7.8%) | 0 | 6 (4.1%) | 3 (10.7%) |

† Data are presented as mean ± SD.
† P < .01.
§ P < .001.
* Ascertained among women with delivery of a live infant.
that were delivered at 35, 36, and 37 weeks of gestation were hypertensive disorders (include worsening of maternal or fetal status and complications of preeclampsia) and rupture of membranes (data not shown). Suspected intrauterine growth restriction was another common medical indication for delivery of hypertensive pregnancies at 37 weeks of gestation. Among women with spontaneous labor, there were no significant differences in neonatal outcome, except for more frequent NICU admission and more prolonged total neonatal stay at 37 weeks of gestation among hypertensive pregnancies (Table 5).

After stratification by disease severity (GH, mild preeclampsia, and severe preeclampsia), infants who were born from hypertensive pregnancies, regardless of severity, had higher rates of NICU admission at 36 weeks of gestation than from normotensive pregnancies (GH vs normotensive, 40% vs 11% [P < .01]; mild preeclampsia vs normotensive, 36% vs 11% [P < .05]; severe preeclampsia vs normotensive, 18% vs 11% [P, not significant]) and at 37 weeks of gestation (GH vs normotensive, 24% vs 9% [P < .01]; mild preeclampsia vs normotensive, 25% vs 9% [P < .05]; severe preeclampsia vs normotensive, 43% vs 9% [P < .05]). The total neonatal stay was longer in infants who were delivered from hypertensive pregnancies at 36 weeks of gestation (GH vs normotensive, 5.4 vs 2.8 days [P < .05]; mild preeclampsia vs normotensive, 6.1 vs 2.8 days [P < .05]; severe preeclampsia vs normotensive, 4.9 vs 2.8 days [P < .01]) and 37 weeks of gestation (GH vs normotensive, 3.3 vs 2.0 days [P < .001]; mild preeclampsia vs normotensive, 5.5 vs 2.0 days [P < .001]; severe preeclampsia vs normotensive, 4.4 vs 2.0 days [P < .05]), regardless of disease severity.

**Comment**

It is accepted generally that pregnancies that experience hypertension have higher rates of neonatal morbidity than normotensive pregnancies. However, there are no data that compare neonatal outcomes from pregnancies that are complicated with hypertension according to gestational week of delivery, particularly at 35, 36, or 37 weeks of gestation. It is not known whether increased morbidity in infants who are born to hypertensive women is due to the hypertensive disorder, the gestational age at delivery, or the process of induction, which is increased greatly in these pregnancies. To our knowledge, ours is the first study that addresses this question. Our study was designed to compare neonatal outcomes of pregnancies with preeclampsia or GH and normotensive pregnancies that delivered at 35, 36, or 37 weeks of gestation by week of delivery.

We found that, compared with normotensive pregnancies, hypertensive pregnancies (preeclampsia/GH) had a higher rate of induction at each gestational week of delivery (35, 36, and 37) and a higher rate of cesarean delivery at 37 weeks of gestation. Hypertensive pregnancies had higher rates of SGA infants who were born at 35 and 36 weeks of gestation, with a greater incidence of respiratory distress syndrome in infants who were delivered at 36 weeks of gestation from hypertensive pregnancies. Regardless of the severity of disease, hypertensive pregnancies had higher rates of NICU admission at 35, 36, and 37 weeks of gestation and longer total neonatal stay at 36 and 37 weeks of gestation than normotensive pregnancies. Among women with induced labor or delivery by cesarean, both hypertensive and normotensive pregnancies had more adverse perinatal outcomes than pregnancies with corresponding hypertensive status and spontaneous labor.

Recently, Chen et al. investigated the effect of pregnancy-induced hypertension on infant mortality rates in different birthweight percentiles and gestational ages (early preterm, late preterm, and full term). In this large population-based study, late preterm gestational age (32-36 weeks of gestation) that was complicated by pregnancy-induced hypertension was associated with higher risk of SGA than normotensive pregnancies (21.9% vs 6.6%; P < .0001). These results are consistent with our study.

Other studies have found that preterm infants who are born to hypertensive pregnancies have a higher rate of NICU admission. Friedman et al. in a matched cohort study (223 women with preeclampsia and 223 control subjects) found a higher rate of NICU admission in infants who were born to preterm (<35 weeks of gestation) preeclamptic women than to normotensive women (78% vs 64%; P < .001). Also, no differences were observed in neonatal deaths or in respiratory morbidities (mechanical ventilation, respiratory distress syndrome) in infants who were delivered at <35 weeks of gestation. The consistently high rates of admission to the NICU in our study may be related to prolonged labor that led to increased incidences of intrauterine infection and/or non reassuring fetal heart rate or mode of delivery (vaginal vs cesarean delivery). On the other hand, the higher rate of respiratory distress syndrome at 36 weeks of gestation (9.5%) in our study could be related to the definition of respiratory distress syndrome that was adopted in the CPEP study, which included the conventional definition and respiratory insufficiency.

Several studies showed that perinatal morbidity and mortality rates are affected by disease severity. Buchbinder et al., in a secondary analysis that compared fetal outcome in hypertensive pregnancies, found that women who experienced severe GH had higher rates of preterm delivery at <37 weeks of gestation (54.2% vs 17.8%; P = .001) and at <35 weeks of gestation (25.0% vs 8.4%; P = .0161) and delivery of SGA infants (20.8% vs 6.5%; P = .024), when compared with women who remained normotensive or those who experienced mild GH. A higher percentage of NICU admission was observed in women who experienced severe preeclampsia (38%), compared with those with mild preeclampsia (24%). There were no statistically significant differences in perinatal outcomes between the normotensive/mild GH and the mild preeclampsia groups. In contrast, our findings suggest that gestational week of delivery rather than severity of disease had greater impact on NICU admission and total neonatal stay. These differences may be attributed to the fact that we studied severity of disease within a specific gestational age week at delivery.
rather than within a range of gestational ages.

Another contributing factor to neonatal morbidity is medical intervention. The rate of intervention for medical indication in late preterm infants (34-36 weeks of gestation) has risen by 12% from 1992-2002, with hypertensive disorders being 1 of the most common medical indications. In the present study, hypertensive disease, premature rupture of membranes, and suspected intrauterine growth restriction were the main indications for induction.

The association of induction with neonatal morbidity in late preterm hypertensive pregnancies compared with normotensive pregnancies, to our knowledge, has not been addressed previously. There were more adverse neonatal outcomes when labor was induced in normotensive or hypertensive pregnancies separately, compared with pregnancies with spontaneous labor. This suggests that the differences in neonatal outcomes between hypertensive and normotensive pregnancies may be related to induction status. As in many studies, it is difficult to differentiate between indicated and iatrogenic medical interventions. Barton et al found that 15% of women with mild GH are delivered between 34 and 36 weeks of gestation, which is similar to our observation that 14.7% of hypertensive pregnancies were delivered between 35 and 37 weeks of gestation. Therefore, the decision to deliver the fetus should be based on careful maternal and fetal assessment.

Overall, the strength of our study is comparison of neonatal outcomes of normotensive pregnancies and pregnancies with GH or preeclampsia by week of delivery and without stratification by induced or spontaneous labor and by disease severity. Other studies have examined these outcomes in relation to severity of disease using ranges of gestational age at delivery and without regard to induction status.

Study weaknesses include the absence of data on important confounding variables, such as chorioamnionitis, duration of labor, and other labor complications. These variables might have affected the rates of NICU admission and the duration of total neonatal stay. Neonatal outcomes such as neonatal sepsis were not recorded in the original study database. This also could have influenced the differences that we observed in neonatal outcome between normotensive and hypertensive pregnancies.

In conclusion, pregnancies with hypertensive disorders that were delivered between 35 and 37 weeks 6 days of gestation were associated with higher rates of SGA and NICU admission and longer duration of neonatal stay than normotensive pregnancies. These differences were seen largely in women who required labor induction and did not appear to be related to the severity of hypertensive disease. The need for medical intervention in late preterm pregnancies with GH or preeclampsia should be evaluated carefully.

ACKNOWLEDGMENTS

We thank the patients who participated in the CPEP study, the CPEP Study Group, and Dr Kai Yu for helpful discussions. The following individuals were members of the CPEP Study Group: J.C. Hauth, R. Goldenberg, B.S. Stofan (University of Alabama at Birmingham); L.B. Curet, G.M. Joffe, V. Dorato (University of New Mexico at Albuquerque); B.M. Sibai, S.A. Friedman, B.M. Mercer, T. Carr (University of Tennessee at Memphis); P.M. Catalan, A.S. Petrulis, L. Barbach (Case Western Reserve University at MetroHealth Medical Center, Cleveland); C. Morris, S.L. Jacobson, K. McCracken (Oregon Health Sciences University, Portland); J.R. Esterlitz, M.G. Ewell, D.M. Brown (The EMMES Corporation, Rockville); R.J. Levine, R. DerSimonian, J.D. Clemens, M.A. Kiebanoff, E.G. Raymond, J.G. Rigau-Perez, H. Shifrin (National Institute for Child Health and Human Development); J.A. Cutler, D.E. Bihl (National Heart, Lung, and Blood Institute); M. Lindheimer, C. Begg, T. Chalmers, M. Druzin, R. Sokol (Data Safety and Monitoring Board).

REFERENCES

A population study of the contribution of medical comorbidity to the risk of prematurity in blacks

Deborah B. Ehrenthal, MD; Claudine Jurkovitz, MD, MPH; Matthew Hoffman, MD, MPH; Charlan Kroelinger, PhD; William Weintraub, MD

OBJECTIVE: The purpose of this study was to test the hypothesis that the higher prevalence of medical comorbidities among black women accounts for their increased risk of prematurity.

STUDY DESIGN: A population-based regional cohort of women receiving obstetric care for singleton pregnancies at a large community hospital between 2003 and 2006 were analyzed using univariate and multivariable logistic regression.

RESULTS: Data for 18,624 consecutive births found increased odds of adverse outcomes for black compared to white women: prematurity OR = 1.6 (1.4-1.8), extreme prematurity OR = 2.5 (2.0-3.2). Logistic regression modeling identified black race, age < 20, preconception diabetes and hypertension, smoking, underweight, and gestational hypertension as the greatest risks for adverse outcomes. Controlling for these risks did not attenuate the higher risk for prematurity among blacks.

CONCLUSION: Though there is a greater burden of health risk among black women, this did not account for the higher rates of low birthweight and prematurity.

Key words: health disparities, low birthweight, preconception health, prematurity


Prematurity is a major driver of infant morbidity and mortality in the US.1 In spite of efforts to expand access to health insurance and improve early entry into prenatal care, rates of prematurity and low birthweight have increased significantly during the past 15 years.2 These rates continue to be markedly higher for blacks regionally and nationally and are a major contributor to higher infant mortality rates in this group.2,3 This disparity in outcomes for blacks has been attributed to socioeconomic status, a higher prevalence of chronic health risks, barriers to accessing health care, increased levels of maternal stress, maternal infection, environmental factors, and genetic factors.4,5 Research examining infant birthweight and subsequent maternal cardiovascular mortality indicates that the size and weight of offspring are significantly inversely associated with maternal cardiovascular disease.6,7 Such analyses suggest that maternal diagnosed or undiagnosed chronic conditions prior to and during pregnancy affect both the weight, gestational age, and size of offspring, and subsequent disease mortality of the mother.

Maternal health at the time of conception has a critical impact on pregnancy outcomes, through a variety of mechanisms, influencing the risk of indicated and spontaneous premature delivery as well as the risk of birth defects.8 There is a new emphasis on the influence of preconception health on obstetric outcomes, with recommendations from the American College of Obstetricians and Gynecologists and the Centers for Disease Control to address health risks prior to conception.9,10 Because the prevalence of these chronic health risks differs among different racial and ethnic groups, it offers a potential explanation for the disparities seen in rates of prematurity, low birthweight, and infant mortality. Understanding the contribution of chronic health risks to outcome disparities is essential in order to adequately identify and target health risks during the preconception period.

Neither prospective clinical studies nor large retrospective cohort studies using administrative databases have shown that maternal health or socioeconomic factors explain disparities in preterm birth or recurrent preterm birth.5,11 However, during the past 20 years we have seen a significant increase in the prevalence of relevant medical risk factors among women of childbearing age in the US. An epidemic rise in the prevalence of obesity, diabetes, and hypertension has been seen across the population.2,12,13 In light of these changes as well as the increasing rates of prematurity, it is important to reexamine the impact of maternal health risks on the disparities in prematurity and low birthweight seen among blacks.

The aim of this study is to understand the current impact of maternal health...
risks on pregnancy outcomes in a representative population, and to determine the contribution of these health risks to the higher rates of prematurity among blacks.

MATERIALS AND METHODS

Subjects and data source

Prior to the initiation of the study, institutional review board approval was obtained. Using a contemporaneously maintained obstetric database we identified all women who received obstetric care at a large regional medical center between May 2003 and September 2006.

Women were excluded if they carried a multiple gestation pregnancy or if there were missing data for race/ethnicity, parity, age, height or weight, gestational age at delivery, and birthweight. Analysis was restricted to the most recent pregnancy if more than 1 occurred during the time frame of the study.

The obstetric database is derived from direct entry by hospital nursing staff during the patients’ hospitalization and is, for the vast majority of data, the patient medical record. This database consists of contemporaneously collected labor and delivery information. When a woman is admitted to labor and delivery, the nursing staff creates an entry for her in the database. Information such as obstetric and medical history is noted in real time by labor and delivery nursing staff. All nursing staff are trained in the preformatted questionnaire as part of their orientation. Daily logic checking occurs to assess for incomplete values and miskeying. Quarterly performed audits have demonstrated that the database that the quality of data exceeds 95%.

The obstetricians providing prenatal care for the patients represent both community and staff physicians. Prenatal comorbidities and pregnancy complications are identified by the patients’ personal obstetricians and noted in the chart at the time of hospital admission. Prenatal comorbidities used in the analysis included chronic hypertension, pregestational diabetes, and asthma. Pregnancy complications included gestational hypertension, gestational diabetes, and anemia. For the purposes of analysis, pregestational diabetes diagnosis represents a combination of type 1 and type 2 diabetes. Prepregnancy weight was self-reported and recorded by the admitting nurse; height was either measured or self-reported. Performance improvement studies in this department at our institution of this database have shown a high degree of reliability of self-reported heights as has been shown in other published studies. Body mass index (BMI) was calculated as weight (kg)/height (m)². Obesity was defined by NHLBI guidelines using BMI 30 kg/m² or greater; underweight was defined as BMI less than 18.5 kg/m². Smoking history was self-reported and women were categorized as nonsmokers or smokers.

Our primary outcome of interest was gestational age at delivery less than 32 weeks. Secondary outcomes were delivery at less than 37 weeks, birthweight less than 2500 g (LBW), and birthweight less than 1500 g (VLBW). Spontaneous and indicated deliveries were combined in the analysis unless specifically stated. The primary exposure of interest was race and ethnicity. Race and ethnicity were self-identified. The categories used by the institution to categorize race are white, black, Hispanic, Asian, American Indian, and other.

Statistical analysis

Chi-square tests were used to compare demographic characteristics, rates of birth outcomes, incidence of pregnancy complications, and prevalence of health risk factors between races. Logistic regression analysis was used to derive adjusted odds ratios (OR) and 95% confidence intervals (CI) for the association between birth outcomes and race after controlling for all the other covariates. Separate models were run for each of the 4 dependent dichotomous variables (gestational age < 32 weeks, gestational age < 37 weeks, LBW, and VLBW). The independent variable of interest was race/ethnicity, which was defined according to 5 categories: white, black, Hispanic, Asian, and American Indian, with white as the reference. The following covariates were included in each model: age, smoking status, BMI, common maternal medical diagnoses including chronic hypertension, asthma, diabetes, and anemia, pregnancy-associated risk factors such as gestational diabetes and gestational hypertension. Age was stratified into 4 categories, < 20 years, 20-29, 30-39 and 40 or older, with the age 20-29 as the reference. BMI was stratified into the following categories: underweight (< 18.5 kg/m²), normal (18.5-24.9), overweight (25.0-29.9), obese (30.0 or greater), with 18.5-24.9 as the reference. Anemia was defined as a hemoglobin < 11 g.

In further logistic regression analyses, the maternal prepregnancy risk factors (smoking, BMI < 18.5, diabetes, asthma, chronic hypertension) were combined into 1 overall risk factor variable (ORF) stratified into 3 categories as follows: no risk factor, 1 risk factor, and 2 or more risk factors, with “no risk factor” as the reference.

To evaluate the confounding effect of socioeconomic status on the association between race and poor pregnancy outcomes, we added marital status and insurance status separately in each of the 4 full models. Marital status is a dichotomous variable with “being married” as the reference. Insurance status includes 3 categories, private insurance, Medicaid/Medicare, and noninsured, with private insurance as the reference category.

Finally, to explore the hypothesis that the effect of race may differ according to the type of labor because of the wide range of practice patterns regarding delivery induction, we further restricted the analysis to those who experienced spontaneous labor.

Data were analyzed using SAS version 9.1 (SAS Institute, Cary, NC).

RESULTS

Data were collected for 25,997 consecutive births between May 2003 and September 2006. Of these, 2634 were excluded for multiple gestation pregnancy, 3289 were excluded due to more than 1 pregnancy during study period, 1018 were excluded due to missing height and/or weight, and 432 were missing other data (diagnoses and outcomes),
leaving 18,624 mother-baby pairs. There were 62.3% white, 22.2% black, 10.1% Hispanic, 4.7% Asian, 0.6% American Indian women. Average age of the cohort was 28.6 ± 6.1 years, average BMI was 25.9 ± 6.4 kg/m². Overall, 48.5% were classified as having a “spontaneous” delivery, 64.0% of the women were married, 67.7% carried private health insurance, 31.7% Medicaid or Medicare, and 0.6% were uninsured. Insurance type and marital status varied significantly by race/ethnicity. The percentage of women who had private health insurance and were married were, respectively, 83% and 76% among whites, 44% and 32% among blacks, 22% and 46% among Hispanic women, and 83% and 93% among Asians.

Rates of prematurity (gestational age < 37 weeks), extreme prematurity (gestational age < 32 weeks), low birthweight (< 2500 g), and very low birthweight (< 1500 g) for the population varied significantly by maternal race and ethnicity (Table 1). Rates of premature delivery and low birthweight were highest for black and lowest for Asian women. The crude odds ratio (OR) for adverse outcomes for black compared to white women were: OR = 1.6 (1.4-1.8) for prematurity, OR = 2.5 (2.0-3.2) for extreme prematurity, OR = 2.2 (2.0-2.5) for low birthweight and OR = 3.0 (2.3-3.7) for very low birthweight.

The prevalence of medical risks factors and the incidence of pregnancy complications also vary by maternal race and ethnic group (Table 2). Because the number of American Indian mothers is very small in our population (n = 119), we chose not to present these results. Overall, 20.8% of the women were obese (BMI ≥ 30 kg/m²), 4.0% were underweight (BMI < 18.5 kg/m²), and 17.3% were smokers. Chronic hypertension was present in 3.3%, diabetes prior to pregnancy in 0.9%, and asthma in 10.0%; gestational hypertension developed in 7.9%, and gestational diabetes in 6.6%. Average age of the mothers differed significantly by race (P < 0.001). The average age in Asian mothers was 30.3 (± 4.7) years, in white women 29.7 (± 5.8) years, in black women 26.2 (± 6.4) years, and in Hispanic women 25.9 (± 5.8) years.

The adjusted odds ratio (aOR) comparing blacks to whites for each of the outcomes was determined after controlling for maternal age, BMI, the presence of chronic hypertension, smoking, pregestational diabetes, asthma, and the development of gestational diabetes and gestational hypertension (Table 3). Even after controlling for all the above risk factors, black mothers were still more likely to exper-

### TABLE 1
Rates of prematurity and low birthweight in the population overall and by race/ethnicity

<table>
<thead>
<tr>
<th>Maternal Risk Factor and Pregnancy Complications</th>
<th>White (n = 11,602)</th>
<th>Black (n = 4138)</th>
<th>Hispanic (n = 1884)</th>
<th>Asian (n = 881)</th>
<th>Overall* (n = 18,624)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gestational age &lt; 32 (%) [n]</strong></td>
<td>1.5 (175)</td>
<td>3.8 (155)</td>
<td>1.4 (27)</td>
<td>1.0 (9)</td>
<td>2.0 (369)</td>
</tr>
<tr>
<td><strong>Gestational age &lt; 37</strong></td>
<td>9.8 (1134)</td>
<td>14.6 (605)</td>
<td>8.0 (151)</td>
<td>6.6 (58)</td>
<td>10.5 (1961)</td>
</tr>
<tr>
<td><strong>Birthweight &lt; 1500 g</strong></td>
<td>1.2 (139)</td>
<td>3.5 (143)</td>
<td>1.4 (26)</td>
<td>0.9 (8)</td>
<td>1.7 (317)</td>
</tr>
<tr>
<td><strong>Birthweight &lt; 2500 g</strong></td>
<td>6.5 (756)</td>
<td>13.3 (551)</td>
<td>6.2 (117)</td>
<td>5.8 (51)</td>
<td>8.0 (1488)</td>
</tr>
</tbody>
</table>

*The total number of white, black, Hispanic, and Asian mothers is 18,505. The results for the 119 American Indians are not shown.

### TABLE 2
Prevalence of chronic health risks and incidence of pregnancy complications, overall and by race and ethnicity

<table>
<thead>
<tr>
<th>Maternal risk factor and pregnancy complications</th>
<th>White (n = 11,602)</th>
<th>Black (n = 4138)</th>
<th>Hispanic (n = 1884)</th>
<th>Asian (n = 881)</th>
<th>Overall* (n = 18,624)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>BMI &lt; 18.5 (%)</strong></td>
<td>3.8</td>
<td>3.7</td>
<td>3.0</td>
<td>9.5</td>
<td>4.0</td>
</tr>
<tr>
<td><strong>BMI ≥ 30</strong></td>
<td>18.5</td>
<td>30.6</td>
<td>21.3</td>
<td>6.2</td>
<td>20.8</td>
</tr>
<tr>
<td><strong>Chronic hypertension</strong></td>
<td>3.0</td>
<td>5.5</td>
<td>1.5</td>
<td>0.9</td>
<td>3.3</td>
</tr>
<tr>
<td><strong>Prepregnancy diabetes</strong></td>
<td>0.8</td>
<td>1.0</td>
<td>1.2</td>
<td>0.8</td>
<td>0.9</td>
</tr>
<tr>
<td><strong>Smoking</strong></td>
<td>20.1</td>
<td>17.7</td>
<td>7.8</td>
<td>2.0</td>
<td>17.3</td>
</tr>
<tr>
<td><strong>Asthma</strong></td>
<td>10.3</td>
<td>12.3</td>
<td>7.0</td>
<td>3.9</td>
<td>10.0</td>
</tr>
<tr>
<td><strong>Gestational hypertension</strong></td>
<td>8.2</td>
<td>9.6</td>
<td>4.7</td>
<td>3.0</td>
<td>7.9</td>
</tr>
<tr>
<td><strong>Gestational diabetes</strong></td>
<td>6.2</td>
<td>5.7</td>
<td>7.4</td>
<td>13.4</td>
<td>6.6</td>
</tr>
</tbody>
</table>

*P < .0001 for overall comparison between race/ethnicity (chi-square).
*Chi-square not significant.
The total number of white, black, Hispanic, and Asian mothers is 18,505. The results for the 119 American Indians are not shown.
perience adverse pregnancy outcomes than whites (Table 3).

Significant risk factors for delivery prior to 37 weeks were gestational hypertension, diabetes prior to pregnancy, chronic hypertension, black race, smoking, underweight, age under 20, and asthma. Significant risk factors for low birthweight were gestational hypertension, chronic hypertension, black race, diabetes prior to pregnancy, smoking, age under 20, and underweight. Obesity, defined as a BMI of 30 kg/m² or greater, was associated with a decreased risk of both low birthweight and prematurity. Anemia was not a significant risk factor for any of the outcomes and was eliminated from analysis. In addition, the clustering of the maternal pregancy risk factors into 1 variable did not alter the OR comparing blacks to whites (Table 4). It is interesting to note that as the number of risk factors increases, the risk of adverse outcome increases.

Insurance status and marital status were both independent predictors of pregnancy outcomes when included in the models described in Table 3. Women with Medicaid/Medicare or unmarried women were more likely to experience worse outcomes when compared with those with private insurance or married women (data not shown). The strength of association between race and outcomes decreased slightly when insurance status was added in the models. The aORs comparing blacks to whites for the respective outcomes of delivery < 32 weeks, delivery < 37 weeks, VLBW, and LBW were 2.1 (1.7-2.7), 1.3 (1.2-1.5), 2.5 (1.9-3.3), and 1.9 (1.6-2.1), respectively. Similar results were obtained when mar-

### Table 3

Adjusted odds ratio for risk of preterm delivery prior to 32 weeks’ and 37 weeks’ gestational age, and birthweight less than 1500 g and 2500 g

<table>
<thead>
<tr>
<th>Maternal risk</th>
<th>&lt; 32 weeks aOR (95% CI)</th>
<th>&lt; 37 weeks aOR (95% CI)</th>
<th>&lt; 1500 g aOR (95% CI)</th>
<th>&lt; 2500 g aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age &lt; 20</td>
<td>1.7 (1.2-2.3)</td>
<td>1.3 (1.1-1.5)</td>
<td>1.3 (0.9-2.0)</td>
<td>1.3 (1.1-1.6)</td>
</tr>
<tr>
<td>Black</td>
<td>2.4 (1.9-3.0)</td>
<td>1.5 (1.4-1.7)</td>
<td>2.8 (2.2-3.6)</td>
<td>2.2 (2.0-2.5)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.1 (0.7-1.7)</td>
<td>0.9 (0.8-1.1)</td>
<td>1.5 (0.9-2.3)</td>
<td>1.1 (0.9-1.4)</td>
</tr>
<tr>
<td>Asian</td>
<td>0.9 (0.4-1.7)</td>
<td>0.8 (0.6-1.0)</td>
<td>1.1 (0.5-2.3)</td>
<td>1.1 (0.8-1.4)</td>
</tr>
<tr>
<td>Smoking</td>
<td>1.6 (1.3-2.1)</td>
<td>1.4 (1.2-1.6)</td>
<td>1.9 (1.4-2.4)</td>
<td>1.9 (1.6-2.1)</td>
</tr>
<tr>
<td>BMI &lt; 18.5</td>
<td>2.0 (1.3-3.1)</td>
<td>1.5 (1.2-1.9)</td>
<td>1.9 (1.2-3.1)</td>
<td>1.7 (1.4-2.1)</td>
</tr>
<tr>
<td>BMI ≥ 30</td>
<td>1.2 (0.9-1.6)</td>
<td>0.9 (0.7-1.0)</td>
<td>1.2 (0.9-1.6)</td>
<td>0.7 (0.6-0.8)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>3.3 (2.6-4.3)</td>
<td>3.4 (3.0-3.9)</td>
<td>4.8 (3.7-6.2)</td>
<td>3.5 (3.0-4.1)</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>2.7 (1.9-3.9)</td>
<td>2.2 (1.8-2.7)</td>
<td>2.8 (2.0-4.1)</td>
<td>2.3 (1.8-2.9)</td>
</tr>
<tr>
<td>Diabetes</td>
<td>1.0 (0.4-2.4)</td>
<td>2.4 (1.6-3.5)</td>
<td>1.5 (0.7-3.4)</td>
<td>2.0 (1.3-3.1)</td>
</tr>
<tr>
<td>Asthma</td>
<td>0.9 (0.6-1.2)</td>
<td>1.2 (1.0-1.4)</td>
<td>0.9 (0.6-1.3)</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>0.7 (0.5-1.2)</td>
<td>1.2 (1.0-1.5)</td>
<td>0.6 (0.4-1.0)</td>
<td>1.0 (0.8-1.2)</td>
</tr>
</tbody>
</table>

### Table 4

Adjusted ORs for race after controlling for preconception maternal health risk factors clustered into an overall variable

<table>
<thead>
<tr>
<th>Maternal risk</th>
<th>&lt; 32 weeks aOR (95% CI)</th>
<th>&lt; 37 weeks aOR (95% CI)</th>
<th>&lt; 1500 g aOR (95% CI)</th>
<th>&lt; 2500 g aOR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Black</td>
<td>2.5 (2.0-3.1)</td>
<td>1.5 (1.4-1.7)</td>
<td>2.9 (2.3-3.7)</td>
<td>2.1 (1.9-2.4)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>1.2 (0.8-1.8)</td>
<td>0.9 (0.8-1.1)</td>
<td>1.6 (1.0-2.4)</td>
<td>1.1 (0.9-1.3)</td>
</tr>
<tr>
<td>Asian</td>
<td>0.9 (0.5-1.7)</td>
<td>0.8 (0.6-1.0)</td>
<td>1.1 (0.5-2.2)</td>
<td>1.1 (0.8-1.5)</td>
</tr>
<tr>
<td>ORF=1</td>
<td>1.8 (1.5-2.3)</td>
<td>1.5 (1.3-1.6)</td>
<td>2.1 (1.7-2.7)</td>
<td>1.9 (1.6-2.1)</td>
</tr>
<tr>
<td>ORF=2 or more</td>
<td>3.6 (2.3-5.7)</td>
<td>3.2 (2.5-4.1)</td>
<td>4.1 (2.6-6.7)</td>
<td>3.9 (3.0-5.1)</td>
</tr>
<tr>
<td>Age &lt; 20*</td>
<td>1.6 (1.1-2.2)</td>
<td>1.3 (1.1-1.5)</td>
<td>1.3 (0.9-1.9)</td>
<td>1.4 (1.2-1.7)</td>
</tr>
<tr>
<td>Gestational hypertension</td>
<td>3.6 (2.8-4.6)</td>
<td>3.5 (3.1-4.0)</td>
<td>5.1 (4.0-6.6)</td>
<td>3.4 (2.9-3.9)</td>
</tr>
<tr>
<td>Gestational diabetes</td>
<td>0.8 (0.5-1.2)</td>
<td>1.2 (1.0-1.4)</td>
<td>0.7 (0.4-1.1)</td>
<td>0.9 (0.7-1.1)</td>
</tr>
</tbody>
</table>

ORF: overall risk factor; ORF=1, presence of 1 risk factor compared to no risk factor; ORF=2, presence of 2 risk factors or more compared to no risk factor.
* The ORs associated with the other age categories (30-39 and ≥ 40) are not significant except for the outcomes gestational age < 32 weeks and birthweight < 2500 g, where the OR associated with age ≥ 40 are, respectively, 1.9 (1.0-3.1) and 1.4 (1.3-1.8).
ital status was added in the models. The aORs comparing blacks to whites for the respective outcomes of delivery < 32 weeks, delivery < 37 weeks, VLBW, andLBW were 1.9 (1.5-2.5), 1.4 (1.2-1.6), 2.3 (1.8-3.0), and 1.9 (1.6-2.1), respectively.

Finally, restricting the analysis to include only women with spontaneous births attenuated the excess risk for blacks across all outcomes. As in the analysis of the entire cohort, controlling for maternal medical risk factors did not significantly diminish the risk associated with black race. The aOR for blacks compared to white for gestational age < 32 weeks was 1.9 (1.3-2.7), < 37 weeks aOR = 1.3 (1.1-1.5), < 1500 g aOR = 2.2 (1.5-3.3), and < 2500 g aOR = 1.3 (1.1-1.5).

**COMMENT**

We found prematurity and low birthweight to be significantly more common among blacks in our population when compared to pregnancy outcomes for white, Hispanic, and Asian women. Disparities were greatest for the most clinically significant outcomes, with almost 3 times the risk for very low birthweight and extreme premature delivery for black mothers compared to white mothers. Overall, the most favorable pregnancy outcomes were seen for Asian and Hispanic women, consistent with national data.2

In addition to maternal race, maternal age (< 20), preconception maternal health conditions, and pregnancy complications were significant risk factors for preterm delivery and low birthweight for the population. The previously known effects of chronic hypertension, smoking, being underweight, and the development of gestational hypertension were reflected in our study as significant independent risk factors for prematurity and low birthweight. Gestational diabetes was associated with a small increase, and obesity a small decrease, in the risk of prematurity and low birthweight. Neither had an effect on the risk of extreme prematurity or very low birthweight. Consistent with prior studies, women with private health insurance and women who were married were at lower risk for prematurity and low birthweight.

Though the prevalence of maternal health risks in our study parallels the variation in infant mortality and prematurity rates in the racial and ethnic groups studied, our statistical models controlling for the presence of maternal comorbidities and health risks failed to reveal an impact on the risk of poor outcomes associated with black race. This suggests that factors beyond the presence of defined medical comorbidities contribute to prematurity and low birthweight. Our study provides evidence that socioeconomic risk factors play an important role, but including these factors also failed to fully account for the disparities in outcomes for blacks in our population. The smaller disparity in risk for blacks in the group of women who present with spontaneous delivery suggests that a detailed exploration of outcomes for women with medically managed delivery would be of value.

The strength of our findings lay in our use of contemporary clinical data to identify maternal health risks in the large and representative regional population. Our data are more accurate than birth certificate data in identifying maternal health risks and the size of our population provides the power to look at effects on all 4 adverse outcomes. Maternal health problems and risks were significantly more prevalent than are reported in vital statistics data and we suspect provide a more accurate view of the burden of maternal health conditions and risks in an obstetric population.2,15-17 The significant variation we see in the prevalence of maternal health conditions and risks among women of different race and ethnicity is consistent with what is seen in the general population.13

This study is not able to fully address the contribution of socioeconomic factors or maternal stress on the maternal risk factors or the outcomes of interest. However, prior studies using varying methods and design have been inconsistent in identifying socioeconomic factors or maternal stress as factors that account for racial disparities in pregnancy outcomes.3,18-20 Population-based vital data studies indicate that although black women are at a higher risk for premature and low birthweight delivery, socioeconomic status is not a major contributor to the racial disparity. Collins et al found the same racial disparities in pregnancy outcomes between black and white women among life-long residents in high income neighborhoods. Earlier initiation of prenatal care among black and white women in Chicago increased the racial disparity in premature delivery, suggesting that access to care is also a minimal contributor to differences in poor outcomes.22

Our main findings are consistent with those of other studies examining the impact of maternal health on disparities in outcomes for blacks. In the study of a high risk cohort of 1491 multiparous women with singleton pregnancies, Goldenberg et al found that controlling for maternal characteristics, including hypertension, diabetes, and anemia, failed to explain the disparities in outcomes for blacks.11 Leslie et al conducted an analysis using 1993-97 linked birth/infant death certificate data from North Carolina.20 Better pregnancy outcomes were seen for Hispanic women in spite of their findings of more favorable prenatal care use and socioeconomic factors among the African American women.

Collins et al conclude that racial disparities in low birthweight delivery are associated with intergenerational differences with racial and ethnic groups, indicating that these differences may be more influential than genetic differences. Observing 3 generations of black women, mean birthweight steadily decreased from first generation immigrants to third generation US born black women. By contrast, moderately low birthweight among third generation US born white women compared to first generation immigrant white women decreased by 10%. Earlier work by Collins and David indicated that the birthweight of infants of African-born black women were closer to those of US-born whites compared with US-born black women. Such findings support the influence of sociodemographic and behavioral factors on birth outcomes rather than genetic factors.
The prevalence of maternal health risks among women of childbearing age is growing and a significant fraction of women enter pregnancy with potentially modifiable health risks. Addressing these risks through improved preconception health care has the potential to significantly improve pregnancy outcomes. However, our study like others suggests that identification of additional approaches is needed if we are to eliminate disparities in outcomes for blacks.

ACKNOWLEDGMENTS

We are indebted to the work of Barbara Temple, RN, for maintaining the quality of the data and to James Bowen, BA, and Milt Gottschall, BS, for assembling the obstetrics registry used for this study.

REFERENCES

Preeclampsia and cardiovascular complications remain significant contributors to maternal morbidity and mortality. The Centers for Disease Control and Prevention (CDC) reported that between 1991 and 1999, 19.3% of maternal mortality during live births was due to pregnancy-related hypertension. Research efforts over the past decade have attempted to elucidate risk factors for cardiovascular disease (CVD) and, increasingly, those specific to women. Strikingly, similar biological mechanisms for preeclampsia and CVD have been proposed, including inflammation, hypercoagulability, and insulin dysregulation. In addition, normal physiologic adaptations of pregnancy, including increased insulin resistance, hyperlipidemia, and hypercoagulable changes, are considered risk factors for CVD, or even pathologic processes, in nonpregnant women. These same changes have been shown to be risk factors for preeclampsia when they occur before pregnancy and, perhaps, when they develop during pregnancy as well.

In the nonpregnant population, aside from functioning as independent risk factors, hypertension, insulin dysregulation, and dyslipidemia also have been shown to convey an aggregate risk for future CVD. To capture the additive nature of these factors and the hypothesized shared pathologic pathway, this relationship has been conceptualized as a metabolic syndrome. The National Cholesterol Education Program—Adult Treatment Panel III Guidelines (NCEP-ATP III) define metabolic syndrome as the presence of 3 or more of the following 5 risk factors: abdominal obesity, triglycerides, high-density lipoprotein (HDL) cholesterol, blood pressure, and fasting glucose. Large-scale clinical trials such as the Women’s Health Study and the Framingham Offspring Study have confirmed the presence of metabolic syndrome in women and its contributing role to CVD. In fact, the presence of metabolic syndrome in women is more predictive of future CVD than its presence in men.

Many of the individual risk factors that compose metabolic syndrome also have been noted to be risk factors in the development of preeclampsia, and the syndrome itself has been posited as the connection between preeclampsia and CVD. Recent research suggests that preeclampsia is a significant risk factor for the development of future metabolic syndrome and that preeclampsia itself may alter the patient’s physiology, predisposing her to future CVD and stroke.

Conversely, the teleologic hypothesis has been made that because pregnancy places stress on the cardiovascular and metabolic systems of women, it may uncover a predisposition for future cardiovascular disease. A large, retrospective cohort study found an adjusted hazard ratio of 2.1 for future cardiovascular disease in women with a history of pre-
eclampsia, a risk that was found to be additive with both the individual components of metabolic syndrome and the composite diagnosis. More severe pre-eclampsia, especially preterm pre-eclampsia, appears to be associated with an even greater risk of long-term cardiovascular morbidity and mortality. In a large retrospective cohort, women with preeclampsia and preterm delivery (same pregnancy) had a 2.71 (1.99, 3.68)-fold increase in mortality and a 8.12-fold increase in (4.31, 15.33) mortality from cardiovascular causes.

Whether preeclampsia predisposes women to future CVD through an intermediate of metabolic syndrome or whether preeclampsia is a pregnancy-induced positive “stress” test and the manifestation of a subclinical susceptibility to future metabolic syndrome and CVD remains unclear. The concept of metabolic syndrome has demonstrated clinical utility in the nonpregnant population for risk stratification and intervention targeting. We posit that using this concept in pregnant women may provide similar benefits and also allow further study of metabolic syndrome as a potential pathophysiological mechanism in the development of preeclampsia.

To date, only individual components of metabolic syndrome have been examined during pregnancy. To begin to assess the role of metabolic syndrome in preeclampsia, we developed a composite measure, or metabolic score, using the 3 factors from the NCEP-ATP III definition as an initial and final presentation. Separate metabolic scores (initial and final) were calculated for both time points by the statistician using the database and not at time of admission or chart review. A separate individual performed data entry. One of the primary investigators (S.K.S.) reviewed all data sheets for completeness, consistency, and accuracy.

Metabolic score was composed of the 3 NCEP-ATP III criteria that could be approximated with clinical criteria only: obesity, blood pressure, and fasting glucose. The diagnosis of CHTN was made by patient self-report or if a patient screened at less than 20 weeks' gestation with 140 mm Hg or greater systolic or 90 mm Hg diastolic or greater blood pressure. The blood pressure cut-points for CHTN were set by the traditional obstetric criteria for the diagnosis of pregnancy-induced hypertension and not by NCEP-ATP III criteria.

The presence of diabetes was used in place of a documented fasting glucose. Diabetes mellitus was defined as the presence of pregestational or gestational diabetes. Given the impracticality of waist circumference measurement, the NCEP-ATP III criterion for abdominal obesity, in the gravid women, body mass index (BMI) was used in the calculation of the metabolic score. Type 2 diabetes, impaired glucose tolerance, and BMI are criteria in the World Health Organization definition of metabolic syndrome, supporting this approach.

Initial pregnancy BMI was calculated based on screening prenatal weight and patient-reported height to assess for an association between baseline adiposity and preeclampsia. Final pregnancy BMI was calculated based on last prenatal weight and patient-reported height to allow assessment of pregnancy-related weight gain in relation to preeclampsia. As in prior studies on metabolic syndrome, BMI was dichotomized as 30 kg/m² or greater or less than 30 kg/m² based on the CDC’s accepted definition of obesity. Each variable (BMI 30 kg/m² or greater, CHTN, and diabetes) was dichotomized as yes/no at the time of the screening visit (initial); BMI and the presence of diabetes were reassessed at final presentation. Separate metabolic scores (initial and final) were calculated for both time points by the statistician using the database and not at time of admission or chart review. A metabolic

Materials and Methods
This study examined a subset of patients within a larger case-control study, Pre-eclampsia: Mechanisms and Consequences. Institutional review board approval was obtained from the University of Pennsylvania School of Medicine prior to enrollment. Cases were women with preeclampsia. Controls were non-motivational women presenting for delivery at term. Cases and controls were collected prospectively between March 2005 and October 2006 at the Hospital of the University of Pennsylvania.

All women admitted to labor and delivery with preeclampsia were eligible for enrollment and invited to participate. Cases were identified based on maternal criteria for preeclampsia. Mild preeclampsia included the diagnosis of gestational hypertension and was defined as elevated blood pressure (140/90 mm Hg or greater on 2 measurements 6 hours or more apart or 160/105 mm Hg or greater on presentation) with a score of 1 or less proteinuria. Severe preeclampsia was defined as blood pressure 160/105 mm Hg or greater on presentation and more than 1 or more proteinuria or blood pressure 140/90 mm Hg or greater on 2 measurements 6 hours or longer apart with any of the following: platelets less than 120,000/mL, aspartate aminotransferase greater than 45 U/L, aminotransferase greater than 60 U/L, or creatinine 1.0 mg/dL or greater; the use of intravenous anti-hypertensive medications prior to delivery; delivery prior to 37 weeks’ gestation secondary to the diagnosis of preeclampsia; or the presence of eclampsia.

Based on these prespecified criteria, subclassification of preeclampsia into mild or severe categories was determined at the time of enrollment by the investigators (S.K.S. and M.A.E.) and not by physician diagnosis. Controls were prospectively enrolled from women presenting for delivery at term (37 weeks or longer) for scheduled induction of labor, scheduled cesarean section, spontaneous rupture of membranes, or term labor. There were no exclusion criteria for either cases or controls in the overall study. Patients with multiple gestations were not included in this analysis.

Trained research nurses collected information on height, race, ethnicity, chronic hypertension (CHTN) history and family history by patient interview at the time of enrollment. Other history including obstetric, demographic, prenatal delivery, and neonatal information were collected from prenatal and hospital chart abstraction by trained research nurse abstractors. A separate individual
The prevalence of the metabolic score (0, 1, 2 or more) among cases and controls was evaluated using Pearson χ² tests of association; prevalence was similarly calculated among women with pre-eclampsia by severity of disease. For both primary and secondary analyses, initial descriptive analyses were performed. Multivariable logistic regression analyses were performed to control for biologic confounders (age [30 years or younger vs older than 30 years], race [African American vs other], and history of pre eclampsia). Because of the case-control design, the outcome in the logistic models was case/control status or severe vs mild pre-eclampsia. Considering the potential biological role of age in the underlying comorbidities, tests for effect modification/interaction between metabolic score and maternal age were also evaluated using stratified analyses and tests of interaction within logistic models. Dichotomization occurred at 30 years of age to identify increased cardiovascular risk (rather than age 35 years to identify genetic/obstetric risk). All analyses were performed using STATA statistical software (version 9.0 Special Edition, College Station, TX).

A priori calculations assumed a population prevalence of the components of metabolic syndrome to be a minimum of 5%. Other assumptions included a type I, alpha, error rate of 5% and 80% statistical power. As predicted in sample size calculations, we have sufficient statistical power to detect odds ratios of 2.6 or larger for the components of metabolic score and the ability to detect smaller associations for more prevalent exposures and the continuously-measured metabolic score (0-3).

**RESULTS**

Two hundred fifty-nine cases and 297 controls were evaluated. A greater number of cases were African American and had CHTN, preexisting diabetes, and a BMI of 30 kg/m² or greater at the time of the initial prenatal care visit as well as a history of prior pre eclampsia (Table 1). Odds ratios were calculated for the components of metabolic score and for age; these were adjusted for race and history of pre-eclampsia. CHTN increased the odds of being a case by 3.04 (1.58, 5.84; P = .001), as did pregestational diabetes (odds ratio 4.21 [1.12, 15.83]; P = .034). Diabetes at any point in pregnancy had an odds ratio of 1.92 (0.89, 4.16; P = .099) but did not reach statistical significance. BMI increased the odds of being a case at either time point but also did not reach statistical significance: initial BMI had an odds ratio of 1.3 (0.87, 1.93; P = .203), and final BMI had an odds ratio of 1.46 (0.98, 2.18), P = .062.

For initial metabolic score, 44.1%, 42.3%, and 13.5% of cases and 61.5%, 33.2%, and 5.3% of controls had a score of 0, 1, and 2 or more, respectively (P < .0001). For final metabolic score, 25%, 57.1%, and 17.9% of cases and 40.1%, 51.1%, and 8% of controls had a score of 0, 1, and 2 or more, respectively (P < .0001).

Controlling for age younger than 30 years, race, and history of preeclampsia, an initial metabolic score of 1 and 2 or more, compared with 0, increased the odds of being a case by 1.67 (1.09, 2.54, P = .017) and 2.96 (1.36, 6.47, P = .006), respectively. A final metabolic score of 1 and 2 or more also increased the odds of being a case by 1.67 (1.09, 2.57; P = .018) and 3.28 (1.64, 6.55; P = .001), respectively.

The potential biological role of age on the underlying comorbidities was explored by including interaction terms between maternal age (older than 30 years of age) and the metabolic score. Although the test of effect modification did not reach statistical significance (P = .32 for initial metabolic score and P = .071 for final metabolic score), the association between metabolic score and pre-eclampsia appeared to be modified by maternal age. In women older than 30 years, an increasing metabolic score carried even greater odds of having pre-eclampsia. In this group, after controlling for race and history of preeclampsia, an initial metabolic score of 1 increased the odds of pre-eclampsia by 2.2 times over women without any of the contributing health conditions but did not reach statistical significance (0.94, 4.91; P = .070). A score of 2 or more increased the odds by 5.4 times over women with a score of zero (1.75, 16.409; P = .003) (Table 2). In women 30 years of age or younger, there was no significant association between the initial metabolic score and preeclampsia.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Demographic characteristics of study population</th>
</tr>
</thead>
<tbody>
<tr>
<td>Characteristic</td>
<td>Controls (%)</td>
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<tr>
<td>African American</td>
<td>72.4</td>
</tr>
<tr>
<td>Age 30 y or younger</td>
<td>66.3</td>
</tr>
<tr>
<td>Chronic hypertension</td>
<td>5.4</td>
</tr>
<tr>
<td>Diabetes</td>
<td></td>
</tr>
<tr>
<td>Pregestational</td>
<td>1.0</td>
</tr>
<tr>
<td>Gestational plus pregestational</td>
<td>4.4</td>
</tr>
<tr>
<td>History of pre eclampsia</td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>7.1</td>
</tr>
<tr>
<td>Primigravida</td>
<td>35.4</td>
</tr>
<tr>
<td>Tobacco</td>
<td>12.8</td>
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</tbody>
</table>

* BMI 30 kg/m² or greater at initial prenatal visit.
for the final metabolic score are shown in Table 3.

To further explore the potential mechanisms for the association between metabolic score and preeclampsia, additional analyses were performed comparing women with mild (n = 95) and severe (n = 164) disease. Within the cases, 52.5%, 37.5%, and 10% of women with mild preeclampsia and 39.4%, 45.1%, and 15.5% of women with severe preeclampsia had an initial metabolic score of 0, 1, and 2 or more, respectively (P = .15). A similar non-significant trend was observed between increasing final metabolic score and severity: 24.4%, 64%, and 11% of mild cases and 25.4%, 52.8%, and 21.8% of severe cases had a final metabolic score of 0, 1, and 2 or more, respectively (P = .09). When the metabolic score was assumed to be a continuous variable (0-3) in unadjusted models, the odds of severe disease increased by nearly 50% for each additional component of the initial metabolic score (odds ratio 1.49 [0.99, 2.24]; P = .058). The odds ratio for final metabolic score was 1.26 (0.83, 1.93; P = .27). These associations were not significant after adjustment for important confounders (age and race) because of insufficient statistical power to fully evaluate these case-case associations.

**TABLE 2**

<table>
<thead>
<tr>
<th>Risk factor</th>
<th>Odds ratio*</th>
<th>95% CI</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Metabolic score and age older than 30 y</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>2.15</td>
<td>0.940, 4.907</td>
<td>.070</td>
</tr>
<tr>
<td>2 or greater</td>
<td>5.36</td>
<td>1.750, 16.409</td>
<td>.003</td>
</tr>
<tr>
<td>Metabolic score and age 30 y or younger</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>1.55</td>
<td>0.953, 2.513</td>
<td>.077</td>
</tr>
<tr>
<td>2 or greater</td>
<td>1.67</td>
<td>0.568, 4.917</td>
<td>.351</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>2.49</td>
<td>1.454, 4.267</td>
<td>.001</td>
</tr>
</tbody>
</table>

**TABLE 3**

<table>
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<tr>
<th>Risk factor</th>
<th>Odds ratio*</th>
<th>95% CI</th>
<th>P value</th>
</tr>
</thead>
<tbody>
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<td>Metabolic score and age older than 30 y</td>
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<td></td>
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<td>1</td>
<td>1.41</td>
<td>0.575, 3.450</td>
<td>.453</td>
</tr>
<tr>
<td>2 or greater</td>
<td>6.08</td>
<td>2.063, 17.932</td>
<td>.001</td>
</tr>
<tr>
<td>Metabolic score and age 30 y or younger</td>
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<td></td>
</tr>
<tr>
<td>1</td>
<td>1.76</td>
<td>1.078, 2.879</td>
<td>.024</td>
</tr>
<tr>
<td>2 or greater</td>
<td>1.66</td>
<td>0.664, 4.143</td>
<td>.278</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>2.65</td>
<td>1.554, 4.507</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

**Comment**

This study demonstrates that the metabolic score, a surrogate measure of metabolic syndrome in pregnancy, is independently associated with developing preeclampsia. Whereas others have posited that metabolic syndrome is a shared biological pathway between preeclampsia and CVD, there are no prospective studies investigating more than 1 component of the conceptualized metabolic syndrome in association with preeclampsia. Although a novel method, the metabolic score was designed with consideration of established World Health Organization and NCEP-ATP III diagnostic criteria. Our results provide evidence that, similar to nonpregnant populations, the conceptualized metabolic syndrome may provide a screening and eventual therapeutic target for risk reduction efforts in the pregnant population for preeclampsia. These studies should prompt more rigorous studies addressing all the components of metabolic syndrome, including not only their relationship to preeclampsia risk but also long-term cardiovascular risk in women with a history of preeclampsia.

Our study has limitations. Despite the relatively large numbers enrolled, this study was unable to elucidate whether the variables that comprise the metabolic score have a greater combined risk than the sum of the individual parts, outlining a path of future study. We also were unable to determine which metabolic components contributed the additionally observed risk because the components were equally weighted in this study. In addition, our study did not incorporate laboratory data and so could not include 2 of the 5 NCEP-ATP III criteria: triglycerides and HDL cholesterol. Both triglycerides and HDL cholesterol are known to increase during pregnancy, and the incorporation of these parameters into our
metabolic score may affect the observed association significantly and, indeed, would likely create a scoring system with a more robust ability to identify those at greatest risk. We intend to explore this with additional studies.

In our study, an increasing metabolic score in women older than 30 years increases the likelihood of having pre-eclampsia. If components of metabolic syndrome increase with maternal age, then this should occur with equal frequency in both cases and controls. However, our findings suggest a more complex interplay between these biologic pathways. Increasing maternal age may further predispose women with preexisting metabolic disturbances to succumb to the stress of pregnancy with the consequent manifestation of preeclampsia.

Like cardiovascular disease, preeclampsia may be the endpoint of many different, diverse processes of disease. Others have argued that metabolic syndrome could be a causal link that explains the increased lifetime risk of cardiovascular disease in women with preeclampsia. These studies support a link between metabolic syndrome and preeclampsia. However, we acknowledge that metabolic syndrome may serve as an explanation for only some forms of preeclampsia and that the odds ratio shown here therefore may be weakened, particularly in a population in which the individual components (eg, BMI) are relatively common. Our definition of severe preeclampsia, slightly different from traditional American College of Obstetricians and Gynecologists criteria, could have resulted in misclassification bias. However, the use of stricter inclusion criteria for severe preeclampsia in our study may be more clinically justified and biologically relevant.

The determination of diagnoses was made by the investigators (S.K.S. and M.A.E.); this could introduce a potential bias. However, we believe the greater potential for bias was using definitions of disease as determined by various attending physicians. This potential bias was removed because diagnoses were determined by a priori defined definitions and not reliant on the caring physician’s assessment. We also examined a predominantly African American inner-city population, and so these results may not be widely generalizable. However, pre-eclampsia does occur more frequently in African American populations, so our population may be representative of those mostly likely to be affected by the disease.

The prevalence of cardiovascular disease, metabolic syndrome, and preeclampsia in the US population brings with it evident morbidity and mortality. Clarifying the relationship between these conditions may help efforts to develop early screening, diagnostic, and treatment modalities. Given the pervasiveness of these illnesses, successful interventions would give obvious benefits to both patient and fetus at multiple points during their lifetimes.

REFERENCES
Randomized comparison of intravenous terbutaline vs nitroglycerin for acute intrapartum fetal resuscitation

Kristin M. Pullen, MD; Edward T. Riley, MD; Sarah A. Waller, MD; Larisa Taylor, MD; Aaron B. Caughey, MD, PhD; Maurice L. Druzin, MD; Yasser Y. El-Sayed, MD

OBJECTIVE: The purpose of this study was to compare terbutaline and nitroglycerin for acute intrapartum fetal resuscitation.

STUDY DESIGN: Women between 32-, 42 weeks’ gestation were assigned randomly to 250 μg of terbutaline or 400 μg nitroglycerin intravenously for nonreassuring fetal heart rate tracings in labor. The rate of successful acute intrapartum fetal resuscitation and the maternal hemodynamic changes were compared. Assuming a 50% failure rate in the terbutaline arm, we calculated that a total of 110 patients would be required to detect a 50% reduction in failure in the nitroglycerin group (50% to 25%), with an alpha value of .05, a beta value of .20, and a power of 80%.

RESULTS: One hundred ten women had nonreassuring fetal heart rate tracings in labor; 57 women received terbutaline, and 53 women received nitroglycerin. Successful acute resuscitation rates were similar (terbutaline 71.9% and nitroglycerin 64.2%; P = .38). Terbutaline resulted in lower median contraction frequency per 10 minutes (2.9 [25-75 percentile, 1.7-3.3] vs 4 [25-75 percentile, 2.5-5]; P < .002) and reduced tachysystole (1.8% vs 18.9%; P = .003). Maternal mean arterial pressures decreased with nitroglycerin (81-76 mm Hg; P = .02), but not terbutaline (82-81 mm Hg; P = .73).

CONCLUSION: Although terbutaline provided more effective tocolysis with less impact on maternal blood pressure, no difference was noted between nitroglycerin and terbutaline in successful acute intrapartum fetal resuscitation.

Key words: terbutaline, nitroglycerin, intrapartum fetal resuscitation, tocolysis, nonreassuring fetal heart rate

M}atal interventions to assist in fetal intrapartum resuscitation include change in maternal position, oxygen by face mask, intravenous fluids, discontinuation of labor induction or augmentation medications, amnioinfusion, and tocolytics. The use of acute tocolysis with various agents has become widespread in clinical practice on the basis of the presumption that uterine relaxation improves uteroplacental blood flow and therefore fetal oxygenation.

The most commonly studied tocolytics for intrapartum fetal resuscitation are betamimetic drugs. However, data from randomized clinical trials that use betamimetic drugs are limited by small numbers, predetermined mode of delivery, and open-label protocols. Based on our PubMed literature search using the key words nitroglycerin, terbutaline, fetal resuscitation, nonreassuring fetal heart rate, and tocolysis, there have been no published randomized studies that have investigated intrapartum administration of nitroglycerin for acute intrapartum fetal resuscitation.

We performed a prospective, randomized trial that compared the efficacy and safety of intravenous terbutaline to intravenous nitroglycerin for acute intrapartum fetal resuscitation.

MATERIALS AND METHODS

This randomized trial was conducted on the labor and delivery unit at Lucile Packard Children’s Hospital at Stanford University Medical Center after approval by the hospital’s institutional review board. Patients were recruited from October 2003-June 2006. Eligible patients for this study included women between 32 and 42 weeks’ gestation with a singleton pregnancy who were admitted in active labor or for an induction of labor. Maternal exclusion criteria included preeclampsia, chronic hypertension, cardiac disease, placental abruption, insulin-dependent diabetes mellitus, hyperthyroidism, maternal age <18 years, and patients with a previous cesarean delivery. Fetal exclusion criteria included intrauterine growth restriction, multiple gestation, and fetal anomalies that were incompatible with life. The criteria for a nonreassuring fetal heart rate tracing (NRFHT) were adapted from Kubli et al and the National Institute of Child Health and Human Development guidelines and included prolonged deceleration (decrease in baseline fetal heart rate to <100 beats/min, with a duration of >2 min), severe variable deceleration (decrease in the fetal heart rate to ≤70 beats/min with a duration of ≥60 seconds but <2 min), late deceleration (a gradual decrease in the fetal heart rate with the nadir of the deceleration occurring after the peak of the contraction),
and tachycardia with reduced variability (baseline fetal heart rate >160 beats/min) without evidence of chorioamnionitis (defined as fetal and/or maternal tachycardia with maternal temperature ≥38°C). Patients were excluded from receiving a study medication if the only indication was fetal tachycardia with reduced variability in the setting of chorioamnionitis. Otherwise, chorioamnionitis was not an exclusion criteria.

All patients had electronic fetal monitoring. If the patient was noted to have an abnormal fetal heart rate tracing, a cervical examination was performed to evaluate for cord prolapse, dilation, and station. Then, the usual methods of fetal resuscitation were initiated. These included position change, intravenous fluid hydration, oxygen administration by face mask, and cessation of augmentation and induction medications. The option of amnioinfusion was left to the individual physician. If these measures of fetal resuscitation did not improve the fetal heart rate tracing, tocolysis with terbutaline 250 µg or nitroglycerin 400 µg was administered intravenously. The study drug was prepared by the labor and delivery nurse who used a prepared, concealed randomization chart that was located in the medication supply room. The randomization chart was created with the use of a random numbers table, and the study drug assignment was available only to the labor and delivery nurse who prepared the medication. The obstetrician who ordered the tocolytic and the patient were both blinded to the medication.

Maternal blood pressure and heart rate were recorded immediately before and at least every 15 minutes for 1 hour after the tocolytic was administered. If the fetal heart rate tracing did not improve after the study medication, the decision to readminister a second tocolytic and to proceed with an urgent delivery was left to the attending obstetrician. If, after initial resolution, there were subsequent episodes of NRFHT, management decisions were left to the attending obstetrician. In our institution, both terbutaline and nitroglycerin are administered routinely as tocolytics for NRFHT; thus, either of these medications could be given for persistent or subsequent episodes of NRFHT per physician preference. All fetal heart rate tracings were later read and interpreted by a single researcher (K.M.P.) who was also blinded to the study medication.

The primary outcome was success or failure of terbutaline vs nitroglycerin tocolysis for acute intrapartum fetal resuscitation. Successful resuscitation including complete resolution of the NRFHT within 10 minutes, no recurrence of the NRFHT or readministration of a tocolytic within 30 minutes, and no operative delivery (forceps, vacuum, or cesarean section) for NRFHT within 1 hour of study drug administration. Assuming a 50% failure rate in the terbutaline arm, we calculated that a total of 110 patients would be required to detect a 50% reduction in failure in the nitroglycerin group (from 50% to 25%), with an alpha value of .05 and a power of 80%. Statistical analysis of the data was performed with χ², Student t, Fisher’s exact, and Mann-Whitney U tests where appropriate.

**RESULTS**

Nine hundred fifty-six women were enrolled preliminarily in this study after written informed consent was obtained. Of these, 110 women (11.5%) were assigned randomly to the terbutaline or nitroglycerin after experiencing NRFHT, thus fulfilling all study inclusion criteria. Fifty-seven women were assigned randomly to the terbutaline group, and 53 women were assigned randomly to the nitroglycerin group (Figure). There were no differences in the baseline maternal characteristics, except that significantly more patients who were assigned randomly to the terbutaline group had oligohydramnios on admission (defined as an amniotic fluid index of <5 cm with intact membranes) as an indication for induction of labor.
(Table 1). There were no cord prolapses in either group.

Successful acute intrapartum fetal resuscitation was achieved in 41 of 57 women who received terbutaline and in 34 of 53 women who received nitroglycerin (71.9% vs 64.2%, respectively; \( P = .38 \); Table 2). Terbutaline tocolysis resulted in lower median contraction frequency in a 10-minute window after drug administration (median, 2.9 \([25-75\text{ percentile, 1.7}-3.3]\) vs 4 \([25-75\text{ percentile, 2.5}-5]\) contractions/10 minutes; \( P = .002 \); Table 3) and better resolution of tachysystole at 10 minutes after administration of the study drug (1.8% vs 18.9%; \( P = .003 \); Table 3). The ultimate operative delivery rates for NRFHT were similar (47.4% vs 47.2%; \( P = .98 \); Table 2).

The effectiveness of the terbutaline and nitroglycerin for acute intrapartum fetal resuscitation remained similar when analyzed by the type of NRFHT, although there was a trend towards an increased effectiveness of terbutaline in the setting of severe variable decelerations (66.7% vs 17.0%; \( P = .13 \)).

No statistically significant differences were noted between groups in reasons for failed acute intrapartum resuscitation, although more women in the nitro-

### Table 1

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Terbutaline (n = 57)</th>
<th>Nitroglycerin (n = 53)</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (yr)*</td>
<td>28.5 ± 6.4</td>
<td>27.8 ± 6.1</td>
<td>.54</td>
</tr>
<tr>
<td>Gestational age (wk)*</td>
<td>39.4 ± 1.6</td>
<td>39.8 ± 1.4</td>
<td>.16</td>
</tr>
<tr>
<td>Multiparity</td>
<td>14 (24.6%)</td>
<td>22 (41.5%)</td>
<td>.06</td>
</tr>
<tr>
<td>Public insurance (n)</td>
<td>30 (52.6%)</td>
<td>30 (56.6%)</td>
<td>.68</td>
</tr>
<tr>
<td>Induction (n)</td>
<td>31 (54.4%)</td>
<td>26 (49.1%)</td>
<td>.58</td>
</tr>
<tr>
<td>Oligohydramnios indication for induction (n)</td>
<td>12 (21.1%)</td>
<td>1 (1.9%)</td>
<td>.002</td>
</tr>
<tr>
<td>Oxytocin augmentation (n)</td>
<td>18 (31.6%)</td>
<td>15 (28%)</td>
<td>.71</td>
</tr>
<tr>
<td>Median cervical dilation (cm)^†</td>
<td>7 (3.5-8)</td>
<td>7 (5-9)</td>
<td>.35</td>
</tr>
<tr>
<td>Ruptured membranes at time of study drug (n)</td>
<td>55 (96.5%)</td>
<td>52 (98.1%)</td>
<td>.10</td>
</tr>
<tr>
<td>Amnioinfusion before study drug (n)</td>
<td>7 (12.3%)</td>
<td>2 (3.8%)</td>
<td>.16</td>
</tr>
<tr>
<td>Study drug within 1 hour of regional anesthesia (n)</td>
<td>7 (12.3%)</td>
<td>3 (5.7%)</td>
<td>.32</td>
</tr>
</tbody>
</table>

* Student \( t \)-Fisher’s exact, and Mann-Whitney \( U \) tests were used, as appropriate.
* Data are presented as mean ± SD.
^ Data are presented as median (25%-75%).

### Table 2

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Terbutaline (n = 57)</th>
<th>Nitroglycerin (n = 53)</th>
<th>( P ) value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall success (n)</td>
<td>41 (71.9%)</td>
<td>34 (64.2%)</td>
<td>.38</td>
</tr>
<tr>
<td>Emergent cesarean within one hour for NRFHT (n)</td>
<td>8 (14.0%)</td>
<td>12 (22.6%)</td>
<td>.24</td>
</tr>
<tr>
<td>Ultimate operative delivery for NRFHT (n)^†</td>
<td>27 (47.4%)</td>
<td>25 (47.2%)</td>
<td>.98</td>
</tr>
<tr>
<td>Ultimate cesarean delivery for NRFHT (n)</td>
<td>19 (33.3%)</td>
<td>17 (32.1%)</td>
<td>.89</td>
</tr>
<tr>
<td>Overall cesarean delivery (n)</td>
<td>30 (52.6%)</td>
<td>29 (54.7%)</td>
<td>.83</td>
</tr>
<tr>
<td>Required second agent at initial NRFHT (n)</td>
<td>2 (3.5%)</td>
<td>2 (3.8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Subsequent tocolytic (n)</td>
<td>12 (21.1%)</td>
<td>9 (17.0%)</td>
<td>.59</td>
</tr>
<tr>
<td>Median estimated blood loss (mL)^‡</td>
<td>600 (300-800)</td>
<td>600 (300-800)</td>
<td>.79</td>
</tr>
<tr>
<td>Uterine atony (n)</td>
<td>5 (8.8%)</td>
<td>3 (5.7%)</td>
<td>.72</td>
</tr>
<tr>
<td>Postpartum hemorrhage (n)</td>
<td>3 (5.3%)</td>
<td>2 (3.8%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Chorioamnionitis (n)</td>
<td>12 (21.1%)</td>
<td>10 (18.9%)</td>
<td>.78</td>
</tr>
</tbody>
</table>

* \( \chi^2 \), Fisher’s exact, and Mann-Whitney \( U \) tests were used, as appropriate.
† Operative delivery included forceps, vacuum, or cesarean section.
‡ Data are represented as median (25%-75%).
TABLE 3
Tocolytic efficacy

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Terbutaline (n = 57)</th>
<th>Nitroglycerin (n = 53)</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median contractions/10 minutes before drug administration†</td>
<td>5 (5-10)</td>
<td>5 (5-10)</td>
<td>.82</td>
</tr>
<tr>
<td>Median contractions/10 minutes after drug administration‡</td>
<td>2.9 (1.7-3.3)</td>
<td>4 (2.5-5)</td>
<td>.002</td>
</tr>
<tr>
<td>Tachysystole at 10 minutes before drug administration (n)</td>
<td>45 (78.9%)</td>
<td>44 (83.0%)</td>
<td>.59</td>
</tr>
<tr>
<td>Tachysystole at 10 minutes after drug administration (n)</td>
<td>1 (1.8%)</td>
<td>10 (18.9%)</td>
<td>.003</td>
</tr>
</tbody>
</table>

* Mann-Whitney U, χ², and Fisher’s exact tests were used, as appropriate.
† Data are presented as median (25%-75%).
‡ H11549

Patriarco et al\textsuperscript{11} conducted a open-label, randomized trial of 20 women with ominous fetal heart rate tracings before a cesarean section delivery. The women were assigned randomly to either subcutaneous terbutaline or no treatment. The women who received terbutaline had better Apgar scores and fewer cases of fetal acidemia. Magann et al\textsuperscript{4} compared intravenous magnesium sulfate to subcutaneous terbutaline in 46 women who were awaiting a cesarean section delivery for fetal distress. They detected an improvement in the fetal heart rate tracings and higher umbilical cord pH values. Although this was an open-label trial, the investigator who retrospectively read the fetal heart rate tracings was blinded to the study drug. Kuller et al\textsuperscript{16,17} studied 37 women who were awaiting a cesarean section delivery for nonreassuring fetal heart rate patterns. The women were assigned randomly to intravenous hexoprenaline or no treatment. There was a trend toward fewer acidotic neonates in the hexoprenaline group and 2 stillbirths in the no treatment arm. This was also an open-label trial, with the investigator who retrospectively read the fetal heart rate tracings blinded to the study drug.

Regarding the hemodynamic parameters, there was a significant decrease in mean arterial blood pressures after the study drug in the nitroglycerin group, but not in the terbutaline group (81-76 mm Hg [P = .02] vs 82-81 mm Hg; P = .73). There were significant mean changes in maternal heart rate in both the terbutaline and nitroglycerin groups (terbutaline 80-99 beats/min; P ≤ .001 vs nitroglycerin 84-93 beats/min; P = .01). Hypotension that required medical intervention did not occur in either group.

Last, there were no differences between the terbutaline and nitroglycerin groups in estimated blood loss, uterine atony, postpartum hemorrhage (defined as ≥ 500 mL for a vaginal delivery; ≥ 1000 mL for a cesarean section delivery), or neonatal outcomes such as Apgar score <7 at 5 minutes, umbilical artery pH <7, or neonatal intensive care admissions (Tables 2 and 4).

**Comment**

Tocolytic agents are potentially valuable tools in the management of intrapartum nonreassuring fetal heart rate tracings, because they may allow for acute intrapartum fetal resuscitation and, with it, labor continuation or a less emergent operative vaginal or cesarean delivery. We were able to identify only 4 randomized control trials that evaluated the use of betamimetics as tocolytics for acute intrapartum fetal resuscitation.\textsuperscript{3,4,11,12}
Our criteria for what constituted success: complete resolution of an NRFHT within 10 minutes, no recurrence of the NRFHT or readministration of a tocolytic within 30 minutes, and no operative delivery for NRFHT within 1 hour of study drug administration. Finally, as best as we are able to determine, our study includes the largest number of women to be assigned randomly to a tocolytic for the purpose of acute intrapartum fetal resuscitation.

Our study had several limitations. Because of the urgent nature of the clinical situation, we were unable to assess and record subjective maternal side-effects, such as headache, dizziness, or palpitations. In addition, because tocolytics are considered routine care at our hospital for acute intrapartum fetal resuscitation, this was not a placebo-controlled trial. Furthermore, although the patient and obstetrician were blinded to the study drug, the labor and delivery nurse who prepared the study drug was unblinded by necessity.

Our power analysis was based on an estimated terbutaline failure rate of 50%. The failure rate of betamimetics in the literature for this indication is very broad, ranging from 10-60%. Future studies should evaluate the use of nitroglycerin in different preparations, such as sublingual or intranasal, and different dosing regimens. There should also be further investigation into treatment that is tailored to specific nonreassuring fetal heart rate patterns.

ACKNOWLEDGMENTS
We thank the excellent Stanford University OB/GYN residents, fellows, and attending physicians and the anesthesia and nursing staff for all their help and dedication to this study.

REFERENCES


14. National Institute of Child Health and Human Development Research Planning Work-
Characterization of a murine model of fetal programming of atherosclerosis

Nima Goharkhay, MD; Elena Sbrana, PhD; Phyllis K. Gamble; Esther H. Tamayo; Ancizar Betancourt, MSc; Karina Villarreal; Gary D. V. Hankins, MD; George R. Saade, MD; Monica Longo, MD, PhD

OBJECTIVE: The objective of the study was to investigate the effect of fetal programming on the development of atherosclerosis in the offspring in a mouse model.

STUDY DESIGN: Male and female mice of the wild type and the knock-out for the apoprotein E (apoE) gene were cross-bred to obtain all 4 possible genetic offspring types. The offspring were kept on regular chow and killed at 8 months of age. Levels of total cholesterol and triglycerides were determined. The aortic arch was examined for the presence and severity of atherosclerosis. Kidney and liver sections were analyzed for pathologic changes.

RESULTS: We found increased total cholesterol levels and incidence of atherosclerosis in offspring born to hypercholesterolemic mothers as compared with genomically similar animals born to wild-type mothers. These animals also showed kidney and liver lesions consistent with chronic hypercholesterolemia.

CONCLUSIONS: There is a strong effect of fetal programming on the development of atherosclerosis in the apoE mouse model.

Key words: atherosclerosis, fetal development, fetal programming, hypercholesterolemia, mouse model

Cardiovascular disease is the number 1 cause for morbidity and mortality in industrialized as well as many developing countries. Atherosclerosis secondary to hyperlipidemia constitutes the main etiologic cause for cardiovascular disease.

A role for developmental programming of atherosclerosis has been suggested by various investigators. A correlation between maternal and fetal cholesterol levels during the first 26 weeks of gestation has been shown in humans. Napoli et al further demonstrated decreased regression of fatty streak lesions in children born to hypercholesterolemic mothers as compared with controls. A rabbit model of fetal programming of atherosclerosis has also been described. In this model, maternal therapy during pregnancy with vitamin E and cholestyramine was shown to decrease plaque formation in the adult offspring to levels found in the litters born to normocholesterolemic mothers. The availability of a murine model would greatly facilitate the study of the effects of fetal programming as well as its underlying mechanisms and possible countermeasures.

The apolipoprotein E (apoE) deficient mouse is a widely used and well-characterized model to study atherosclerosis secondary to hypercholesterolemia. When fed a regular chow, homozygous apoE knockout animals display total cholesterol levels that are 5 times higher than those observed in wild-type animals. In addition, feeding these animals a high-fat diet leads to a further 3-fold increase in circulating cholesterol levels. In contrast, heterozygous mice possess 1 functional copy of the apoE gene and do not show a significant increase in cholesterol levels.

The objective of the current study was to investigate whether maternal hypercholesterolemia during pregnancy correlates with elevated cholesterol levels and early development of atherosclerosis in the adult offspring in a mouse model. The secondary outcomes of interest were to determine the effects of fetal programming on the severity of atherosclerosis, and the nature and extent of secondary organ damage in the liver and kidney of offspring born to hypercholesterolemic mothers.

MATERIALS AND METHODS

Mature cycling female and male mice (4-6 weeks old) homozygous for disruption of the apoE gene (apoE<sup>−/−</sup> KO, strain B6.129P2-Apoel<sup>−/−</sup>KOJ/0)
Cross-breeding pattern used to obtain the 4 genetic variations of offspring

Heterozygous

- apoE+/WT
- apoE+/KO

Homozygous

- apoE+/+WT
- apoE+/-WT
- apoE+/-KO
- apoE+-/-KO

Sections were stained with oil red O and evaluated for presence and size of atherosclerotic plaques using a modification of the en face method with an image analysis software package (ImageJ, v 1.37h, National Institutes of Health, http://rsb.info.nih.gov/ij/).

- Areas of aortic lesions that stained orange and red were classified as advanced plaques. The specimens containing areas of advanced plaques were considered positive for the presence of atherosclerosis. We also measured areas that showed yellow discoloration as compared with the background. These areas were considered preatherosclerotic and categorized as early lesions.

- Livers and kidneys were removed at the time the animals were killed and fixed in 10% buffered formalin solution, prepared as paraffin blocks, and stained with hematoxylin–eosin. These samples were reviewed by a clinical pathologist, who was blinded to the sample origin. In the specimens in which a pathologic change was noted, the severity was graded using the following scheme: grade 0 = no pathologic change, grade 1 = negligible or nonspecific change, grade 2 = mild change, grade 3 = moderate change, and grade 4 = severe change.

Statistical analyses were performed using unpaired t test, χ² test, analysis of variance, and Fisher’s protected least significant difference as appropriate. All tests were 2 tailed, and a P value of less than .05 was considered statistically significant. Data are expressed as mean ± SE.

RESULTS

Overall, 51 offspring were included in the study: 12 apoE+/+WT (7 females, 5 males); 7 apoE+/-mat (3 females, 4 males); 18 apoE+/-pat (8 females, 10 males); and 14 apoE+-/-KO (7 females, 7 males).

- Cholesterol levels were higher in both apoE+-/-mat (289 ± 47 mg/dL) and apoE+-/-KO (396 ± 62 mg/dL) offspring as compared with apoE+/-pat (105 ± 8 mg/dL) and apoE+/+WT (105 ± 11 mg/dL).

dL) offspring (P = .0021; Figure 2). Circulating cholesterol levels in the apoE+/−/mat group of offspring were significantly higher than in the apoE+/−/WT (P = .037) and apoE+/−/pat offspring mice (P = .027). No significant variation between sexes was noted, except in the apoE+/−/WT group in which the male offspring had higher total cholesterol levels than females (136 ± 10 vs 89 ± 9 mg/dL, P = .038).

No significant difference in the triglyceride levels were found among the study groups (172 ± 42 mg/dL in the apoE+/−/WT, 148 ± 14 mg/dL in the apoE+/−/pat, 152 ± 14 mg/dL in the apoE+/−/mat, and 135 ± 13 mg/dL in the apoE+/−/KO offspring). Male offspring had higher levels of triglycerides than females in the apoE+/−/KO (167 ± 16 vs 99 ± 7 mg/dL, P = .037) but not in the other groups of animals (data not shown).

There was a significant difference in the incidence of atherosclerosis (advanced lesions) among the groups: 78.6% in apoE+/−/KO, 72.2% in apoE+/−/mat, 28.6% in apoE+/−/pat, and 9.1% in apoE+/−/WT (P < .0001). The offspring sex did not influence the rate of plaque formation (data not shown). Figure 3 depicts an example of moderately large atherosclerotic plaques found in the aortic arch of an apoE+/−/mat mouse after oil red O staining.

As for the extent of atherosclerosis, the ratio of the surface area of the advanced plaques to the total surface of the aortic arch was significantly different among the offspring groups: 0.16 ± 0.16% in apoE+/−/WT, 0.22 ± 0.16% in apoE+/−/pat, 2.99 ± 0.89% in apoE+/−/mat, and 5.10 ± 1.39% in apoE+/−/KO (P = .0031). We further analyzed the total surface area covered by the plaque combining the early and advanced lesions within each group. The mean total area of atherosclerotic changes in the apoE+/−/WT offspring was 5.96 ± 1.43% vs 7.57 ± 2.65% for the apoE+/−/pat, 15.3 ± 2.07% for the apoE+/−/mat, and 28.9 ± 4.98% for the apoE+/−/KO groups (P < .0001). Sex did not account for any significant difference in advanced or combined area of atherosclerosis (data not shown).

We found an association between cholesterol levels and the presence of atherosclerosis. Animals showing atherosclerotic plaques had mean cholesterol levels of 385 ± 41 mg/dL as compared with 104 ± 4.9 mg/dL in those with no evidence of such lesions (P < .0001). This relationship also held true when analyzing each subgroup individually, as shown in Table 1.

The histological examination of the kidneys did not show any pathologic changes in either apoE+/−/WT or apoE+/−/pat offspring. The predominant lesions found in the kidneys of apoE+/−/mat and apoE+/−/KO animals were glomerulosclerosis with Kimmelstiel-Wilson–type lesions as well as proteinaceous tubular casts (Figure 4). Such changes were found in 50% of apoE+/−/mat and 69.2% of apoE+/−/KO offspring mice. The distribution of the severity of the anomalies found is depicted in Table 2.

In the liver of the animals examined, the predominant pathologic change was multifocal hepatocyte necroapoptosis (Figure 5). Although we did not encounter such lesions in apoE+/−/WT mice, they were present in 28.6% of apoE+/−/pat, 55.5% of apoE+/−/mat and 76.9% of

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**FIGURE 3**
Portion of aortic arch of an apoE+/−/mat mouse depicting atherosclerotic lesions

**FIGURE 4**
Examples of kidney lesions observed in apoE+/−/mat and apoE+/−/KO offspring mice

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**TABLE 1**
Total cholesterol levels (milligrams per deciliter, means ± SE) by presence or absence of advanced plaques within each study group

<table>
<thead>
<tr>
<th>Group</th>
<th>Plaque</th>
<th>No plaque</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE+/−/WT</td>
<td>145</td>
<td>97 ± 10</td>
<td>n/a</td>
</tr>
<tr>
<td>apoE+/−/pat</td>
<td>117 ± 15</td>
<td>99 ± 9</td>
<td>.35</td>
</tr>
<tr>
<td>apoE+/−/mat</td>
<td>307 ± 65</td>
<td>117 ± 10</td>
<td>.065</td>
</tr>
<tr>
<td>apoE+/−/KO</td>
<td>484 ± 54</td>
<td>101 ± 10</td>
<td>.003</td>
</tr>
</tbody>
</table>

Left and insert, glomerulosclerosis with Kimmelstiel-Wilson–type lesion; right, proteinaceous tubular casts.

apoE<sup>−/−</sup>KO offspring mice. Most of these lesions were of low severity (Table 3).

**Comment**

We found that, despite being genetically similar, heterozygous offspring that developed in a hypercholesterolemic environment had higher cholesterol levels and were more predisposed to atherosclerosis as compared with heterozygous offspring growing in a normal maternal environment. In accordance with previous reports of normal triglyceride levels in apoE knockout animals, the triglyceride levels were not different among the groups of offspring. Although the variation in cholesterol levels and atherosclerosis between homozygous wild-type and homozygous knockout animals is expected, the discrepancy found between the heterozygous apoE<sup>+/−</sup>pat and apoE<sup>+/−</sup>mat groups cannot be explained on the basis of genetic differences. Hence, this finding is likely attributable to the influence of the altered intrauterine environment because of the maternal hypercholesterolemia present during development in the apoE<sup>+/−</sup>mat and apoE<sup>+/−</sup>pat offspring but not in the apoE<sup>+/+</sup>WT offspring.

Our findings strongly support a role for developmental programming of hyperlipidemia and atherosclerosis. The underlying mechanism for this phenomenon is currently unknown. Palinski et al<sup>4,6</sup> and Napoli et al<sup>5</sup> have shown an association between maternal and fetal cholesterol levels in humans and a reversal of the effect of fetal programming when treating high cholesterol levels in pregnant rabbits. More recently, Yoshida and Wada<sup>10</sup> provided evidence for transplacental transfer of cholesterol in mice during early and late gestation. One may hypothesize that exposure of the fetus to elevated cholesterol levels during early development permanently alters cholesterol homeostasis. The validity of this hypothesis, as well as its possible cellular and molecular basis, needs to be further studied. An alternative hypothesis may relate the changes in cholesterol levels to possible differences in genetic imprinting between apoE<sup>+/−</sup>pat and apoE<sup>+/−</sup>mat offspring.

A previous study on the apoE model of atherosclerosis did not show a comparable effect of fetal programming.<sup>11</sup> We believe the discrepancy in the outcome is mainly caused by 2 differences in study design. In the report by Madsen et al,<sup>11</sup> the offspring were maintained on an atherogenic Western (high-fat) diet. This resulted in elevated cholesterol levels in all study groups, much higher than those found in our investigation. The other difference lies in the age at which the animals were killed. We chose 8 months of age in contrast to 6 months in the study by Madsen et al.<sup>11</sup> This more closely corresponds to middle-aged humans, a time when variations in the predisposition to the development of atherosclerosis are more likely to become manifest.

The role of developmental programming is highlighted by the consistent effect of the uterine milieu across the various observations in our current study, ranging from the effect on cholesterol levels, the incidence, and the severity of atherosclerotic lesions, to the secondary organ damages seen in the kidney and the liver. This model seems to offer an adequate tool to study the effects of fetal programming on the development of atherosclerotic disease later in life.

One of the limitations of the present study was the lack of data on cholesterol levels in the pregnant dams. Because all animals were maintained on the same diet, one would expect the cholesterol levels in the mothers to be similar to those in the offspring of the same genetic makeup. This, of course, will have to be confirmed in our future studies. Another important question is the degree to which high cholesterol levels in mother reach the fetal compartment in utero. This topic has partially been addressed in previous studies by Palinski et al<sup>8</sup> and Yoshida and Wada,<sup>10</sup> as mentioned earlier. We plan direct measurement of lipid levels in fetuses or neonates to more specifically address this question.

Another subject that needs to be addressed is the extent to which the observed

---

**TABLE 2**

Severity distribution of histopathologic kidney changes in each litter group

<table>
<thead>
<tr>
<th>Study group</th>
<th>Grade 0, %</th>
<th>Grade 1, %</th>
<th>Grade 2, %</th>
<th>Grade 3, %</th>
<th>Grade 4, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE&lt;sup&gt;+/+&lt;/sup&gt;WT</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>apoE&lt;sup&gt;+/−&lt;/sup&gt;pat</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>apoE&lt;sup&gt;+/−&lt;/sup&gt;mat</td>
<td>50</td>
<td>22.2</td>
<td>16.7</td>
<td>—</td>
<td>22.2</td>
</tr>
<tr>
<td>apoE&lt;sup&gt;−/−&lt;/sup&gt;KO</td>
<td>30.8</td>
<td>61.5</td>
<td>15.4</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Total number of samples available for examination of kidney histology was 45.

---

**FIGURE 5**

Examples of liver lesions observed in the animals examined

Left, hepatocellular necrosis; right, acidophilic bodies (arrows).

findings may be due to a postnatal effect during the neonatal period. Such an effect would have to be centered on breastfeeding because all litters were kept on the same chow after 21 days of age. Although we hypothesize that this contribution is not significant, cross-fostering studies will specifically allow targeting this topic.

Assuming that the major effect of developmental programming occurs during gestation, it will be important to define the “critical window period” during which the fetus is most susceptible to noxious stimuli. Manipulation of maternal cholesterol metabolism during different time periods of pregnancy, with use of diets or pharmacologic agents, may give further clues as to the importance of timing in fetal programming. Transfer of embryos in between low-risk and high-risk mothers can be utilized to study other aspects of fetal programming of atherosclerosis, including possible epigenetic mechanisms leading to the observed effects.

The primary event leading to the increased development of atherotic lesions in developmentally programmed offspring appears to be an elevation in circulating cholesterol levels. It remains unclear how fetal programming causes a dysregulation of cholesterol metabolism in the affected offspring. Further studies are clearly needed to evaluate the effect of the fetal environment on cholesterol regulatory pathways.

REFERENCES


### TABLE 3

<table>
<thead>
<tr>
<th>Study group</th>
<th>Grade 0, %</th>
<th>Grade 1, %</th>
<th>Grade 2, %</th>
<th>Grade 3, %</th>
<th>Grade 4, %</th>
</tr>
</thead>
<tbody>
<tr>
<td>apoE^+/+WT</td>
<td>100</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>apoE^+/-pat</td>
<td>71.4</td>
<td>28.6</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>apoE^+/-mat</td>
<td>44.4</td>
<td>44.4</td>
<td>11.1</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>apoE^-/-KO</td>
<td>23.1</td>
<td>61.5</td>
<td>15.4</td>
<td>—</td>
<td>—</td>
</tr>
</tbody>
</table>

Total number of samples available for examination of liver histology was 44.
Gender-specific effect of overexpression of sFlt-1 in pregnant mice on fetal programming of blood pressure in the offspring later in life

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OBJECTIVE: The purpose of this study was to determine whether fetal programming of adult blood pressure is altered in a previously characterized mouse model of preeclampsia that was induced by sFlt-1.

STUDY DESIGN: CD-1 mouse mothers at day 8 of gestation were injected with an adenovirus carrying Flt 1-3 (10⁹ plaque-forming units) or with an adenovirus carrying mFc as control (10⁹ plaque-forming units). The resulting pups were followed until 6 months of age, at which time blood pressure (BP) was recorded continuously for 6 days. The offspring weight was also recorded from weaning until adulthood.

RESULTS: BP was significantly higher in the male offspring that were born to sFlt-1–treated mothers compared with the controls. Male offspring from sFlt-1–treated mothers were significantly smaller from weaning until adulthood. However, there were no significant differences in BP and postweaning weight in female offspring between the 2 groups.

CONCLUSION: Our findings highlight the role of the intrauterine environment in the developmental origin of adult disease.

Key words: CD-1 mice, preeclampsia, sFlt-1, fetal programming, developmental origin


The Barker hypothesis states that the intrauterine environment is involved in the well being of the fetal development. Insults that occur during critical periods of fetal development lead to fetal programming, which is a series of adaptations that allow the fetus to survive in altered uterine conditions. The resulting fetal adaptations may have permanent specific short- and long-term effects on the development of various organ systems that include the cardiovascular and metabolic systems.1-4 In support of this hypothesis, Barker et al found that systolic blood pressure (BP) in adults is related inversely to birthweight and that this inverse relationship was independent of gestational age at birth, which suggests that the intrauterine environment can influence BP in adult life. Since then, several studies have confirmed the association between low birthweight and cardiovascular diseases later in life, which includes coronary artery disease, stroke, and hypertension.5

The milieu in which the fetus develops is created by the interactions between the fetal genome and the intrauterine environment; this interaction may be operational in pregnancy that is complicated by diabetes mellitus, hypertension, and preeclampsia. However, the observed association between an unfavorable/hostile fetal environment and diseases in later life may be confounded by the presence of a genetic predisposition for the particular disease in both mother and offspring. This is particularly applicable to cardiovascular and endocrinologic/metabolic disorders because genetic predispositions for these disorders may affect the fetal environment and the risk for adult diseases when present in both mother and offspring. In these cases, epidemiologic studies may not differentiate or apportion the risk of disease in later life between the unfavorable uterine environment and the hereditary predisposition that is common to both mother and fetus.6 Animal models may be of significant help in addressing such an issue.

Preeclampsia figures prominently among the various causes of altered uterine and fetal environment. Preeclampsia is thought to result from an imbalance between placental perfusion and placental metabolic needs that lead to placental hypoxia.7,8 The hypoxic placenta releases factors into the maternal circulation that result in maternal endothelial dysfunction, maternal hypertension, maternal end organ damage, and fetal growth restriction. During normal pregnancy, vascular remodeling occurs in the placental bed, although pregnancies that are destined to experience preeclampsia have abnormal placental structure and perfusion, which lead to a hostile intrauterine environment in which the fetus is developing.9,10 Thus, the uteroplacental insufficiency that is present in preeclampsia potentially can lead to altered
fetal vascular programming and the risk of cardiovascular disease later in life.

We have shown previously in our laboratory that pregnant mice that overexpress the soluble fms-like tyrosine kinase 1 (sFlt-1) are hypertensive, deliver growth-restricted fetuses, have lower platelet counts compared with controls, and have other manifestations of a preeclampsia-like syndrome. The association between the overexpression of sFlt-1 and a preeclampsia-like condition was also shown by other researchers in pregnant rats. Therefore, we decided to test the hypothesis that elevated levels of sFlt-1 result in an adverse intrauterine environment that leads to an altered fetal vascular programming that manifests as hypertension in the offspring later in life.

**Materials and Methods**

**Animals**

Pregnant CD-1 mice at day 6 of gestation were purchased from Charles River (Wilmington, MA). The mice were housed separately in temperature- and humidity-controlled quarters with constant 12-hour light:dark cycles and provided with food and water ad libitum. At day 8 of gestation, pregnant CD-1 mice were divided randomly into 2 groups and injected through the tail vein with either adenovirus carrying sFlt-1 (10⁹ plaque-forming units in 100 μL; sFlt-1 group) or adenovirus carrying the murine immunoglobulin G2α Fc fragment (10⁹ plaque-forming units in 100 μL; mFc), which was used as a control group for the virus. The mice were maintained in the animal care facility at the University of Texas Medical Branch. All surgical procedures were carried out by trained personnel according to the Institutional Animal Care and Use Committee guidelines with ketamine (Ketalar; Parke-Davis, Morris Plains, NJ) and xylazine (Gemini, Rugby, Rockville Center, NY). The same environment was used in our laboratory room to accommodate the mice during blood-pressure recording. The room was maintained at 12-hour light:dark cycles; the temperature and humidity were maintained as suggested by the Institutional Animal Care and Use Committee guidelines.

**Amplification and purification of sFlt-1 and mFc virus vector**

We used the adenovirus vectors’ stock Ad-Flt-1 (1-3) as the active vector and Ad-mFc as the adenovirus control (first generation, E1 and E3 deleted) that were prepared and titrated by the Research Vector Core, Harvard Medical School. We used the 293 cells to grow and transfect the virus. The cells were cultured in 150-mm plates, were seeded for 2-3 days, and were considered ready for transfection when the cells reached 70%-80% confluence. The transfection medium that contained the virus (transfection medium: 1% penicillin/streptomycin and 2% fetal bovine serum; Invitrogen/Gibco, Carlsbad, CA) was added to each plate; 20 hours later, approximately 50% of the cells started to detach from the plates and showed the cytopathic effect. At this point, the cells were collected and spun twice; the supernatant was removed, and the cells were stored at −80°C until purification. For the purification process, 2 mL of sterile 10 mmol/L Tris buffer, pH 8 was added to the cell pellet, which was thawed in a water bath, then vortexed briefly, and put on the dry slurry to be frozen. This cycle was repeated 6 times, after which the cells were lysed after spinning, and the supernatant was collected. The supernatant that contained the virus was added to a cesium chloride step gradient (d = 1.43 and 1.34). This preparation was again centrifuged, at which point, the adenovirus band was apparent and collected easily with an 18-gauge needle and 5 mL syringe and added to a tube that 1.34 g/mL cesium chloride. The sample was centrifuged again at 35,000 rpm for 16 hours at 4°C, and the adenovirus band, which was now more evident, was loaded into Spectra/Per membrane (molecular weight cut-off, 3500; Spectrum Laboratories Inc., Rancho Dominguez, CA) dialysis tubing that had been rinsed with water and then equilibrated with dialysis buffer. The concentration of adenovirus particles was determined by spectrophotometric analysis of an appropriate dilution of the test sample (typically 1/10) in a solution of 10 mmol/L Tris/1 mmol/L ethylenediaminetetraacetic acid/0.1% sodium dodecylsulfate.

**Offspring weight**

After delivery, the pups from the 2 groups were weighed weekly until early adulthood (9 weeks of age). The offspring were followed and separated into female and male groups after they were weaned (4 weeks of age). After being weaned (4 weeks of age). After being

**FIGURE 1**

**Postweaning weight of offspring**

<table>
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<tr>
<th>Weeks of Age</th>
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A. Postweaning weight of male offspring, at 6 months of age, that were born to pregnant mothers that were treated with adenovirus carrying sFlt-1 (n = 19) or adenovirus carrying mFc (n = 8). Data are shown as mean ± SEM. B. Postweaning weight of female offspring, at 6 months of age, that were born to pregnant mothers that were treated with adenovirus carrying sFlt-1 (n = 19) or adenovirus carrying mFc (n = 12). Data are shown as mean ± SEM.

Lu. Gender-specific effect of overexpression of sFlt-1 in pregnant mice on fetal programming of blood pressure in the offspring later in life. AJOG 2007.
weaned, offspring of the same gender were weighed weekly until 9 weeks. Adult offspring at 6 months of age that were born from sFlt-1– and mFc-treated mothers were used for BP measurement.

**In vivo BP measurement**

Telemetric transducers were used to measure BP in the unrestrained conscious offspring at 6 months of age. The BP catheters were inserted through the left carotid artery into the aortic arch and tunneled to a telemetric transmitter (Data Systems International, Overland Park, KS) that transmitted the signal to a receiver pad that was connected to an on-line computer. After a recovery period, BP was recorded for 6 days continuously.

**Data analysis**

Dataquest software (A.R.T. 3.1; DSI Telemetry & Dataquest Software, St. Paul, MN) was used for data acquisition and analysis of mean, systolic, and diastolic BP. Mean BP over 6-hour or 12-hour intervals was calculated for each animal and used in the final analysis. The overall averages for systolic and diastolic BPs for the entire measurement period and during day and night time were calculated. Data are expressed as mean ± SEM. The Student t test was used for statistical analysis. A probability value of <.05 was considered significant.

**RESULTS**

**Weight after weaning**

The male and female offspring from sFlt-1– and mFc-treated pregnant mice were followed until adulthood. The weight from weaning until adulthood of male offspring that were born to sFlt-1–treated pregnant mice was significantly lower than male offspring that were born to mFc-treated pregnant mice (Figure 1A). There was no significant difference in the postweaning weight in female offspring between the 2 groups (Figure 1B).

**BP results in male offspring**

Mean BP was significantly higher during the entire measurement period in male offspring that were born to sFlt-1–treated mothers (day 1, 146.33 ± 4.98 mm Hg; day 6, 136.54 ± 2.17 mm Hg) compared with offspring that were born to mFc-treated mothers (day 1, 120.76 ± 2.88 mm Hg; day 6, 113.54 ± 2.17 mm Hg; Figure 2A). In addition, the average systolic and diastolic BPs for the entire measurement period were significantly higher in the male offspring that were born to sFlt-1–treated mothers, compared with offspring that were born to mFc-treated mothers both during the daytime and nighttime periods. However, BP did not differ between day and night within each group (Figure 2D).

**BP results in female offspring**

There were no significant differences in mean, systolic, or diastolic BP in female offspring that were born to sFlt-1– and mFc-treated mothers. This lack of difference was also evident during daytime and nighttime measurement (Figures 2B and C, 3A and B).
Comment

We found that systolic, diastolic, and mean BPs of male offspring at 6 months of age that were born to pregnant mothers that were injected with adenovirus carrying sFlt-1 were significantly higher than the similarly aged male offspring from mFc-treated pregnant mothers (adenovirus carrying the murine immunoglobulin G2α Fc fragment as a control) and that these differences were not present in female offspring at the same age that were born to sFlt-1- and mFc-treated mothers. There were no differences in plasma sFlt-1 levels in these offspring born either to sFlt-1- or mFc-treated mothers at 3 growth courses (at birth, 3 months of age, and 6 months of age; data not shown). We have shown previously that this animal model is characterized by overexpression of sFlt-1 and hypertension in the pregnant animal and by growth restriction of the pups. The hypertension in the male offspring in association with altered postweaning growth and its absence in the female offspring where postweaning growth is normal supports the developmental origin of the hypertension. Our results also confirm a gender-sensitive developmental programming of BP in this particular animal model of adverse uterine environment. These findings are consistent with previous studies of altered uterine environment that was induced by manipulation of maternal diet in which fetal vascular programming and hypertension later in life were more pronounced in male vs female offspring. In addition, prenatal glucocorticoids can program adulthood cardiovascular and metabolic physiologic condition in a gender-specific pattern.

The origins of several adult diseases (such as hypertension, cardiovascular disease, and diabetes mellitus) can be tracked back to fetal development in utero. Stimuli or insults can slow fetal growth during critical periods of fetal development and lead to adaptive responses that are aimed at ensuring fetal survival. These adaptive responses lead to a process termed fetal programming. Fetal programming permanently alters fetal structure, physiologic conditions, and metabolism and consequently leads to a long-lasting effect that can manifest as early onset of cardiovascular diseases in adult life. Pregnancy-associated hypertension is 1 of the maternal factors that can affect the fetus indirectly through the altered intrauterine environment.

In the past few years, significant research efforts have been directed towards unraveling the underlying mechanism of the fetal origins of adult disease or, as stated today, the development origin of health and disease. Because it is somewhat challenging to conduct this type of research in humans, laboratories have turned to animal models. Species that have been studied include rat, mouse, guinea pig, sheep, and nonhuman primates. Experimental methods used to produce an unfavorable uterine environment and induce fetal growth restriction have included dietary, pharmacologic, and surgical manipulations. Protein restriction in rats during pregnancy (such as low protein diet supplemented with methionine) has been found to result in hypertension in the offspring later in life. Reduction of the uterine perfusion by placing a silver clip around the aorta below the renal arteries during mid-late gestation in pregnant rats caused low-birthweight offspring that were predisposed for the development of hypertension. Another example is the transgenic animal model that was used by Longo et al., in which vascular reactivity was altered in adult heterozygous offspring that were born to mothers with an abnormal uterine environment that had been induced by nitric oxide deficiency, compared with genomically similar heterozygous offspring that were born to normal mothers.

Preeclampsia has long been suspected of being a placental disorder. Maternal hypertension, either chronic or acute, leads to inadequate vascular adaptations during pregnancy, alteration in the circulation at the uteroplacental interface, and consequently poor perfusion of the placenta-fetal unit. These maladaptations lead to fetal hypoxemia and reduction in fetal perfusion, which can convey long-lasting physiologic and structural alterations that predispose the fetus to diseases in later life.

There is growing evidence that an imbalance between the active circulating proangiogenic factors (vascular endothelial growth factor [VEGF] and placental growth factor [PIGF]) and antiangiogenic factors (sFlt-1) plays an
important role in the pathogenesis of preeclampsia. 23, 24 Particularly, soluble Flt1 as a splice variant of the VEGF receptor Flt-1 seems to be involved centrally in the pathogenesis of preeclampsia. 11, 12 Indeed high levels of circulating sFlt-1 in early pregnancy are associated with later onset of preeclampsia. 25, 26 These increased levels of sFlt-1 are accompanied by decreased levels of free VEGF and PI GF in the maternal circulation, which suggests that sFlt-1 inhibits the functions of VEGF and PI GF by binding VEGF and PI GF and thereby prevents them from binding their endothelial cell receptor, which leads to abnormal angiogenesis and altered circulation at the uteroplacental interface and consequently poor perfusion of the placenta-fetal unit.

Our results strongly support the hypothesis that the elevated levels of sFlt-1 leads to an altered fetal vascular programming that manifests as hypertension in the offspring in adult life. Moreover, the increased BP levels occurred only in male offspring that were born to pregnant mothers that were treated with adenovirus carrying sFlt-1, which suggested gender sensitivity to fetal programming. Gender-specific difference has already been noted in several other animal models of fetal programming. Kwong et al 14 found increased systolic animal models of fetal programming. Gender-specific difference suggested gender sensitivity to fetal programming. Gender-specific difference in male rat offspring that were born to pregnant mothers that were treated with adenovirus carrying sFlt-1, which suggested gender sensitivity to fetal programming and the onset of hypertension in the male offspring later in life. In addition, it demonstrates the role that is played by gender in the fetal programming of adult hypertension later in life.

REFERENCE

12. Maynard SE, Min JY, Merchant J, et al. Excess placental soluble fms-like tyrosine kinase 1 (sFlt1) may contribute to endothelial dysfunc-


SMFM Meeting Papers www.AJOG.org
OBJECTIVE: To determine: 1) placental eNOS mRNA concentration across gestation in normal ovine pregnancy and in an ovine model of intrauterine growth restriction (IUGR), and 2) placental eNOS protein concentration in early ovine pregnancy.

STUDY DESIGN: A total of 24 sheep were studied with 12 ewes placed in hyperthermic (HT) conditions to induce IUGR and 12 were kept in control conditions. HT and control animals underwent euthanasia at 3 developmental time points (55, 95, & 130 days gestational age; dGA) in ovine placental & fetal development.

RESULTS: Compared to controls, HT pregnancies showed 1) no differences in fetal weights at 55 dGA and 95dGA with significant reductions at 130 dGA, 2) significantly smaller placentae at 95 and 130 dGA with a trend for a reduction at 55 dGA, 3) significant decreases in cotyledon eNOS mRNA at 95 and 130 dGA, 4) a significant increase in caruncle eNOS mRNA expression at 130 dGA, 5) significant increase in eNOS protein in the caruncle, but not in the cotyledon at 55 dGA.

CONCLUSION: Placental eNOS concentration is transcriptionally regulated at mid-gestation, while additional post-transcriptional regulation is also involved during early and late gestation in this model of placental and fetal growth restriction.

Key words: endothelial nitric oxide synthase, IUGR, ontogeny

Ontogeny of endothelial nitric oxide synthase mRNA in an ovine model of fetal and placental growth restriction

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Intrauterine growth restriction (IUGR) is a significant complication of pregnancy associated with an increased risk for morbidity and mortality. Placental insufficiency is the most common cause of IUGR, which is characterized by altered expression of vasoactive factors including nitric oxide (NO). Endothelial nitric oxide synthase (eNOS) is an enzyme that catalyzes the production of the potent vasodilator NO, and eNOS is an important regulator of vascular tone and blood flow. Previous studies have demonstrated that inhibition of NO production results in inhibition of NO, and eNOS is an important regulator of vascular tone and blood flow. In an ovine model of IUGR with abnormal placental blood flow resistance and fetal systemic hypertension, we have shown that placental eNOS is decreased at midgestation, but increased near-term when fetuses are growth restricted and hypoxic. Placental eNOS concentrations in this animal model have not been examined during early pregnancy.

While alterations in eNOS abundance may be a determinant of altered blood flow and vasoreactivity in our IUGR model, the mechanism for the altered eNOS protein concentrations has not been examined. In addition, eNOS protein concentrations have only been assessed at midgestation and near-term. In the present study, we determined the following: 1) placental eNOS mRNA ontogeny across gestation, and 2) eNOS protein concentration during early gestation. This model is characterized by reduced umbilical and uterine flows, fetal hypoxia, reduced placental amino acid and glucose transfer, abnormal placental resistance, and systemic hypertension. We hypothesize that eNOS mRNA concentration will be decreased at midgestation (95 days of gestation; dGA) and increased near-term (130 dGA), thus matching the previously reported eNOS concentration data that we have previously reported. However, we do not expect changes in placental eNOS mRNA or protein during early gestation (55 dGA).

MATERIALS AND METHODS

Animals and tissue preparation

This study was approved by the University of Colorado Health Sciences Center Animal Care and Use Committee. Animal care was performed as previously described by Galan et al. A total of 24 sheep were studied, with 12 of these placed in HT conditions beginning at 40 dGA (term = 147 dGA) to induce IUGR and 12 animals placed in thermoneutral...
conditions as controls. HT and control animals underwent euthanasia at 3 time points representing early gestation (55 dGA), when placentation rate is maximal, midgestation (95 dGA), when placentation size is at its maximum, and near-term (130 dGA), where the rate of placental growth is greatest. Animals were equally divided among each time point so that there were 4 animals per HT and control group for each gestational age. At necropsy, the fetal and placental weights were recorded. Placentomes were separated into the maternal (caruncle) and fetal (cotyledon) components, and frozen in liquid nitrogen for further analysis. A time line for these studies is shown in Figure 1 depicting hyperthermic exposure and the timing of the necropsies as they relate to placental development.

**RNA extraction**

Total RNA was extracted from the collected tissues using the TRI REAGENT method. One hundred mg of each tissue were homogenized in 1 mL of TRI REAGENT (Sigma, St Louis, MO). After homogenization, samples were centrifuged, chloroform extracted, precipitated, and washed in cold 75% ethanol before being further purified with a Qiagen RNeasy Mini Kit (Qiagen, Valencia, CA). To quantify the RNA, sample absorbance was measured at 260 and 280 nm using a GE Healthcare Ultraspec 4300 Pro UV-VIS spectrophotometer (GE Healthcare, Piscataway, NJ).

**cDNA synthesis**

cDNA was produced through reverse transcription using the First-Strand cDNA Synthesis protocol from the SuperScript III kit by Invitrogen (Invitrogen, Carlsbad, CA). Five μg of total RNA was mixed with 50 μmol/L of oligo (dT) primers, 10 mmol/L dNTP mix and DEPC-treated water. Next, the samples were incubated at 65°C for 5 minutes and then placed on ice for at least 1 minute. Ten μL of cDNA Synthesis mix (10× RT buffer, 25 mmol/L MgCl₂, 0.1 mmol/L DTT, RNase out, and SuperScript III RT) was added to each sample and then incubated at 50°C for 60 minutes. Reactions were terminated by incubation at 70°C for 15 minutes. RNase H (1 μL) was added to each sample and then incubated at 37°C for 20 minutes. The samples were stored at −20°C until needed.

**Reverse transcriptase-PCR and sequencing of eNOS**

RT-PCR was performed using cDNA generated as explained. Ovine eNOS forward (5’-TGC ATG ACA TTG AGA GCA AAG GGC −3’) and ovine eNOS reverse (5’-ATG TCC TCG TGA TAG CGT TGG TGA −3’) primers were used at an annealing temperature of 60.3°C during RT-PCR. The eNOS PCR product (391 bp) was purified using a QIAquick PCR Purification kit and sequenced for authenticity. The 391 bp RT-PCR product was 99% identical to the published ovine eNOS nucleotide sequence (DQ015701). Our eNOS cDNA exhibited no significant homology with nNOS (U766739) or iNOS (AF223942) sequences confirming the specificity of our eNOS product.

**Real time PCR**

Quantitative real time PCR was used to quantify mRNA concentrations in our samples. Ten ng of each sample cDNA was subjected to real time PCR using our eNOS forward/reverse primers (same as above), and compared to a standard curve generated by known quantities of eNOS cDNA to determine starting quantity. To normalize our eNOS data, sample cDNA were subjected to real-time PCR using primers (forward 5’-TCA ACC AGG TGG AGA TCA ACG −3’ and reverse 5’-TGC TTT ACG GGC TTG TAG GTG −3’) for ribosomal protein S15, and a standard curve of known quantities of S15 cDNA. The amplification efficiencies were 98% and 99% for eNOS and S15, respectively.

**Western blot analysis**

Cotyledons and caruncles of 55 dGA pregnancies were homogenized in protein lysis buffer (10 mmol/L of PMSF, 10 mmol/L of Na3VO4, 1× triton X-100, 150 mmol/L NaCl, 20 mmol/L Tris Base, 5 mmol/L AEBSF, 5 mmol/L EDTA, 10 nmol/L E-64, 10 nm Leupeptin, and 10 ng/mL Aprotinin). Protein tissue lysates (50 μg) were separated on 10% SDS-PAGE and transferred to a nitrocellulose membrane. Membranes were incubated with a mouse HRP-conjugated antibody against eNOS (Santa Cruz Biotechnology, Santa Cruz, CA). The membranes were incubated with chemiluminescent substrate (Pierce, Rockford, IL) for 5 minutes and exposed to x-ray film. To determine loading consistencies, each membrane was stripped of antibodies and reincubated with antibody against mouse beta-actin (dilution 1:4000) (MP Biomedicals, Aurora, OH). The abundance of these proteins was quantified by densitometry.
No differences in fetal weights were observed at 55 dGA and 95 dGA; however, significant decreases were observed in at 130dGA.


Statistical analysis

Data are presented as mean ± SE. Comparisons were made between control and HT pregnancies for fetal and placental weights, eNOS mRNA, and protein concentrations within a given tissue (cotyledon or caruncle) and gestational age (55, 95, or 130 dGA). The t-test was used to assess equality of variance and the appropriate corresponding t test was used to compare the endpoints between groups statistically. \( P \leq .05 \) was considered significant.

Results

There was no difference in fetal weights between HT and control pregnancies (Figure 2) at 55 dGA (29 ± 2.3 g vs 32 ± 2.6 g; \( P = .22 \)) or 95 dGA (682 ± 205 g vs 715 ± 11 g; \( P = .79 \)). However, as previously reported, HT-induced IUGR pregnancies demonstrated significant reductions in fetal weight compared to controls near-term (1.8-fold; 1718 ± 433 g vs 2914 ± 201 g; \( P \leq .008 \)). At 55 dGA (Figure 3), HT pregnancies exhibited a trend towards a smaller placenta (1.3-fold; 135 ± 3 g vs 181 ± 20 g; \( P = .053 \)), and by 95 dGA this trend became statistically significant (2.4-fold; 186 ± 18 g vs 440 ± 50 g; \( P \leq .003 \)). The difference in placental weights persisted near-term (2.0-fold; 169 ± 43 g vs 349 ± 21 g; \( P = .004 \)).

Figure 4 presents eNOS mRNA concentrations at each gestational age sampled for the fetal cotyledon. At 55 dGA, cotyledon eNOS mRNA concentration was not impacted by HT treatment. However, at 95 dGA there was a significant reduction (1.7-fold; \( P = .04 \)) in HT fetal cotyledon eNOS mRNA concentration, and this reduction was more pronounced near-term (18.8-fold; \( P = .03 \)).

Similar to the fetal cotyledons, at 55 dGA there was no difference due to treatment in maternal caruncle eNOS mRNA concentration (Figure 5), and by 95 dGA there was a trend (3.3-fold; \( P = .052 \)) for reduced mRNA concentrations in caruncles derived from HT pregnancies. In contrast, near-term there was a significant increase (5.5-fold; \( P \leq .008 \)) in maternal caruncle eNOS mRNA concentration in IUGR pregnancies.

Figure 6 presents the Western blot analysis of eNOS at 55 dGA. Similar to the mRNA concentration data (Figure 4) there was no effect of treatment on cotyledon eNOS concentrations. However, in contrast to the maternal caruncle mRNA concentration data (Figure 5), the concentration of eNOS was significantly increased (\( P < .04 \)) by HT treatment at 55 dGA.

Comment

Alterations in eNOS regulation in placental and vascular tissues may lead to reduced uterine-umbilical blood flows, increased placental flow resistance, and systemic hypertension in growth restricted pregnancies. Our previous studies of eNOS concentrations in our ovine IUGR model led us to explore 2 primary objectives representing new information and allowing a more complete eNOS expression profile across gestation. In our previous studies, we found cotyledon eNOS concentration to be decreased at


Caruncle eNOS mRNA concentration was significantly increased at 55 dGA in IUGR animals compared to control animals. A trend for a decrease in eNOS mRNA expression was observed at 95 dGA. Near-term, caruncles (130 dGA) of HT-exposed animals showed a significant increase in eNOS mRNA expression as compared to controls.

95 dGA but increased at 130 dGA when the fetus is known to be hypoxic. In this study, we completed the eNOS concentration profile by assessing eNOS at 55 dGA. We found no differences between HT and control pregnancies suggesting that at least early HT exposure does not impact the cotyledonary (fetal side) eNOS concentration. In contrast, 55 dGA caruncle eNOS was increased in the HT pregnancies, suggesting perhaps that the early effects of HT is to enhance eNOS on the maternal side of the placenta.

In order to gain knowledge of the underlying mechanism of placental eNOS regulation, we determined eNOS mRNA concentrations in placental cotyledonary and caruncular tissues at different gestational ages (55 dGA, 95 dGA, and 130 dGA). When comparing HT and control groups, 55 dGA eNOS mRNA concentration data did not indicate differences for either the cotyledon or caruncle tissue in our IUGR animals compared to our controls. When compared with eNOS protein at this gestational age, we also observed no significant difference due to treatment. In contrast, there was a significant increase observed for caruncular eNOS protein at 55 dGA, suggesting posttranscriptional regulation of eNOS in early gestation. At midpregnancy (95 dGA), eNOS mRNA concentration was significantly decreased in cotyledons from IUGR pregnancies, and there was also a trend for a decrease in the caruncle eNOS mRNA concentration. Near-term (130 dGA) eNOS mRNA was reduced in the cotyledon and increased in the caruncle. The reason for the difference between cotyledon and caruncle eNOS mRNA concentrations at 130 dGA, as a function of IUGR, is not readily apparent. Hypoxia is known to increase eNOS mRNA and protein in the lungs. Interestingly, other reports have shown eNOS mRNA to be decreased in endothelial cells in the rat under hypoxic conditions. Thus, hypoxia appears to have different effects depending on the tissues, species, and experimental conditions. It is known that during early gestation embryos and placentas exist in a hypoxic environment and the HT-IUGR fetuses are hypoxic at birth; thus, hypoxia may be the stimulus for increased eNOS mRNA and protein in the HT placenta at this time point.

When placental eNOS mRNA is compared to the previously published protein concentrations at the same time point in our model, a significant decrease in cotyledon eNOS mRNA and a decrease in caruncle eNOS mRNA matched the reductions in eNOS at 95 dGA in these tissues. This suggests that eNOS in these tissues appears to be transcriptionally regulated at midgestation in the HT ovine IUGR model. At 130 dGA, placental eNOS mRNA showed differential expression in the IUGR pregnancies with a decrease in the cotyledon and an increase in the caruncles. The increase in caruncle (maternal placental component) eNOS mRNA concentration matches prior studies showing an increase in caruncle eNOS concentration, suggesting transcriptional regulation for this protein. In contrast, concentrations of mRNA and protein are discordant in the cotyledon (fetal side), suggesting a posttranscriptional or translational control of eNOS synthesis.

With the exception of the near-term cotyledon, we conclude that eNOS concentration is transcriptionally regulated in the placenta of HT exposed ovine pregnancies. The posttranslational mechanisms of regulation of eNOS in the near-term cotyledon (fetal side) are unknown and remain to be defined. The profile of eNOS mRNA concentration in the placenta of normal and IUGR ovine pregnancies has been outlined in this study and represents the first report of placental eNOS mRNA concentration across gestation in the ovine placenta.

REFERENCES
Liver position and lung-to-head ratio for prediction of extracorporeal membrane oxygenation and survival in isolated left congenital diaphragmatic hernia

Holly L. Hedrick, MD; Enrico Danzer, MD; Aziz Merchant, MD; Michael W. Bebbington, MD; Huaqing Zhao, MA; Alan W. Flake, MD; Mark P. Johnson, MD; Kenneth W. Liechty, MD; Lori J. Howell, RN, MS; R. Douglas Wilson, MD; N. Scott Adzick, MD

OBJECTIVE: The purpose of this study was to determine the ability of liver position and lung-to-head ratio to predict outcome in isolated left congenital diaphragmatic hernia.

STUDY DESIGN: We reviewed prenatal studies and postnatal outcomes of congenital diaphragmatic hernia between January 1996 and January 2006.

RESULTS: Eighty-nine patients received prenatal and postnatal care at 1 institution. In fetuses with liver up, extracorporeal membrane oxygenation was required in 39 of 49 fetuses (80%), compared with 10 of 40 fetuses (25%) for those with liver down (P < .0001). Overall survival rate was 45%, compared with 93% for those with liver down (P < .00005). Low lung-to-head ratio (<1.0) predicted increased incidence of extracorporeal membrane oxygenation (75%; P = .036) and lower survival (35%; P = .0003). However, when measured at <24 weeks of gestation, lung-to-head ratio was not predictive of outcome (extracorporeal membrane oxygenation, P = .108; survival, P = .150); liver position remained highly predictive (extracorporeal membrane oxygenation, P = .006; survival, P = .001).

CONCLUSION: Liver position is the best prenatal predictor of outcome in isolated left congenital diaphragmatic hernia. Lung-to-head ratio alone should not be used to counsel families regarding mid gestational management choices.

Key words: congenital diaphragmatic hernia, extracorporeal membrane oxygenation, liver herniation, lung area-to-head circumference ratio, prenatal diagnosis


The first challenge in the management of congenital diaphragmatic hernia (CDH) is to understand the natural history of the disease. CDH occurs in 1 in 2500 live births¹ and has a spectrum that varies from mild disease to severe and refractory to all therapies. Depending on the severity of the CDH, families may face many difficult choices, which include termination of pregnancy, experimental fetal intervention, or standard postnatal therapy with or without extracorporeal membrane oxygenation (ECMO). Several prognostic factors for poor postnatal outcome have been proposed, such as early gestation diagnosis, severe mediastinal shift, polyhydramnios, a small lung-thorax transverse area ratio, left heart underdevelopment, and the presence of stomach in the chest.²⁻⁴ Unfortunately, none of these features has been predictive consistently of outcome.

Currently, the most widely used prenatal predictors of postnatal outcome are liver position and the right lung area-to-head circumference ratio (LHR). Liver position is determined most accurately by ultrafast fetal magnetic resonance imaging (MRI) by the rapid half-Fourier acquisition single-shot turbospin echo (HASTE) technique.⁵ Ultrasound and color flow Doppler imaging can also visualize bowing of the ductus venosus to the left of the midline or the coursing of the branches of the portal or hepatic veins to the lateral segment of the left lobe above the diaphragm. The LHR is used to estimate the size of the contralateral right lung and the degree of mediastinal shift at the level of the atria on transverse scan of the fetal thorax.⁶ We sought to determine the ability of liver position and LHR to predict the need for ECMO and survival in fetuses with isolated left CDH that receive both prenatal and postnatal care at a single institution.

MATERIALS AND METHODS

Patient population
We examined all prenatal patient records with a referral diagnosis of CDH to the Center for Fetal Diagnosis and Treatment at the Children’s Hospital of Philadelphia from January 1996 through January 2006. The initial evaluation included detailed fetal ultrasonography and fetal ultrafast MRI. Assessment included confirmation of diagnosis, the type of CDH, liver position, LHR, and the presence of other anomalies. Fetal echocardiography and Doppler flow measurements were performed to assess...
cardiac anatomy and function. After evaluation, all patients underwent non-directive counseling for pregnancy management options. Data collected retrospectively from patient charts included gestational age (GA) at evaluation, results of prenatal imaging studies, chromosomal studies, surgical interventions, and clinical outcomes. The principal outcome variable was survival. Secondary measures were the need for ECMO, type of repair, days of ventilator support, and days of initial hospitalization. This study was approved by the Committee for Protection of Human Subjects Institutional Review Board at the Children’s Hospital of Philadelphia.

Liver position and LHR

Fetal liver position was determined by ultrasound evaluation and confirmed by MRI (Figure 1). Liver position was intrathoracic in 49 fetuses (80%), compared with 10 of 49 fetuses (20%) for those with liver down (range, 8 months to 10 years). No patient died after 1 year of age. Overall survival was 66.3% at median follow up of survivors at 57.8 months (range, 8 months to 10 years). No patient died after 1 year of age.

Liver position was intrathoracic in 49 of 89 fetuses (55%). In fetuses with liver up, ECMO was required in 39 of 49 fetuses (79%), compared with 10 of 49 fetuses (20%) for those with liver down (P < .0001). Survival rate was 45% for fetuses with liver up, compared with 93%
for fetuses with liver down (P < .00005; Figure 3). Liver position was highly predictive of both need for ECMO (P = .006) and survival (P = .001) at all GAs of evaluation. In addition, the liver up patients had a longer ventilator duration (median, 29 days) than did liver down patients (median, 18.5 days; P = .0016). There were no significant differences in length of stay between the liver up and liver down groups.

Median LHR was 1.2 mm (range, 0.5-3.6 mm). Mean LHR among survivors was 1.45 ± 0.56 mm; mean LHR among nonsurvivors was 1.1 ± 0.36 mm (P = .0028). Independent of GA at time of initial measurement, low LHR (<1.0 mm) predicted increased incidence of ECMO (75%; P = .036) and lower survival (35%; P = .0003; Figure 4). When measured before 24 weeks of gestation (n = 41), LHR was not predictive of outcome (need for ECMO, P = .108; survival, P = .150). When measured after 24 weeks of gestation (n = 48), LHR was predictive of the need for ECMO (P = .027) and correlated with survival but did not reach statistical significance (P = .07). The incidences of need for ECMO and survival for the variables liver position and LHR are summarized in the Table. In addition, when liver position was analyzed with LHR, LHR was not predictive of the need for ECMO (P = .277) or survival (P = .129).

Overall GA at delivery (cesarean delivery, 17/89 patients; 19%) was 37.8 ± 1.4 weeks and did not differ significantly between survivors and nonsurvivors (P = .5). Deaths were the result of multisystem organ failure in the setting of pulmonary hypertension (n = 9), central nervous system bleed (n = 10), and persistent pulmonary hypertension (n = 11). Seven patients died before surgery to repair the diaphragm. Gore-Tex patch repair was performed in 57 of 82 of those fetuses that underwent surgery (70%). Four additional patients underwent muscle flap repair. One patient who underwent primary repair experienced recurrence at 1 month of age and subsequently underwent Gore-Tex patch repair. Days ventilated did not correlate with survival (P = .952)

**TABLE**

The incidence of need for ECMO and survival for the variables liver position and LHR

<table>
<thead>
<tr>
<th>Variable</th>
<th>ECMO (%)</th>
<th>Survival (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Liver up (49/89; 55%)</td>
<td>80</td>
<td>45</td>
</tr>
<tr>
<td>Liver down (40/89; 45%)</td>
<td>25</td>
<td>93</td>
</tr>
<tr>
<td>Lung-to-head circumference ratio &lt;1</td>
<td>75</td>
<td>35</td>
</tr>
<tr>
<td>(20/89)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Lung-to-head circumference ratio &gt;1</td>
<td>49</td>
<td>75</td>
</tr>
<tr>
<td>(69/89)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

* P < .05, comparison of liver up vs liver down and lung-to-head circumference ratio <1 vs >1.

† Need for ECMO compared by Fisher’s exact test.

‡ Kaplan-Meier curve.

**COMMENT**

The ability to determine prognosis accurately for CDH impacts prenatal counseling, possible selection for fetal intervention, and decisions for postnatal management. This study represents a 10-year experience of prenatal and postnatal care at a single high-volume institution with standardized methods of prenatal assessment and postnatal management. We conclude that liver position is the best prenatal predictor of both need for ECMO and survival in isolated left CDH. Others have described the importance of liver position. In this series with the largest number of patients from a single institution with standardized prenatal and postnatal care, liver position was highly predictive of outcome, regardless of GA or LHR. The advantage to the liver up-down approach if measured by ultrafast fetal MRI is that it is simple, requires no subjectivity, and is highly reproducible. In the future, if we accurately quantitate the degree of liver herniation, we may be able to further risk stratify for patient outcomes.

LHR has been controversial in the past because of variability between observers. Although subsequent retrospective and prospective reviews have continued to demonstrate a significant relationship between survival and LHR, fetuses with lower LHRs are now surviving. Other groups are showing an inability of LHR to predict survival. In our recent experience, LHR alone was predictive of survival and the need for ECMO but was not predictive when used in conjunction with liver position or at GA of <24 weeks. The reasons for the inability...
of LHR to predict outcome early in gestation may be related to the relative small size of the lungs. As the fetus grows, the relative differences between lung sizes become more pronounced. Timing of prophylaxis is of paramount importance for families making decisions regarding termination or a potential fetal intervention. Although we continue to use LHR in our prenatal counseling as an additional piece of information that is related to contralateral lung size, we are cautious in making any conclusions regarding its significance at <24 weeks. In summary, LHR alone should not be used to counsel families regarding mid gestational management choices that are related to termination or fetal intervention.

Prenatal diagnosis for CDH requires a functional measure early in gestation. Bab-ies who die of CDH succumb to severe pulmonary hypertension. Although measures of lung size indirectly assess the size of the vascular bed, they are unable to determine vascular reactivity. Recent work has focused on pulmonary artery diameter,24 pulmonary artery pulsatility index,22 pulmonary artery acceleration time/ejection ratio of fetal pulmonary artery,21 and maternal hyperoxegenation testing24 as predictors of outcome in CDH. Pulmonary artery measurements are promising yet technically difficult and require validation in larger patient numbers and observer groups. Because the fetal pulmonary circulation is unresponsive to oxygen at <26 weeks of gestation, the maten-al hyperoxegenation test cannot be applied during the critical decision-mak-ing period at <24 weeks of gestation. Ulti-mately, we may need a combination of predictors, a pulmonary hypoplasia “bi-ophysical profile” that incorporates measures of lung size and pulmonary vascular function from a variety of modalities.

ACKNOWLEDGMENTS

We thank Beverly Coleman, MD, Steven Horii, MD, Anne Hubbard, MD, and Ann Johnson, MD, for their radiologic expertise.

REFERENCES

Breastfeeding confers significant health, social, and economic benefits to mothers, infants, and society.\(^1\,^2\) Promotion of breastfeeding has been strongly supported by several national organizations and has led to an observed increase in breastfeeding initiation rates from 24.7% in 1971 to 72.9% in 2005.\(^3\) In spite of this increase in breastfeeding initiation, few women continue to breastfeed exclusively at 6 months postpartum (14%) as recommended by the American College of Obstetricians and Gynecologists and the American Academy of Pediatrics.\(^4\)

The American College of Obstetricians and Gynecologists has identified breastfeeding as a top priority and recently published a Committee Opinion calling on health care professionals, hospitals, and employers for their support and promotion of women who choose to breastfeed.\(^4\) However, several obstacles exist that predispose breastfeeding women to early weaning including insufficient milk supply, lack of lactation education, pain or discomfort, and lack of partner and/or employer support.

Pain associated with breastfeeding is the second most commonly cited reason for cessation of breastfeeding.\(^5\) Breast pain may result from engorgement, nipple pain, or mastitis. Nipple pain is commonly reported with the initiation of breastfeeding, but this is usually temporary and relieved with proper positioning of the infant. Persistent nipple pain (after the first 2 weeks) described as sharp and burning and associated with radiating breast pain during and after feedings requires prompt evaluation to avoid early cessation of breastfeeding. Evaluation of breast pain is based primarily on clinical signs, symptoms, and exam. After mastitis has been ruled out, insufficient scientific data exist with regard to the diagnosis and treatment of breast pain.

The epidemiology of breastfeeding-associated pain is not well characterized, and consequently, there is a paucity of treatment guidelines. The medical and lay literature consists of case reports and studies with conflicting microbiological results.\(^6\,^7\) Empiric treatment frequently involves either topical/systemic antifungal or antibiotic therapy or both.

Our objective of this prospective cohort study was to investigate the frequency with which Candida or bacterial pathogens are present on the superficial breast (areola, nipple) and within the ductal architecture in lactating women with and without symptoms of breastfeeding-associated pain. We also examined the incidence of oral thrush in the newborn infants of women with and
This prospective cohort study was conducted between May 2004 and July 2006 at the University of Iowa Hospitals and Clinics (Iowa City, IA). The study was approved on April 8, 2004, by the Institutional Review Board (protocol 200403114). Postpartum women who were planning to breastfeed were recruited as controls by clinical research nurses and lactation specialists on the postpartum unit and gynecology clinic. Symptomatic women were asked to participate if they described sharp, shooting breast pain in the absence of fever, breast redness, or other evidence of mastitis. Women were included in the study if they were 18 years old or older and their infant was less than 3 months of age. We planned to continue enrollments until a minimum of 20 symptomatic women were enrolled.

Women were excluded from the study for the following reasons: clinical evidence of mastitis, insulin-dependent diabetes mellitus, exclusively feeding via a breast pump, supplementing with more than 2 feedings per day, use of antibiotic or antifungal preparations in the prior 14 days, and a history of breast procedures including augmentation, reduction, or radiation.

After completing informed consent and enrolling in the study, participants completed a questionnaire. Questions elicited demographic data, breastfeeding methods (eg, pumping and/or supplementing), medication use including antibiotics, and over-the-counter antifungal preparations since delivery. Participants experiencing breastfeeding-associated pain were asked to describe and rate their pain on a scale from 0 (none) to 10 (most intense pain imaginable). A breast examination was performed and cultures were obtained by a gynecologic nurse practitioner. Cultures were obtained from expressed breast milk, areolae, and the infants’ oropharynx.

### Specimen collection

Paired specimens were collected from both the infant and the mother at the time of the clinic visit. A dry, cotton-tipped swab was gently rolled over the surface of the oral mucosa and tongue of the infant and placed in a transport culturette to conserve specimen moisture. Specimens were collected from the mother by using 2 cotton-tipped culturette swabs that were moistened with sterile saline and separately rolled over each of the right and left areolae and nipples and then placed in culturette sleeves for transport. In addition, approxi-

---

### TABLE 1
Characteristics of women with breastfeeding associated pain, compared with pain-free breastfeeding women

<table>
<thead>
<tr>
<th>Variable</th>
<th>Pain (n = 20)</th>
<th>Pain free (n = 78)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (mean y)</td>
<td>29.6</td>
<td>31.3</td>
<td>.19</td>
</tr>
<tr>
<td>Parity [median (range)]</td>
<td>2 (1-4)</td>
<td>2 (1-4)</td>
<td>.61</td>
</tr>
<tr>
<td>Gestational age [mean wks (range)]</td>
<td>39 (37-40)</td>
<td>39 (34-41)</td>
<td>.53</td>
</tr>
<tr>
<td>Infant age (wks)</td>
<td>6.5</td>
<td>7.0</td>
<td>.29</td>
</tr>
<tr>
<td>Cesarean section delivery (n, %)</td>
<td>2 (10)</td>
<td>22 (28)</td>
<td>.14</td>
</tr>
<tr>
<td>Twin delivery</td>
<td>1 (5)</td>
<td>3 (4)</td>
<td>.82</td>
</tr>
<tr>
<td>Breast pump use</td>
<td>18 (90)</td>
<td>68 (88)</td>
<td>.83</td>
</tr>
<tr>
<td>Formula supplements</td>
<td>5 (25)</td>
<td>19 (25)</td>
<td>.98</td>
</tr>
<tr>
<td>History of skin conditions of breast or areola</td>
<td>0 (0)</td>
<td>3 (4)</td>
<td>.37</td>
</tr>
</tbody>
</table>

### Antimicrobial use variables

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Antibiotics during delivery</td>
<td>4 (20)</td>
<td>25 (35)</td>
</tr>
<tr>
<td>Month prior to delivery</td>
<td>0 (0)</td>
<td>7 (9)</td>
</tr>
<tr>
<td>Since delivery</td>
<td>3 (15)</td>
<td>10 (13)</td>
</tr>
<tr>
<td>Infant antimicrobial receipt</td>
<td>3 (15)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Over-the-counter yeast treatment</td>
<td>1 (5)</td>
<td>2 (3)</td>
</tr>
</tbody>
</table>

### Exam findings: maternal breast

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</tr>
</thead>
<tbody>
<tr>
<td>Normal skin exam of breast</td>
<td>8 (40)</td>
<td>66 (87)</td>
</tr>
<tr>
<td>Fissures/cracks present</td>
<td>4 (20)</td>
<td>0 (0)</td>
</tr>
<tr>
<td>Erythema present</td>
<td>15 (75)</td>
<td>15 (20)</td>
</tr>
</tbody>
</table>

### Exam findings: infant

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral thrush present</td>
<td>4 (20)</td>
<td>4 (6)</td>
</tr>
<tr>
<td>Diaper rash present</td>
<td>5 (25)</td>
<td>18 (24)</td>
</tr>
</tbody>
</table>

### Microbiology findings

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
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</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>6 (30)</td>
<td>6 (8)</td>
</tr>
<tr>
<td><em>Staphylococcus aureus</em></td>
<td>5 (25)</td>
<td>15 (20)</td>
</tr>
<tr>
<td>Coagulase-negative <em>Staphylococcus</em></td>
<td>20 (100)</td>
<td>78 (100)</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>13 (65)</td>
<td>52 (68)</td>
</tr>
</tbody>
</table>

### Infants oropharynx

<p>| | | |</p>
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Yeast</td>
<td>7 (35)</td>
<td>9 (12)</td>
</tr>
<tr>
<td><em>S. aureus</em></td>
<td>2 (10)</td>
<td>9 (12)</td>
</tr>
<tr>
<td>Coagulase-negative <em>Staphylococcus</em></td>
<td>18 (90)</td>
<td>61 (79)</td>
</tr>
<tr>
<td>Gram-negative rods</td>
<td>16 (80)</td>
<td>57 (74)</td>
</tr>
</tbody>
</table>
mately 3-5 mL of breast milk was expressed from each breast into 2 separate sterile containers. The labeled specimens were immediately transported to the laboratory for processing.

Specimen processing
In the laboratory, oral and areolar swab specimens were plated directly to both CHROMagar Candida medium (Hardy Diagnostics, Santa Maria, CA) and blood agar (5% sheep blood, Remel, Inc, Lenexa, KS) using a semiquantitative method. The swabs were then placed into tubes containing 1 mL brain heart infusion (BHI) broth and vortexed for 10 seconds. The semiquantitative plates were incubated for 72 hours at 35°C and then examined for bacterial or yeast growth. The BHI broth was incubated for 48 hours at 35°C with rotary agitation. Following incubation 10 μL of a 1:100 dilution of broth was plated onto both CHROMagar Candida and blood agar and incubated for a further 72 hours.

For each of the left and right milk samples, 250 μL was plated directly to both CHROMagar Candida and blood agar and then incubated for 72 hours at 35°C. In addition, each of the left and right milk samples was further divided into 2 aliquots. The first aliquot was added to an equal volume of BHI broth (1:1) supplemented with fresh iron sulfate (FeSO₄·7H₂O) stock for a final concentration of 300 μg/mL iron in the BHI-milk mixture. The second milk aliquot was processed in a manner similar to the first but without the addition of iron. The BHI-milk mixtures were incubated for 48 hours at 35°C with gentle shaking, whereupon 10 μL of a 1:100 dilution of broth was plated onto both CHROMagar Candida and blood agar and incubated for an additional 72 hours. The plates were then examined for bacterial or yeast growth.

Bacterial colonies visually identified as probable Staphylococcus aureus were isolated and subcultured to blood agar, with identification confirmed by Gram stain and coagulase test. Other bacterial colonies were also presumptively identified using standard microbiological methods. Yeast colonies were collected from CHROMagar and identified to the species level using YBC cards (Vitek; bioMerieux, Inc, Durham, NC). All S. aureus were banked in tryptic soy broth with 15% glycerol and stored at −20°C. Yeasts were banked in sterile water at ambient room temperature for later retrieval and further molecular characterization.

We hypothesized that Candida and bacterial colonization would not differ between women experiencing breastfeeding associated pain and asymptomatic breastfeeding women. χ² and Fisher’s exact test were used, as appropriate, to examine for differences in categorical variables between symptomatic and asymptomatic women, whereas Student t test or Mann Whitney U test was used, as appropriate, to examine for differences in the continuous variables between symptomatic and asymptomatic women. All P values were 2 sided, and alpha was set at 0.05.

<p>| TABLE 2 | Yeast types isolated from women and infant pairs |
|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Pain status</th>
<th>Culture growth</th>
<th>Infant</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pain</td>
<td>Candida albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>No yeast growth</td>
<td>Rhodotorula spp.</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>No yeast growth</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. parapsilosis</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. guilliermondii</td>
</tr>
<tr>
<td>Pain free</td>
<td>Yeast NOS</td>
<td>No yeast growth</td>
</tr>
<tr>
<td>Pain free</td>
<td>No yeast growth</td>
<td>Yeast NOS</td>
</tr>
<tr>
<td>Pain free</td>
<td>No yeast growth</td>
<td>C. albicans</td>
</tr>
<tr>
<td>Pain free</td>
<td>No yeast growth</td>
<td>C. lusitaniae</td>
</tr>
<tr>
<td>Pain free</td>
<td>C. albicans</td>
<td>C. albicans</td>
</tr>
</tbody>
</table>

NOS, not otherwise associated.

RESULTS
Demographic characteristics and clinical data for all 98 women are summarized in Table 1. There were no significant differences in age, parity, gestational age, type of delivery, or antimicrobial use (during or following delivery) between breastfeeding women who reported pain and those who were pain free. As shown in Table 1, examination of the breasts revealed that women who were reporting pain were also more likely to have erythema (75% vs 20%, P < .001) and fissures (20% vs 0%, P = .001) present. Although oral thrush was more common in infants of women with breastfeeding associated pain than asymptomatic women (20% vs 6%, P = .08), this finding did not reach statistical significance.

Six of 20 symptomatic women had breast milk cultures positive for yeast, compared with 6 of 78 asymptomatic women (Table 1: 30% vs 7.7%, P = .015). The relative risk of yeast culture
positivity among symptomatic vs asymptomatic women was 3.9 (95% confidence interval, 1.4 to 10.8). Among the 12 subjects from whom yeast was isolated, 11 grew Candida albicans (Table 2). In each woman from whom yeast was isolated, yeast also grew from infant oropharyngeal cultures. There was no difference in yield between routine broth cultures of breast milk and those supplemented with iron. Incidence of lactoferrin in breast milk,12 we found no benefit from iron supplementation of our breast milk cultures. In addition, the primary fungal organism isolated was C. albicans.

We found an association between the presence of Candida, but not bacterial organisms such as S. aureus, and breastfeeding associated pain. However, it is not clear from this study whether Candida played an etiologic role in the pain syndrome, and the majority of patients with pain (70%) had no Candida isolated from breast milk or areola/nipple cultures. Our findings support the need for a randomized, placebo-controlled trial of antifungal therapy in the management of breastfeeding associated pain.

ACKNOWLEDGMENT
The authors gratefully acknowledge Karen Johnson, Gretchen Cress, Deborah Hubbard, and Vicki Guzman and the General Clinical Research Center at University of Iowa for their assistance in recruiting and the collection of data.

REFERENCES
Does knowledge of cervical length and fetal fibronectin affect management of women with threatened preterm labor? A randomized trial

Amen Ness, MD, MSCP; John Visintine, MD; Emily Ricci, MD; Vincenzo Berghella, MD

OBJECTIVE: The purpose of this study was to estimate the effect of sonographic cervical length (CL) and fetal fibronectin (FFN) on length of evaluation and outcomes in women with preterm labor (PTL).

STUDY DESIGN: Women with threatened PTL were randomized to either a knowledge group (results of CL and FFN available and used according to study protocol), or a standard group (blinded to CL and FFN). Primary outcome was length of evaluation in triage.

RESULTS: One hundred women were randomized. There was no significant difference between groups in length of evaluation, but in women with CL ≥ 30 mm, the mean time for evaluation was significantly shorter in the knowledge group (1:58 h ± 0:50 vs 2:53 h ± 0:50, P = .004). Incidence of spontaneous preterm birth (SPTB) in the knowledge group was significantly reduced (13.0 vs 36.2%, P = .01).

CONCLUSION: The knowledge of CL and FFN was associated with reduction in length of evaluation in women with CL ≥ 30 mm and in incidence of SPTB in all women with PTL.

Key words: cervical length, fetal fibronectin, preterm labor

From the Division of Maternal Fetal Medicine, Department of Obstetrics and Gynecology, Stanford University Medical Center, Palo Alto, CA (Dr Ness); and the Division of Maternal–Fetal Medicine, Department of Obstetrics and Gynecology, Jefferson Medical College, Thomas Jefferson University, Philadelphia, PA (Drs Visintine, Ricci, and Berghella).

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Materials and Methods

We performed a randomized trial of women evaluated for PTL before treatment at the Thomas Jefferson University Hospital, Philadelphia, PA, between November 2004 and April 2006. This study was approved by the Institutional Review Board. Women were eligible for the study if they were between 24 and 33 weeks 6 days; had ≥ 6/contractions/hour, or symptoms suggestive of PTL such as cramping, pressure; were < 3 cm dilated and < 100% effaced and had intact membranes. Exclusion criteria were: known congenital anomaly, triplets or greater, persistent vaginal bleeding that might have affected clinical management, cerclage, or known short CL. Gestational age was assigned by last menstrual period (LMP) or an ultrasound at least 20 weeks. If at < 14 weeks the LMP and ultrasound-based dating differed by ≥ 7 days, or at 14-20 weeks by ≥ 10 days, preference was given to the ultrasound-based date.

Eligible women who consented to participate were randomized to either management by a protocol in which physicians were aware of the CL and FFN results (“knowledge group”) or to a stan-
standard management group in which physicians were blinded to those results. Standard management on our labor and delivery unit usually involves observation for cessation of contractions with or without hydration, and reexamination in 1-2 hours to detect cervical change.

All women were monitored in the triage area on labor and delivery, and had a speculum exam to rule out ruptured membranes and obtain a specimen for rapid FFN, followed by a digital cervical exam. Specimens for rapid FFN were obtained with a Dacron swab from the posterior vaginal fornix and placed in buffer, which was then held on labor and delivery until the patient was randomized. If the patient had intercourse, a pelvic exam, or a TVU within the previous 24 hours, FFN collection was delayed for 24 hours but the patient was still randomized at the time of evaluation. Women who did not return for an FFN were included in the analysis for all outcomes. Once randomized, all FFN samples were sent to the lab for analysis but results were made available only to physicians of women in the knowledge group. Samples were delivered to our in-house laboratory and results were available within 1 hour. A value of $\geq 50$ ng per milliliter was designated as positive.

Physicians managing women in the knowledge group were instructed to follow a study protocol based on the Ohio State University algorithm\(^5\) to determine a woman’s eligibility for treatment (Figure 1). This protocol was reviewed with each physician by a study investigator for each patient randomized to the knowledge group. Once eligible for treatment, management was left to the discretion of the physician. Randomization was performed by means of computer generated blocked randomization, stratified by gestational age $< 28$ weeks and $\geq 28$ weeks. Numbered, sealed, opaque envelopes were opened sequentially within each gestational age strata by 1 of the study investigators.

Following randomization, TVU assessment of the CL was performed in all participants by sonographers not involved in clinical management using a 5-7.5 MHz transvaginal probe placed in the anterior fornix after emptying the bladder, using standard technique.\(^6\) The probe was withdrawn slightly until the image was blurred, and enough pressure then reapplied to restore the image. At least 3 measurements were recorded over 5 minutes and the shortest best measurement was communicated to the managing obstetricians if the patient was in the knowledge group.

The primary outcome was time from initial evaluation to discharge from the triage area. Women admitted at the time of entry into the study, by definition, had no evaluation to discharge interval. Since it was not possible to determine exactly when evaluation ended and admission began, these women were excluded from the primary analysis but were included with regard to admission to delivery interval and preterm delivery rates. Using a 2-sided $t$ test with alpha of 0.05, we calculated that a sample size of 35 patients in each group would be needed for a 90% power to detect a 1 hour (33%) difference in the time for evaluation on labor and delivery. These assumptions were based on a survey taken from the labor and delivery triage database for the 6 months prior to the study, which showed an average time for evaluation of about 3.0 hours with a standard deviation of 1.27 hours.

Secondary outcomes included admission for PTL and tocolysis, SPTB $< 34$ and $< 37$ weeks, and the median interval to delivery (ITD). We also evaluated the
performance characteristics of FFN and CL for the prediction of SPTB < 37 weeks.

Data were analyzed according to intent-to-treat using SPSS software (version 13; SPSS Inc, Chicago, IL). Demographic and clinical characteristics of the 2 groups were compared with the use of the 2-sample t test for continuous variables and the χ² or Fisher exact test for categorical variables. Medians were compared with the Wilcoxon rank sum test. A Kaplan-Meier survival analysis with log rank test was used to compare the interval to delivery between the randomized groups, women with a positive versus a negative FFN and those with a CL < 20 mm and ≥ 20 mm.

**RESULTS**

One hundred women were randomized: 49 to the standard management (blinded) group and 51 to the knowledge group (Figure 2). TVU CL results were obtained in 97 women (2 declined after randomization and 1 CL measurement was not available). FFN results were obtained in 96 women (3 women initially ineligible for FFN did not return for FFN assessment, and 1 was unreadable). FFN results were available within 1 hour at the initial evaluation in 85 women. One woman assigned to the knowledge group who should have been discharged according to the study protocol was admitted and treated. Final delivery data were not available in 6 women who delivered elsewhere, but 4 reached a gestational age of at least 34 weeks.

Both groups were similar with respect to maternal age, parity, race, prior SPTB, smoking status, public insurance, and GA at evaluation (Table 1). The median digital cervical dilatation in both groups was 0.5 cm (range 0-2), and there were no significant differences in the frequency of a CL < 20 mm, 20-29 mm, ≥ 30 mm, a positive FFN, or the number of contractions (Table 2). The mean gestational age at randomization for the entire study population was 29 weeks ± 2.7 and the median ITD was 56 days (4-122). The overall rate of SPTB < 37 weeks was 24.5% (23/94) and SPTB < 34 weeks was 10.4% (10/96). Overall, 54 women

**TABLE 1**  

Demographic characteristics and risk factors for preterm birth

<table>
<thead>
<tr>
<th>Patient characteristics</th>
<th>Knowledge of FFN/CL (n = 51)</th>
<th>Standard (blinded) (n = 49)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>26.5 ± 6.3</td>
<td>25.6 ± 6.4</td>
</tr>
<tr>
<td>Nulliparas</td>
<td>14 (27.5%)</td>
<td>13 (28.9%)</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>27 (52.9%)</td>
<td>36 (73.5%)</td>
</tr>
<tr>
<td>Non-Hispanic white</td>
<td>17 (33.3%)</td>
<td>8 (16.3%)</td>
</tr>
<tr>
<td>Hispanic</td>
<td>6 (11.8%)</td>
<td>5 (10.2%)</td>
</tr>
<tr>
<td>Other</td>
<td>1 (2%)</td>
<td>0</td>
</tr>
<tr>
<td>Smoker</td>
<td>9 (17.6%)</td>
<td>6 (12.2%)</td>
</tr>
<tr>
<td>Public insurance</td>
<td>31 (60.8%)</td>
<td>31 (63.3%)</td>
</tr>
<tr>
<td>Gestational age at evaluation*</td>
<td>29.4 ± 2.6</td>
<td>29.9 ± 2.7</td>
</tr>
<tr>
<td>Gestational age &lt; 28 wk</td>
<td>12 (23.5%)</td>
<td>12 (24.5%)</td>
</tr>
<tr>
<td>Prior PTB</td>
<td>16 (31.4%)</td>
<td>13 (26.5%)</td>
</tr>
<tr>
<td>Prior spontaneous PTB</td>
<td>16 (31.4%)</td>
<td>11 (22.5%)</td>
</tr>
<tr>
<td>Multiple gestation</td>
<td>2 (3.9%)</td>
<td>1 (2%)</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SD.

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(55.7%) had a CL > 30 mm. Of these, 9 (17.0%) had an SPTB < 37 weeks, 3 (5.7%) had an SPTB < 34 weeks, and 1 was lost to follow-up. Among the women with a CL > 30 mm and FFN results, 5/51 (9.8%) had a positive FFN; 1 was a twin pregnancy who had PPROM and delivered within 14 days (33.6 weeks), 1 had an indicated PTB (preeclampsia), and 3 delivered at term.

Table 3 shows the outcomes for each study group. The mean time from evaluation to discharge in the knowledge group was less than in the standard group. In addition, significantly more women were admitted for PTL in the knowledge group with a long CL who should not have been admitted, there were still more admissions in the knowledge group, but the significance decreased to P = .05.

Tocolysis was used in 10/12 women admitted, and 9 received steroids. Notably, of the 12 women admitted for PTL, 11 delivered at term (1 lost to follow-up). All 9 patients who had an SPTB < 34 weeks received steroids, except 1, who had an abortion.

The sensitivity, specificity, positive, and negative predictive values for the FFN results and for a CL < 20 mm are shown in Table 4. The rates of SPTB based on CL and FFN are shown in Table 5. CL < 20 mm was the only significant predictor of SPTB < 37 weeks (P = .015) but both tests had similar negative predictive values. Women with a CL < 20 mm were significantly more likely (P = .008) and women with a CL ≥ 30 mm significantly less likely (P = .002) to have a positive FFN.

Kaplan-Meier survival analyses with log rank test were performed to assess the effect of knowledge of FFN and CL, FFN, and a CL < 20 mm on the time from evaluation to delivery (Figures 3A-C). Women in the knowledge group and women with a CL ≥ 20 mm had a significantly longer ITD than women in the standard group or those with a CL < 20 mm, respectively. No difference was noted between women with a positive versus a negative FFN.

### TABLE 2

<table>
<thead>
<tr>
<th>Cervical length (CL) (n = 97)</th>
<th>Knowledge of FFN/CL</th>
<th>Standard (blinded)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt; 20</td>
<td>11 (22.0%)</td>
<td>7 (14.9%)</td>
<td>.36</td>
</tr>
<tr>
<td>20-29</td>
<td>12 (24.0%)</td>
<td>13 (27.7%)</td>
<td>.68</td>
</tr>
<tr>
<td>&gt; 30</td>
<td>27 (54.0%)</td>
<td>27 (57.4%)</td>
<td>.73</td>
</tr>
<tr>
<td>Fetal fibronectin (FFN) (n = 96)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Positive</td>
<td>10/50 (20%)</td>
<td>10/46 (21.7%)</td>
<td>.83</td>
</tr>
<tr>
<td>Negative</td>
<td>40/50 (80%)</td>
<td>36/46 (78.3%)</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 3

<table>
<thead>
<tr>
<th>Outcomes</th>
<th>Knowledge of FFN/CL</th>
<th>Standard (blinded)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time from evaluation to discharge (hr)*</td>
<td>2:24 ± 1:09</td>
<td>2:49 ± 1:09</td>
<td>.14</td>
</tr>
<tr>
<td>Gestational age at delivery*</td>
<td>38.3 ± 2.1</td>
<td>37.1 ± 2.9</td>
<td>.03</td>
</tr>
<tr>
<td>SPTB ≤ 37 weeks (n = 93)</td>
<td>6/46 (13.0%)</td>
<td>17/47 (36.2%)</td>
<td>.01</td>
</tr>
<tr>
<td>SPTB ≤ 34 weeks (n = 97)</td>
<td>3/49 (6.1%)</td>
<td>6/48 (12.5%)†</td>
<td>.32</td>
</tr>
<tr>
<td>Delivery within 7 d (n = 94)</td>
<td>1 (2.1%)</td>
<td>1 (2.1%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Delivery within 14 d (n = 94)</td>
<td>1 (2.1%)</td>
<td>2 (4.3%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Delivery within 28 d (n = 94)</td>
<td>6 (13.0%)</td>
<td>7 (14.9%)</td>
<td>.78</td>
</tr>
<tr>
<td>Median interval from evaluation to delivery (d)</td>
<td>66.2 (6-122)</td>
<td>49.0 (4-99)</td>
<td>.001</td>
</tr>
<tr>
<td>Admission for PTL (n = 51)</td>
<td>10/51 (19.6%)‡</td>
<td>2/49 (4.1%)</td>
<td>.03</td>
</tr>
</tbody>
</table>

* Values are given as mean ± SD.
† One was a twin pregnancy (the other 2 twin pregnancies had indicated deliveries < 34 weeks).
‡ One of 10 patients was a protocol deviation.

Comment

We found that knowledge of CL and FFN using a study protocol similar to that proposed by Iams et al was not associated with a significant overall effect on length of time for evaluation. But among women with a CL ≥ 30 mm, those in the knowledge group had a significant decrease in the time for evaluation compared to women with a CL ≥ 30 mm in the standard group. According to our protocol, only women with a CL ≥ 30 mm were discharged immediately. Since women with a CL ≥ 30 represent over...
50% of patients evaluated for PTL, the ability to discriminate them from women with a shorter CL would appear to be important in reducing time for evaluation, and as well as avoiding unnecessary admissions and interventions. Importantly, the decreased evaluation time was not associated with an increased rate of SPTB or with a failure to administer steroids prior to delivery in those who delivered before 34 weeks.

The lack of overall effect on time for evaluation is consistent with the results of 3 other randomized trials that studied the effect of knowledge of FFN results on patient treatment decisions by physicians.\textsuperscript{2,7,8} None of these studies utilized a protocol or algorithm for the use of FFN. In the study by Grobman et al\textsuperscript{7} there was also no difference in the number of admissions or the total medical and non-medical costs even when controlling for the presence of cervical dilatation or public versus private insurance. But Lowe et al\textsuperscript{8} found fewer admissions and shorter length of stays in women with a negative FFN. Studies evaluating the use of TVU CL in women with preterm labor, none of which were randomized, have also shown reductions in admissions and shorter lengths of stay.\textsuperscript{5,10}

In our study, a number of factors may have partially masked a potential difference in the minimum time needed for evaluation. Although the average time required for study enrollment, and to obtain results of the FFN and CL was similar in both groups, this time overlapped with the usual time of evaluation for evidence of cervical change in the standard group, but in the knowledge group, management was exclusively based on the results of the FFN and CL. Therefore, their time for evaluation was more dependent on this additional time than the standard group. Furthermore, due to the nature of a busy labor and delivery floor, not all patients were sent home as soon as the information allowing them to leave was obtained. On the other hand, compared to other studies where the median time spent for evaluation on labor and delivery was either 3-4\textsuperscript{4} or 8-9 hours,\textsuperscript{11} the mean time from evaluation to discharge in the standard group was relatively short. While managing obstetricians were not aware of the primary outcome of the study, a ‘Hawthorne effect’ (when people are observed in a study, their behavior or performance temporarily changes) cannot be ruled out. Thus, it may not have been possible to further reduce this short time in the triage area despite implementation of our protocol.

### Table 4

<table>
<thead>
<tr>
<th>SPTB &lt; 37 wk</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Positive predictive value</th>
<th>Negative predictive value</th>
</tr>
</thead>
<tbody>
<tr>
<td>FFN</td>
<td>18 (8-34)</td>
<td>81 (78-86)</td>
<td>24 (10-44)</td>
<td>75 (72-80)</td>
</tr>
<tr>
<td>CL &lt; 20 mm</td>
<td>36 (22-51)</td>
<td>87 (87-92)</td>
<td>47 (28-66)</td>
<td>81 (77-85)</td>
</tr>
<tr>
<td>SPTB &lt; 14 d</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>FFN</td>
<td>67 (21-94)</td>
<td>82 (81-83)</td>
<td>11 (14-16)</td>
<td>99 (97-100)</td>
</tr>
<tr>
<td>CL &lt; 20 mm</td>
<td>33 (6-79)</td>
<td>82 (81-84)</td>
<td>6 (1-14)</td>
<td>97 (96-99)</td>
</tr>
</tbody>
</table>

### Table 5

| Rates of SPTB for fetal fibronectin, cervical length, and/or both together |
|-----------------------------|-----------------------------|-----------------------------|-----------------------------|
| FFN and/or CL               | SPTB < 37 wk P value        | SPTB < 34 wk P value        |
| FFN positive                | 4/17 (23.5%) .01            | 3/20 (15.0%) .36            |
| FFN negative                | 18/73 (24.7%) 5/74 (6.8%)   |                            |
| CL < 20 mm                  | 8/17 (47.1%) .015           | 4/18 (22.2%) .053           |
| CL ≥ 20 mm                  | 14/74 (18.9%) 5/77 (6.5%)   |                            |
| CL < 20 mm or FFN positive  | 9/27 (35%) .179*            | 5/30 (16.7%) .109*          |
| CL < 20 mm and FFN positive | 3/7 (42.9%) .340            | 2/8 (25.0%) .110            |
| CL < 20 mm and FFN negative | 5/10 (50.0%) 2/10 (20.0%)   |                            |
| CL ≥ 30 mm                  | 9/49 (18.4%) .092           | 3/53 (5.7%) .179            |
| CL < 30 mm                  | 13/38 (34.2%) 6/42 (14.3%)  |                            |
| CL ≥ 30 mm and FFN positive | 1/5 (20.0%) 1.0             | 1/5 (20.0%) .199            |
| CL ≥ 30 mm and FFN negative | 7/44 (15.9%) 1/45 (2.2%)    |                            |

Knowledge of cervical length and fetal fibronectin shortens the time for evaluation for women with suspected preterm labor without increasing the rate of preterm birth.

* Compared to women with neither a CL < 20 mm nor a positive FFN.
The significant reduction in the incidence of PTB in the knowledge group compared to the standard group was unexpected and occurred despite any significant differences in baseline variables, including cervical dilatation on admission, and results of the TVU CL and FFN tests. The number of admissions is the only documented intervention that differed between the groups. Because 10 of 11 women admitted for PTL delivered after 37 weeks, it might be tempting to speculate that admission and/or tocolysis may have accounted for at least some of the difference in the rates of PTB. Women admitted for PTL are also more likely to have been told to stop work, and have their activity restricted. Nevertheless, these interventions have not been definitively shown to reduce the rate of PTB, at least as studied in the past.12,13

Unfortunately, populations included in prior studies were heterogeneous, and did not have the benefit of standardized tools for risk assessment such as CL and FFN.13 It is unknown if any of these interventions are beneficial in symptomatic women with either a short CL and/or a positive FFN. Since our study was small, this finding may still be due to chance.

As might be expected based on our protocol, there was no difference in admissions between FFN positive and negative women or those with a CL < 20 mm in the standard management (blinded) group, but there were significantly more admissions among FFN positive women and women with a CL < 20 mm in the knowledge group. It would appear that knowledge of the FFN result and CL encouraged more admissions than standard evaluations based on clinical examination alone.

In our population, CL was a better predictor of PTB < 37 weeks than FFN but the negative predictive values were similar. A recent prospective blinded study by Schmitz et al14 in 359 women admitted with PTL confirmed the potential value of the strategy proposed by Iams et al,5 and used in our study. They demonstrated that when FFN is used only in patients selected on the basis of TVU CL the sensitivity and specificity of the FFN is maintained while reducing the number of FFN tests performed by 55%. Our study did not demonstrate any significant improvement in predictive values using FFN. This may have been due to the combination of the low positive predictive value of a positive FFN,13,15 and the limited time (usually about 2 weeks) for which the negative predictive value of the FFN test is valid. Because the majority of SPTD occurred between 35-37 weeks, with a mean ITD of 4.7 ± 2.9 weeks, the FFN obtained at the initial evaluation may not accurately predict PTB more than a month later.

It has been emphasized15,16 that the value of these tests lies in their negative predictive values, especially for delivery within 14 days. But this benefit is limited by the prevalence of the outcome in the particular population to which it is applied and by a physicians’ willingness to avoid interventions when test results indicate a low risk of PTD. In our population the short-term negative predictive value (delivery within 14 days) of both CL and FFN was only marginally better than the baseline predictive value before the tests were done since 96.9% (94/97) of women did not deliver within 14 days.

Our study has several limitations. First is its small size. Although we enrolled a population at high risk for PTB, the rate of SPTD within 14 days was low, and our sample size therefore did not allow for robust evaluation of these short-term outcomes. Second, delivery data were not available on all women. Third, although we encouraged all women who were evaluated for PTL to be approached for enrollment in the study, not all were. Strengths of the study are that it was a randomized trial and that it included only women being evaluated for PTL before admission or tocolysis, and thus represents real-life clinical use of FFN and CL as experienced by most practitioners. It is the only trial that randomizes women to knowledge or no knowledge of both FFN and CL tests, and that standardized the use of FFN and CL through a protocol.

In summary, although we were unable to demonstrate a statistical decrease in the overall time for evaluation for PTL, the time for evaluation was shorter when using FFN and CL, especially in women with a CL ≥ 30 mm. On the other hand,
knowledge of FFN and CL was associated with increased rate of admissions for PTL and a decreased rate of SPTB. Adding FFN did not seem to improve the predictive values of CL < 20 mm. These findings require further study in larger randomized trial.

REFERENCES
Does length of labor vary by maternal age?

Mara B. Greenberg, MD; Yvonne W. Cheng, MD, MPH; Margaret Sullivan, MPH; Mary E. Norton, MD; Linda M. Hopkins, MD; Aaron B. Caughey, MD, PhD

OBJECTIVE: The purpose of this study was to examine lengths of first and second stages of labor across maternal age groups to determine whether different norms should be established.

STUDY DESIGN: We conducted a retrospective cohort study of all laboring, term, singleton, and cephalic deliveries at a single institution between 1980-2001. Median lengths of labor were compared among 6 maternal age groups. Statistical comparisons were made using Kruskal–Wallis and Wilcoxon rank sum tests. Multivariable linear and logistic regression models were performed.

RESULTS: Among 31,976 births, length of labor differed significantly by maternal age for both nulliparous and multiparous women. Younger nulliparous women (age, <20 yrs) had a shorter median second stage by up to 97 minutes (P < .001) than older nulliparous women (age, >39 yrs). After we controlled for potential confounders, we found that older women had a persistently higher likelihood of experiencing longer labor and prolonged labor than younger women.

CONCLUSION: Length of labor and prolonged labor increases with increasing maternal age.

Key words: length of labor, maternal age


The rising cesarean delivery rate is the subject of intense scrutiny and debate, both nationally and globally. In 2003, this rate was noted to reach an all-time high of 30%.1 Given that the indication of failed progress of labor is responsible for a large proportion of primary cesarean deliveries,2 a better understanding of risk factors for abnormal labor progression may impact the timely diagnosis of dystocia, active management of labor, and ultimately the method of delivery. Also important to consider is not only the rate of cesarean in the index pregnancy but also the impact on maternal and fetal well-being in subsequent pregnancies that may be at increased risk because of a previous cesarean delivery.3,4

In addition to the impact on cesarean delivery rates, the study of labor progression and mode of delivery is also crucial to our understanding of how demographic characteristics contribute to pregnancy outcome and how obstetric management can maximize maternal and fetal health. Labor management parameters in current use draw from Friedman’s studies that were performed 40-50 years ago on small, homogeneous groups of women5-7; more current studies of labor have challenged the assumption that 1 labor curve can be applied to all women. A recent investigation by our group showed that the length of labor varies by maternal ethnicity8; others have studied labor progression and mode of delivery as related to maternal weight and body mass index, gestational age, and other parameters.9-12 Another important demographic characteristic that has received increasing attention is maternal age.

In the United States, particularly in urban areas, median maternal age has increased over the past 2 decades. In 2005, birth rates for women in all age categories >30 years of age continued to rise to their highest levels yet; birth rates to teens showed slight decreases, and childbearing in women aged 25-29 years remained stable.1 Likely contributors to these trends include changes in the landscape of socioeconomic factors on the timing of starting a family and developments in reproductive technology and improvements in prenatal and obstetric care.13,14 To adapt to the changing demographics of the obstetric patient, it is important to understand the effects of maternal age on perinatal outcomes. Previous studies of maternal age-related differences in perinatal outcome have showed higher rates of some adverse conditions, which include preterm delivery, low birthweight, gestational hypertension, diabetes mellitus, failure of trial of labor after cesarean, fetal death,15-17 and increased rates of cesarean delivery in the older cohort.18-21 It is imperative therefore that we consider the effects of maternal age as we seek to improve the characterization of factors that contribute to progress in labor and rate of cesarean deliveries.

Our objective was to study length of labor with relation to maternal age, to better understand a multifactorial redefinition of the labor curve. Although this type of descriptive study does not speak to causality, it is aimed at contributing to
the developing descriptions of rate of progress in labor and the effects of maternal age.

Materials and Methods
We performed a retrospective cohort study of all laboring, singleton, cephalic deliveries at ≥37 weeks’ gestation at the University of California, San Francisco (UCSF), between 1980-2001. Data were collected concurrently with the creation of the prenatal record and during each patient’s hospital stay for labor and delivery and were entered by research staff into a computerized database. Maternal age categories were defined as <20 years, 20-24 years, 25-29 years, 30-34 years, 35-39 years, or ≥40 years.

Median lengths of the first and second stages of labor were compared among these 6 maternal age groups. In addition, comparisons were made of proportions of women who had a first stage of labor for >18 hours, a second stage of labor for >3 hours, and a prolonged second stage of labor. The latter was defined as >3 hours for nulliparous women with an epidural, >2 hours for multiparous women with an epidural, >2 hours for nulliparous women without an epidural, and >1 hour for multiparous women without an epidural.22 The onset of first stage of labor was defined as the time when regular, painful contractions that lead to cervical change began occurring every 3-5 minutes per patient history. The second stage of labor was defined as the time between complete cervical dilation and delivery of the infant. Women without this information available were excluded from the study.

Statistical comparisons were made with the Kruskal–Wallis and Wilcoxon rank sum tests. Further, the lengths of first and second stages of labor were compared with the use of survival analysis. Multivariable linear and logistic regression models were used to control for potential confounders that included race/ethnicity, parity, use of regional anesthesia, chorioamnionitis, gestational age, birthweight, year of delivery, body mass index, Medicaid insurance status, fetal occiput posterior position, intrapartum cesarean delivery, and labor management techniques that included the use of oxytocin, artificial rupture of membranes, and prostaglandins for labor induction. Body mass index was calculated with the use of the height and weight at the first prenatal visit. Fetal position was determined at time of delivery of the fetal vertex. Additionally, subgroup analyses were conducted in women with and without induction of labor, epidural use, and cesarean delivery. Because of concern about using standard linear regression to analyze the length of labor data with a nonnormal distribution, the models were run as log-transformed data as well. Two-tailed $P$ values <.05 were considered statistically significant. Institutional review board approval was obtained from the Committee on Human Research at UCSF.

Results
Overall, 31,976 women with a term, singleton, cephalic labor met study inclusion criteria. There were 16,899 nulliparous and 15,077 multiparous births studied. Of these, 8.8% were <20 years of age; 19.7% were ages 20-24 years; 28.7% were ages 25-29 years; 27.1% were ages 30-34 years; 13.2% were ages 35-39 years, and 2.5% were aged ≥40 years (Table 1). For both first and second stages of labor, there were statistically significant differences between the median lengths of labor among the different age groups (Table 2). In the first stage, nulliparous women had increasing median lengths of labor, from 9.2 hours in the youngest age group to a peak of 11.0 hours in women aged 35-39 years. For multiparous women in the first stage, younger women had slightly longer labors than older women, which decreased from 6.1 hours in those aged <20 years to 5.7 hours in those aged ≥40 years ($P$ = .02). The 95th percentile values for first stage lengths among nulliparous women ranged from 23-29 hours in women aged <20 years to those aged ≥40 years but were consistently 18 hours in multiparous women across age groups. When data were analyzed by epidural status, significant increases in the length of first stage among nulliparous women and decreases among multiparous women of advancing age groups were limited to those women with epidural analgesia (Table 2).

In the second stage of labor, nulliparous women had lengths that increased from 51 minutes for women who were <20 years old to 148 minutes in women who were ≥40 years old ($P$ ≤ .001). Similarly, length of second stage among multiparous women increased from 16 minutes in women who were <20 years old to 26 minutes in women who were >39 years old ($P$ < .001). These differences remained significant for both nulliparous and multiparous women, regardless of epidural status (Table 2). The 95th percentile of length of second stage ranged from 232-381 minutes in nulliparous women and 120-197 minutes in multiparous women, comparing women who were <20 years old in comparison with women who were ≥40 years old. With regard to univariable threshold comparisons, older nulliparous women were more likely to experience a first stage of labor >18 hours than were younger nulliparous women, and both nulliparous and multiparous women were more likely to experience prolonged second stage of labor in the older than in the younger age groups (Table 3).

In the multivariable analysis, age-associated differences in the length of the first stage of labor persisted for nulliparous women when we controlled for epidural status, induction of labor, ethnicity, gestational age, Medicaid insurance status, birthweight, occiput posterior position, body mass index, intrapartum cesarean delivery, year of delivery, and chorioamnionitis. Compared with women who were <20 years old, nulliparous women in older age categories had first stage lengths that ranged from no difference in 20- to 24-year-old women up to 88.1 minutes longer in women who were 35-39 years old ($P$ < .001). These differences were most pronounced in women with epidurals, compared with nulliparous women without epidurals, and in women who labored spontaneously compared with those who received induction of labor (Table 4). For multiparous women in the first stage, differences in length did not remain significant in the multivariable model.
### TABLE 1
Demographics and intrapartum characteristics

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maternal age (y)</th>
<th>&lt;20</th>
<th>20-24</th>
<th>25-29</th>
<th>30-34</th>
<th>35-39</th>
<th>≥40</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nulliparous (%)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiparous (%)</td>
<td></td>
<td>3.4</td>
<td>17.1</td>
<td>28.4</td>
<td>30.7</td>
<td>17.1</td>
<td>2.2</td>
<td></td>
</tr>
<tr>
<td>Gestational age of ≥41 wks (%)</td>
<td></td>
<td>26.5</td>
<td>27.0</td>
<td>25.4</td>
<td>24.8</td>
<td>23.1</td>
<td>24.5</td>
<td>.001</td>
</tr>
<tr>
<td>Birthweight ≥4 kg (%)</td>
<td></td>
<td>7.9</td>
<td>9.9</td>
<td>11.5</td>
<td>14.4</td>
<td>14.9</td>
<td>16.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>White (%)</td>
<td></td>
<td>21.7</td>
<td>31.6</td>
<td>41.8</td>
<td>49.8</td>
<td>55.6</td>
<td>58.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Black (%)</td>
<td></td>
<td>40.6</td>
<td>22.5</td>
<td>11.6</td>
<td>6.8</td>
<td>6.3</td>
<td>5.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Latina (%)</td>
<td></td>
<td>18.4</td>
<td>16.8</td>
<td>11.2</td>
<td>8.4</td>
<td>7.4</td>
<td>9.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Asian (%)</td>
<td></td>
<td>19.3</td>
<td>29.2</td>
<td>35.4</td>
<td>35.0</td>
<td>30.7</td>
<td>25.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Induction of labor (%)</td>
<td></td>
<td>14.6</td>
<td>12.7</td>
<td>12.9</td>
<td>14.4</td>
<td>16.2</td>
<td>19.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Epidural (%)</td>
<td></td>
<td>56.3</td>
<td>48.7</td>
<td>48.4</td>
<td>51.9</td>
<td>54.4</td>
<td>57.2</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Medicaid insured (%)</td>
<td></td>
<td>45.6</td>
<td>46.0</td>
<td>37.9</td>
<td>26.4</td>
<td>21.3</td>
<td>18.9</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Occiput posterior (%)</td>
<td></td>
<td>14.8</td>
<td>13.1</td>
<td>14.6</td>
<td>15.6</td>
<td>16.0</td>
<td>18.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Body mass index &gt;29 (%)</td>
<td></td>
<td>10.8</td>
<td>9.6</td>
<td>8.2</td>
<td>6.9</td>
<td>8.2</td>
<td>9.4</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Chorioamnionitis (%)</td>
<td></td>
<td>8.2</td>
<td>6.7</td>
<td>6.8</td>
<td>7.3</td>
<td>7.3</td>
<td>7.4</td>
<td>.2</td>
</tr>
<tr>
<td>Cesarean delivery (%)</td>
<td></td>
<td>8.7</td>
<td>9.4</td>
<td>11.0</td>
<td>13.2</td>
<td>16.0</td>
<td>16.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Total n</td>
<td></td>
<td>2437</td>
<td>5456</td>
<td>7926</td>
<td>7501</td>
<td>3636</td>
<td>689</td>
<td></td>
</tr>
</tbody>
</table>

### TABLE 2
Univariable analyses, median lengths of stages 1 and 2

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maternal age (y)</th>
<th>&lt;20</th>
<th>20-24</th>
<th>25-29</th>
<th>30-34</th>
<th>35-39</th>
<th>≥40</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Stage 1</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nulliparous women*</td>
<td></td>
<td>550</td>
<td>555</td>
<td>595</td>
<td>630</td>
<td>660</td>
<td>585</td>
</tr>
<tr>
<td>With epidural*</td>
<td></td>
<td>630</td>
<td>670</td>
<td>705</td>
<td>720</td>
<td>760</td>
<td>715</td>
</tr>
<tr>
<td>Without epidural (P = .14)</td>
<td></td>
<td>435</td>
<td>450</td>
<td>465</td>
<td>457</td>
<td>470</td>
<td>395</td>
</tr>
<tr>
<td>Total multiparous women (P = .02)</td>
<td></td>
<td>368</td>
<td>365</td>
<td>355</td>
<td>345</td>
<td>345</td>
<td>343</td>
</tr>
<tr>
<td>With epidural*</td>
<td></td>
<td>478</td>
<td>485</td>
<td>475</td>
<td>465</td>
<td>436</td>
<td>465</td>
</tr>
<tr>
<td>Without epidural (P = .25)</td>
<td></td>
<td>308</td>
<td>310</td>
<td>300</td>
<td>300</td>
<td>300</td>
<td>290</td>
</tr>
<tr>
<td><strong>Stage 2</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total nulliparous women*</td>
<td></td>
<td>51</td>
<td>65</td>
<td>89</td>
<td>118</td>
<td>127</td>
<td>148</td>
</tr>
<tr>
<td>With epidural*</td>
<td></td>
<td>83</td>
<td>102</td>
<td>136</td>
<td>152</td>
<td>174</td>
<td>192</td>
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<tr>
<td>Without epidural*</td>
<td></td>
<td>29</td>
<td>40</td>
<td>51</td>
<td>70</td>
<td>70</td>
<td>75</td>
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<tr>
<td>Total multiparous women*</td>
<td></td>
<td>16</td>
<td>15</td>
<td>18</td>
<td>20</td>
<td>24</td>
<td>26</td>
</tr>
<tr>
<td>With epidural*</td>
<td></td>
<td>34</td>
<td>31</td>
<td>42</td>
<td>50</td>
<td>55</td>
<td>58</td>
</tr>
<tr>
<td>Without epidural*</td>
<td></td>
<td>12</td>
<td>11</td>
<td>13</td>
<td>14</td>
<td>15</td>
<td>17</td>
</tr>
</tbody>
</table>

Median times are reported in minutes, with 5th/95th percentile in parentheses. All comparisons are to ages <20 years.

*P ≤ .001
In the second stage of labor, older nulliparous women had significantly longer lengths than younger women when we controlled for potential confounders, regardless of epidural status and whether labor was spontaneous or induced (Table 5). Compared with women who were <20 years old, differences ranged from 9.4 minutes longer in women who were 20-24 years to 56.5 minutes longer in women who were >39 years old (P < .001). For multiparous women, significantly longer length of second stage compared with women who were <20 years old was limited primarily to women who were ≥30 (Table 5).

Also in the multivariable analysis, older nulliparous women had higher rates of first stage of labor of >18 hours than younger nulliparous women, which was most pronounced in 35- to 39-year-old women, compared with women who were <20 years old (odds ratio, 1.53; 95% CI, 1.23-1.91). Older nulliparous and multiparous women had a higher likelihood of experiencing prolonged second stage of labor than did younger women, after we controlled for potential confounders, with an odds ratio to 3.90 for nulliparous women who were >39 years old, compared with women who were <20 years old (95% CI, 2.70-5.62), and an odds ratio to 2.28 for multiparous women who were 35-39 years old, compared with women who were <20 years old (95% CI, 1.48-3.53).

When the regression models were conducted in women with and without cesarean deliveries, the findings among those without a cesarean delivery were similar to the overall group. In the small subgroup of women who were delivered by cesarean, maternal age–related differences in neither first nor second stage of labor reached statistical significance. These differences reported earlier were of similar direction and statistical significance when analyses were conducted with the log-transformed lengths of labor (data not shown).

**Comment**

We have demonstrated a significant difference in the length of labor by maternal age.
TABLE 5
Second stage of labor, by multivariable analyses

<table>
<thead>
<tr>
<th>Variable</th>
<th>Maternal age (y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>20-24</td>
</tr>
<tr>
<td>Nulliparous women</td>
<td></td>
</tr>
<tr>
<td>Overall*</td>
<td>+9.4</td>
</tr>
<tr>
<td>Stratified</td>
<td></td>
</tr>
<tr>
<td>With epidural†</td>
<td>+7.2</td>
</tr>
<tr>
<td>Without epidural**‡</td>
<td>+11.8</td>
</tr>
<tr>
<td>Induction of labor‡</td>
<td>+8.8</td>
</tr>
<tr>
<td>Spontaneous labor†</td>
<td>+9.6</td>
</tr>
<tr>
<td>Multiparous women</td>
<td></td>
</tr>
<tr>
<td>Overall</td>
<td>-1.2</td>
</tr>
<tr>
<td>Stratified</td>
<td></td>
</tr>
<tr>
<td>With epidural†</td>
<td>-3.4</td>
</tr>
<tr>
<td>Without epidural**‡</td>
<td>-0.1</td>
</tr>
<tr>
<td>Induction of labor‡</td>
<td>+4.1</td>
</tr>
<tr>
<td>Spontaneous labor†</td>
<td>-1.8</td>
</tr>
</tbody>
</table>

Reported as difference in minutes, compared with age group <20 years.

* P ≤ .001, controlled for epidural, induction of labor, ethnicity, gestational age, Medicaid insurance status, birthweight, occiput posterior position, body mass index, intrapartum cesarean delivery, year of delivery, and chorioamnionitis.

† Controlled for all of the above, except epidural status.

‡ Controlled for all of the above, except the use of augmentation or induction of labor that included pitocin, prostaglandin analogues, and artificial rupture of membranes.

§ P < .05, controlled for epidural, induction of labor, ethnicity, gestational age, Medicaid insurance status, birthweight, occiput posterior position, body mass index, intrapartum cesarean delivery, year of delivery, and chorioamnionitis.

‖ P ≤ .01, controlled for epidural, induction of labor, ethnicity, gestational age, Medicaid insurance status, birthweight, occiput posterior position, body mass index, intrapartum cesarean delivery, year of delivery, and chorioamnionitis.

age in a large, diverse cohort of women. When we controlled for potential confounders, older women had persistently longer first and second stages of labor than did younger women and higher rates of prolonged first and second stages of labor. These differences are primarily clinically significant in the second stage for both nulliparous and multiparous women. For first-stage lengths, clinical applicability may be limited to 95th percentile values, which demonstrate variation among women of different ages, as does the likelihood of experiencing prolonged labor.

This retrospective cohort analysis is a descriptive study that was aimed at aiding practitioners in understanding the influence of maternal age on length of labor and how various confounders impact the effect of a woman’s age on labor progression. Factors of interest that may vary by maternal age include differences in maternal habitus and fetal size, measures of general maternal health, or iatrogenic factors such as labor management or differential effect of epidural on progress in labor. Although it is notable in the demographic description of our cohort that maternal body mass index, infant birthweight, and occiput posterior presentation do differ across maternal age categories, the fact that age-related increases in length of labor remained significant in the multivariable model after these factors were controlled for minimizes their potential candidacy as the sole causative influences on the observed variation. Although epidural anesthesia use is one modifiable factor that may be discussed with an older pregnant woman when her individual risks for prolonged labor are considered, our finding that older women experienced longer labors and higher rates of prolonged labors than did younger women, regardless of epidural status, may lessen the impact of directive counseling toward or against epidural use, especially in context with other personal maternal preferences and concerns.

It has been demonstrated by numerous investigators that older women have higher rates of cesarean deliveries. Different models have shown considerable variation in the rates of possible medical and obstetric influences on increases in intrapartum cesarean delivery in particular, such as rates of leiomyomata, obesity, gestational diabetes mellitus, large for gestational age infants, and preeclampsia. It is our concern that, in addition to these influences, increased intrapartum cesarean rates may also be due to the way in which providers respond to natural variation in length of labor. The use of a one-size-fits-all labor curve may lead to truncating labor in both the first and second stage in older women and lead to higher rates of cesarean delivery. In the setting of a reassuring fetal heart tracing, it may be prudent for
clinicians to exhibit more patience with “slow” labors in older women to achieve lower cesarean delivery rates.

Directions for further investigation in this area may be modeled on the prospective work of Rouse et al,24 who demonstrated that, by modifying and possibly extending our limits of the definition of prolonged labor, more vaginal deliveries will be achieved without demonstrable neonatal injury. Cheng et al25 performed a similar investigation in 2004 that showed that the extension of the limits of the second stage beyond 3 hours can lead to further spontaneous vaginal deliveries without compromising neonatal outcomes, but with some effect on maternal outcomes such as perineal trauma. In this vein, important questions to consider include whether maternal age-related differences in labor length impact perinatal and neonatal outcomes, and if so, should practitioners medically intervene to facilitate older women reaching a labor curve that more closely approximates that of younger women?

One hypothesis regarding the influence of maternal age on labor is the idea that the myometrium, in particular, is less effective and/or responsive to oxytocic agents or prostaglandins with age. Our work confirms findings that previously were published by Main et al19 in 2000 that characterized 8500 term, nuliparous births. This study reported not only increased length of second stage of labor in older parturients but described these increases as continuous rather than as a jump in labor length after a particular age cutoff. Adashek et al20 in 1993 and Treacy et al26 in 2006 and Main et al19 showed the increased use of oxytocin in older women, compared with younger women, and increased rates of cesarean delivery for labor dystocia. It is important to consider these findings with respect to physician willingness to induce or augment labor in older women, because the use of oxytocin and other agents of induction, combined with physician acknowledgement of a shallower slope to the labor curve in older women, may be 2 of the few modifiable factors that contribute to the increased intrapartum cesarean delivery rate in this cohort.

Regarding induced and augmented labors in particular, we chose to include those patients in our final analysis. In the multivariable model, we demonstrated that length of labor remains tied to maternal age even after controlling for the use of labor induction. However, we can also see in the subgroup analyses for nuliparous women that, in looking at induced labors separately from labors that progressed without the use of pitocin or prostaglandins, significant differences across age groups are eliminated in the first stage of labor and persist only in the second stage for women who undergo induction. This implies that, although there are limitations to the impact of oxytocin and other agents in the face of an apparently strong influence of age on progression in the second stage of labor, the impact of maternal age on first-stage length may be ameliorated by the use of such agents. Thus, despite the fact that the examination of induced labors may not contribute directly to the objective of redefining the normal labor curve for older women, it points to the need to further characterize the effects of induction agents on labor across the age spectrum.

Future studies in this area may aim to determine more precisely the contractility of older vs younger myometrial tissues and the action of oxytocin and prostaglandins on these tissues. Although there is a paucity of published investigation into the effects of age on the myometrium in particular, there is evidence that skeletal muscular strength declines with age, even among women of reproductive age. One can hypothesize that this decrease in muscle strength contributes to a more prolonged pushing effort in older women in the second stage of labor.27 Another hypothetic influence on changes in maternal pushing efficacy with age may be rooted in a differential response to pain. At least 1 group of investigators has demonstrated differences in pain thresholds that are associated with age, and even that pressure pain thresholds decrease relative to other types of pain thresholds as the age of study subjects increases.28

Our study is not without limitations. We attempted to control for many identifiable confounders using multivariable regression models. However, there may be other confounders that we did not conceive of or include in our model. Another possible issue is that of generalizability, given that our population was drawn from a single institution. It may be that there are differences that were seen in our local population that may not exist in other populations. However, the population of women who were cared for at UCSF represents a wide variety of racial/ethnic groups, socioeconomic strata, and age groups. Another issue that is related to generalizability is that labor management was performed by 1 group of providers at a single institution. Although we controlled for augmentation of labor, we had no way to control for how aggressively such augmentation was used. Whether such management style should differ by maternal age is at the crux of this discussion, and we are limited in our ability to describe in detail the degree to which such strategies were used in different populations within our cohort.

Despite these limitations, the present analysis contributes to the growing body of evidence that supports multifactorial redefinition of labor curves. Based on our findings, we believe that future studies should examine labor norms with relation to important perinatal outcomes by maternal age, to determine whether varying cutoffs should be used to maximize maternal and neonatal health.

REFERENCES

The validity of cervical dilation as an indication of true labor between 32 and 36 weeks 6 days of gestation

Sangeeta Jain, MD; Angela Earhart, MD; Nicole Ruddock, MD; Tony Wen, MD; Gary D. V. Hankins, MD; George R. Saade, MD

OBJECTIVE: Cervical dilation with regular contraction traditionally has been used to differentiate between true and false labor. This diagnostic criterion has not been tested as most patients receive tocolytics. Our objective was to determine the time from admission to delivery in women with preterm contractions and advanced cervical dilation without tocolytics.

STUDY DESIGN: We reviewed the records of patients with preterm labor on the basis of regular contractions and cervical dilation ≥3 cm between 32 and 36 weeks 6 days of gestation. Chi-square analysis was performed for delivery at >1 week.

RESULTS: In the records, 68.8% of the patients remained pregnant at >1 week without tocolysis. Between 32 and 34 weeks of gestation, the use of tocolysis did not help to prolong pregnancy >1 week (81% vs 88%; \( \alpha = 0.05; \) power = 0.65).

CONCLUSION: Cervical dilation with preterm contraction cannot be used as an indication of true labor. More accurate methods to diagnose true preterm labor and direct management decisions are needed.

Key words: cervical dilation, delivery, preterm labor, tocolysis

Cite this article as: Jain S, Earhart A, Ruddock N, Wen T, Hankins GDV, Saade GR. The validity of cervical dilation as an indication of true labor between 32 and 36 weeks 6 days of gestation. Am J Obstet Gynecol 2007;197:431.e1-431.e3.

Traditionally, preterm labor has been defined as persistent uterine contractions that are accompanied by dilation and/or effacement of the cervix that is detected by digital examination. This definition was extrapolated from evidence at term at which time progressive cervical dilation is an indication of entry into the phases of true labor and used to differentiate it from false labor. In symptomatic women, the best clinical signs of preterm delivery within 1 week of presentation are said to be cervical dilation of ≥3 cm or effacement of ≥80%, vaginal bleeding, and ruptured membranes. However, the natural progression of cervical dilation in preterm gestations has not been studied adequately because most of these patients receive some form of tocolysis.

Preterm labor and its inhibition has been a major subject of research as early as 1970s. Most investigations of tocolytics have not included a placebo arm. The reported delays of delivery for 24 hours, 48 hours, and 1 week have been used by some investigators as evidence of tocolytic effectiveness. However, without knowledge of how many women would have delivered within these limits if no tocolytics were used, the efficacy of tocolysis remains questionable.

The aim of tocolysis is to improve perinatal outcome, but at times, at the cost of adverse maternal and neonatal effects. The critical point is the gestational age at which the adverse effects outweigh the benefit. A recent study has shown that tocolysis that is used after 32 weeks of gestation does not reduce the duration of neonatal hospital stay. Based on the lack of clear evidence of benefit at >32 weeks of gestation, several providers at our center stopped using tocolysis in this group of patients. After this change, we started noticing that several patients with advanced cervical dilation remained undelivered for unusually long periods. Hence, a less aggressive use of tocolysis after 32 weeks of gestation allowed us to observe the progression of cervical dilation without the confounding effects of tocolytics. We therefore hypothesized that cervical dilation in preterm patients does not have the validity to predict preterm labor.

The aim of this retrospective review was to determine the time from admission to delivery in women with preterm contractions and advanced cervical dilation (≥3 cm) at 32 weeks to 36 weeks 6 days of gestational age. Because some of the providers in our center continued to use tocolysis up to 34 weeks of gestation, we also aimed to compare the admission to delivery time in preterm labor at 32-34 weeks gestation with and without the use of tocolysis.

METHODS

After we obtained approval from the Institutional Review Board, records of pregnant women at the John Sealy Hospital labor and delivery suite from January 1, 2003, to June 30, 2006, with preterm contractions were reviewed. Routinely, patients in triage with preterm contractions are evaluated by the monitoring of fetal heart rate, frequency of contractions, and cervical dilation (manually). Oral and intravenous hydration and narcotics for pain relief are
administered as needed. If contractions persist and on reexamination a cervical change is demonstrated, the patient is admitted for preterm labor. All patients >18 years old with singleton or twin pregnancy between 32 weeks and 36 weeks 6 days gestational age, with regular contractions (10/hr) and progressive cervical dilation to ≥3 cm during the observation were included in the study. For patients with 3 cm cervical dilation, only those whose cervix was 80% effaced were included, thereby excluding patients whose cervix may have been dilated to 3 cm at baseline. Subjects who were suspected of having cervical incompetence, premature ruptured membranes, presence of an acute event that necessitated urgent delivery (such as severe pre-eclampsia, chorioamnionitis, and hemorrhage), presence of fetal anomalies or any surgery done on the cervix (such as conization, loop electrosurgical excision procedure, cerclage) were excluded from the study.

**Statistical analysis**

The primary outcome was delivery within 1 week vs >1 week after admission. Chi-squared and Mann-Whitney rank sum tests were used for statistical analysis as appropriate. A probability value of <.05 was considered significant.

### Results

A total of 153 patients met the inclusion criteria during our study period. Of these, 26 patients received tocolysis, and 127 patients did not. Delivery data were not available for 5 subjects, all of whom were in the no tocolysis group, which left 122 patients in the final data analysis.

Table 1 shows the demographic data, pregnancy outcomes, and potential confounding factors of the 122 patients who did not receive tocolysis. Of these, 84 patients (68.8%; 95% CI, 60%-77%) remained pregnant beyond 1 week.

In the subset of 69 patients between 32 and 34 weeks of gestation, 26 patients received tocolysis, and 43 patients were treated expectantly. Both the groups received intravenous hydration, analgesia, and treatment of any infections that were detected. Expectant treatment included either stay in hospital or domicile, which is a dormitory for pregnant women that is located close to the hospital. Table 2 shows demographic data and admission-

---

**Table 1**

Demographic profile of patients between 32 weeks and 36 weeks 6 days of gestation without tocolysis

<table>
<thead>
<tr>
<th>Demographic</th>
<th>Delivery at ≤1 week (n = 38)</th>
<th>Delivery at &gt;1 week (n = 84)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>25 (18-40)</td>
<td>24 (18-43)</td>
<td>NS</td>
</tr>
<tr>
<td>Ethnicity (n)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>7 (18.4%)</td>
<td>20 (24%)</td>
<td></td>
</tr>
<tr>
<td>African American</td>
<td>6 (15.8%)</td>
<td>9 (10.7%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>25 (65.7%)</td>
<td>54 (64.2%)</td>
<td></td>
</tr>
<tr>
<td>Others</td>
<td>0</td>
<td>1 (1.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Gravidity (n)*</td>
<td>2 (1-7)</td>
<td>3 (1-8)</td>
<td>NS</td>
</tr>
<tr>
<td>Parity (n)*</td>
<td>1 (0-6)</td>
<td>1 (0-5)</td>
<td>NS</td>
</tr>
<tr>
<td>Previous preterm delivery (n)</td>
<td>12 (31.6%)</td>
<td>21 (25%)</td>
<td>NS</td>
</tr>
<tr>
<td>Bacterial vaginosis (n)</td>
<td>8 (21%)</td>
<td>22 (26.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Urinary tract infection (n)</td>
<td>16 (42%)</td>
<td>25 (29.8%)</td>
<td>NS</td>
</tr>
<tr>
<td>Group B beta streptococci+ (n)</td>
<td>11 (28.9%)</td>
<td>22 (26.2%)</td>
<td>NS</td>
</tr>
<tr>
<td>Chlamydia: present/past (n)</td>
<td>7 (18.4%)</td>
<td>17 (20.2%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

* Data are given as median (range).

**Table 2**

Demographic profile and admission-to-delivery time in the group of patients between 32 and 34 weeks of gestation, comparing with and without tocolysis

<table>
<thead>
<tr>
<th>Variable</th>
<th>With tocolysis (n = 26)</th>
<th>Without tocolysis (n = 43)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (y)*</td>
<td>24 (18-38)</td>
<td>23 (18-39)</td>
<td>NS</td>
</tr>
<tr>
<td>Gravidity (n)*</td>
<td>2 (1-6)</td>
<td>2 (1-6)</td>
<td>NS</td>
</tr>
<tr>
<td>Parity (n)*</td>
<td>1 (0-4)</td>
<td>1 (0-4)</td>
<td>NS</td>
</tr>
<tr>
<td>History of preterm delivery (n)</td>
<td>7 (26.9%)</td>
<td>9 (20.9%)</td>
<td>NS</td>
</tr>
<tr>
<td>Mean cervical dilation (cm)</td>
<td>4</td>
<td>4</td>
<td>NS</td>
</tr>
<tr>
<td>Mean cervical effacement (%)</td>
<td>75</td>
<td>65</td>
<td>NS</td>
</tr>
<tr>
<td>Effacement &gt;75% (n)</td>
<td>13</td>
<td>12</td>
<td>NS</td>
</tr>
<tr>
<td>Twin pregnancies (n)</td>
<td>7 (27%)</td>
<td>10 (24%)</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery within 48 hours of admission (n)</td>
<td>3 (11.5%)</td>
<td>2 (4.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery ≤1 week (n)</td>
<td>5 (19.2%)</td>
<td>5 (11.6%)</td>
<td>NS</td>
</tr>
<tr>
<td>Delivery at &gt;1 week (n)</td>
<td>21 (80.8%)</td>
<td>38 (88.4%)</td>
<td>NS</td>
</tr>
</tbody>
</table>

NS, not significant.

* Data are given as median (range).
to-delivery time. There is no significant difference in the number of patients who remained pregnant >1 week in the tocolysis group (81%; 95% CI, 61%-93%) and the expectant treatment group (88%; 95% CI, 75%-96%). When twin pregnancies were compared in each group, there was no significant difference in the patients who remained pregnant >1 week (86% vs 80%).

**COMMENT**

We found that 68% (95% CI, 60%-77%) of the patients who were admitted with preterm labor and advanced cervical dilation remained pregnant for at least 1 week, despite expectant treatment. We therefore conclude that, unlike at term, cervical dilation in preterm gestation is not a good indicator of true labor, because most women with progressive cervical dilation do not deliver within 1 week. Any perceived benefit of tocolysis at 32-34 weeks of gestation and most cases of “tocolysis success” are likely due to patients who would have become quiescent, even without tocolysis. This also points to a huge health cost of keeping these patients in the hospital or at nearby facilities until delivery. It would be interesting to make multiple comparisons to see how many patients delivered within 48 hours and how many patients went to term, but our numbers were limited. Even though these results cannot be translated into general practice, because this was a retrospective study, it certainly does inspire us to look further into the pathophysiologic condition of preterm labor, because the known clinical markers, frequency of contractions and cervical dilation, are not useful to predict timing of delivery.

Studies that demonstrated the use of aggressive tocolysis for preterm labor with advanced cervical dilation with a 50%-75% success in achieving a 72-hour delay and a 35% success in 1 week’s delay in pregnancies with cervical dilation of >3 cm have been reported. They also report a greater neonatal morbidity in the advanced cervical dilation group, despite tocolysis. However, they did not have a control group for comparison and did not address the risk of delivery in the late preterm patient with advanced cervical dilation. They did acknowledge the large false-positive rate for diagnosis of “actual” preterm labor, even in patients with documented cervical change.

Gyetvai et al,9 in their metaanalysis, included 18 randomized controlled trials that compared the effect of a tocolytic with a placebo in women in preterm labor. Tocolytics prolonged pregnancy but were associated with maternal side-effects that necessitated the discontinuation of tocolysis and no improvement in perinatal outcome. Delaying delivery after 32 weeks of gestation should be aimed at improving neonatal outcome, and any potential delay from tocolysis should be put to good use. Other than corticosteroid administration between 32 and 34 weeks of gestation and group B beta streptococci prophylaxis, there are no interventions that have been shown to improve neonatal outcome. However, if the majority of patients, even those with advanced cervical dilation, remain pregnant for a period of time that will allow for these beneficial interventions, then the benefit of tocolysis is not apparent.

Our study was a retrospective review, with the shortcoming of a possible selection bias. Information regarding the exact contraction frequency, transvaginal cervical lengths, fetal fibronectin test, and maternal requirement of pain medications could not be compared. Cervical length measurements to rule out preterm labor and fetal fibronectin are not done at our institution. We cannot exclude a selection bias in the use of tocolysis. Therefore, we cannot comment conclusively on the efficacy of tocolytics, because this would be best addressed through a randomized placebo-controlled trial. We also accept that our sample size for the comparison between tocolysis and no tocolysis was small. However, the proportion of patients who delivered within 1 week in the no tocolysis group was low, which implies that 882 women would have been needed in each group to show a one-third reduction in the proportion of women who delivered within 1 week with tocolysis with an 80% power at a 5% type I error. These numbers should be included in future consideration for trials that involve patients between 32 and 34 weeks of gestation with advanced cervical dilation. Despite this limitation, the strength of our study is the large sample size of women with advanced cervical dilation who were not treated with tocolytics. Our results that relate to the proportion of women who remained undelivered after 1 week in this group are robust, as evidenced by the relatively tight 95% confidence index, particularly in the 32-34 weeks of gestation group.

Our study highlights another important issue in the management of preterm labor. Future research that aims at reducing the prevalence of preterm labor and improving the understanding of preterm labor at the molecular level is needed so that novel pharmacologic methods for prevention can be instituted.

**REFERENCES**

Value of HPV testing in follow-up of treated high-risk CIN1: a study by Alonso et al

Premal Thaker, MD; George A. Macones, MD, MSCE, Associate Editor

In the roundtable that follows, clinicians discuss a study published in this issue of the Journal in light of its methodology, relevance to practice, and implications for future research. Article discussed:


DISCUSSION QUESTIONS

■ Can you summarize the objectives of the study?
■ How would you characterize the design of the study?
■ Were the study objectives met?
■ When is the receiver operating characteristic curve useful?
■ What are the strengths and weaknesses of the stepwise method used in the multivariate models?
■ Can you comment on choice of regression model?
■ What are the strengths and weaknesses of the study?
■ Do you think that study design and limitations influenced the results?

■ Can you summarize the key findings in Tables 1 through 4?
■ What are the conclusions of the study?
■ How does this study help us clinically?
■ Will the data change your practice management and if so, how?
■ What future studies would you suggest in the management of low-grade squamous intraepithelial lesions?

Introduction over 50 years ago, cervical cytologic screening is considered to be one of the greatest success stories in cancer screening. However, women continue to die from the disease, particularly in poor countries where testing and treatment are not easily obtained.1 In 2002, nearly 500,000 women around the globe were diagnosed with cervical cancer, and in that year, it was the third leading cause of cancer death among women worldwide.2 This year, an estimated 11,150 women in the United States will be diagnosed with cervical cancer, and 3,670 women will succumb to the disease.3

Even so, optimism is warranted. The correlation between high-risk human papillomavirus and the development of cervical cancer is now well-understood. Much is also known about the progressive steps of dysplasia prior to cancer. HPV typing for high-risk forms of the virus can be performed through detection of viral DNA in liquid cytology.

Typically, research has focused on treatment of cervical intraepithelial neoplasia (CIN) 2/3, since it has a significant risk of progressing to cancer. Alonso and colleagues examine high-risk HPV testing in a prospective cohort of patients who have a CIN 1/low-grade squamous intraepithelial lesion (L-SIL) and who have the following characteristics: unsatisfactory colposcopy, positive endocervical curettage, age over 40, or persistence of CIN1/L-GSIL or high-risk HPV infection for over 2 years.

A QUESTION OF RELEVANCE

It is important to understand the significance of the clinical question being proposed and the clinical outcome. More than 1 million women are diagnosed with CIN1/L-GSIL in the United States each year. In most cases, these patients will clear the infection and will not develop progressive cervical dysplasia. Only persistent infections will progress to CIN3 and perhaps, to cervical cancer. The probability of a patient with biopsy-proven CIN1 progressing to high-grade...
dysplasia is low; approximately 9-16%.\textsuperscript{4,5} Furthermore, we know that in the absence of immunosuppression or other host-specific risk factors, further progression from severe dysplasia to invasive cancer takes several years, providing time to intervene.

Prior studies suggest that when a loop electrosurgical excision procedure is performed for patients with L-SIL and biopsy-proven CIN1, the chance of discovering a high-grade histologic lesion is approximately 10%. The 77 patients studied by Alonso and colleagues all had low-grade dysplasia in addition to risk factors for persistent HPV infection. Among these women, the incidence of CIN2/3 was 22%, and in this subgroup, only 24% went on to persistent or progressive disease. The finding indicates that only a very small percentage of patients with low-grade dysplasia would benefit from pre-treatment prognostic testing for high-risk HPV and an excisional procedure.

Still, Journal Club members felt that screening for high-risk HPV subtypes with quantitative methods will eventually become a standard of care. In the meantime, a more stringent approach to treatment, such as that proposed by Alonso et al for women with high pre-treatment HR-HPV loads, might one day be beneficial in a population at high risk for poor compliance with follow-up. Construction of an effective strategy for this group, though, would require high throughput analyses with rapid turn-around of results, and this is not yet readily available.

**Step by Step**

Understanding statistics is critical to assuring the validity of the results. In this session of the Journal Club, the strengths and limitations of stepwise regression were examined. The final covariate set in the stepwise model is determined automatically through a series of tests. Usually, investigators use a statistics program to select variables for inclusion based on preset criteria, such as a p-value. Forward selection adds variables one at a time, while backward elimination begins with all variables, subsequently deleting each one by one. A combination of these 2 methods can also be employed.

Use of stepwise regression methods is controversial. Proponents note that the method produces valid models, but critics state that it removes much of the thinking or investigator judgment from the process of building an explanatory model. In many cases, a stepwise analysis is driven by the presets in a statistical program rather than the careful considerations of the investigative team. Additional concerns include potential problems spurred by collinear variables and artificially-narrow confidence intervals. When stepwise regression is used, authors should present pertinent details of the model, including the technique used for including and retaining variables and how variables were selected for inclusion and retention and whether that model is based on forward or backward stepwise regression.

**Future Directions**

Our understanding of the genetic and immunologic factors that influence host response to HPV infection is continually evolving. A longitudinal cohort study with molecular and immunologic characterization, as well as HPV subtype analysis, might supply further details of disease biology, allowing us to improve surveillance programs and fine-tune interventions for women with mild cytologic abnormalities. Ideally, we have to develop biomarkers and technologic advances that would also benefit the population beyond our borders; a worldwide population in critical need.

**References**

A diagnostic challenge

Nadia Kabli, MD; Jocelyne Arseneau, MD; Togas Tulandi, MD, MHCM

CASE NOTES

A 43 year-old woman with uterine fibroids, an ovarian cyst, and increasing menorrhagia, pelvic pressure, and pain was scheduled to undergo a laparoscopic hysterectomy and left salpingo-oophorectomy. At laparoscopy, the uterus was enlarged with fibroids, and the left ovary contained a cyst with a smooth surface. The omentum was vascular and thick, covered by numerous structures of variable sizes (Figure 1). These structures were also found in the posterior cul-de-sac (Figure 2) and under the diaphragm (Figure 3).

Laparoscopy provided a view of the distorted omentum.


CONCLUSIONS

Because disseminated malignancy was suspected, biopsies were taken from the peritoneal and omental lesions. Definitive surgery was postponed for a later date. A histopathologic examination revealed multiple small nodules of mature smooth muscle surrounded by omental fat (Figure 4). The findings were consistent with leiomyomatosis peritonealis disseminata. No pulmonary, hepatic, or retroperitoneal nodules were found on computed tomography scan. Final histology confirmed the diagnosis of leiomyomatosis, uterine fibroids, and an ovarian dermoid cyst.

Leiomyomatosis peritonealis disseminata is a rare condition characterized by the presence of multiple nodules on the peritoneum, omentum, and bowel serosa. It mimics metastatic cancer but is benign. Cases mainly occur in women of reproductive age, and the disorder is usually diagnosed during a laparotomy. Metastatic ovarian cancer, peritoneal carcinomatosis, benign metastasizing leiomyoma, and intravenous leiomyomatosis are included in the differential diagnosis.

Multicentric development of leiomyomata has been suggested, though no defin-
itive etiology has been identified. The characteristic masses are estrogen-dependent and contain estrogen and progesterone receptors. Our patient subsequently underwent abdominal hysterectomy and bilateral salpingo-oophorectomy. She has been well ever since.

---

**FIGURE 4**

Small nodules of smooth muscle surrounded by omental fat were seen on histopathologic examination.
The new extended regimen for the management of premenstrual symptoms needs to be properly assessed

TO THE EDITORS: Coffee et al1 deserve credit for their study to assess the incidence and severity of premenstrual-type symptoms in patients converted from a 21/7 oral contraceptive (OC) regimen to an extended regimen. Their conclusion that an extended 168 day regimen of drospirenone and ethinyl estradiol (DRSP/EE) led to a decrease in premenstrual symptoms, compared with the 21/7 day regimen, was striking. But there are a few drawbacks of the study that need consideration.

The division of patients into high cyclic variables and low cyclic variables begs further clarification. Moreover, only the patients with high cyclic variability showed statistically significant improvement in premenstrual symptoms, a fact about which the gynecologists and general practitioners might make unhappy. With increased duration of the extended regimen of DRSP/EE OC, there had been a significant progressive reduction of premenstrual-type symptoms leaving the clinician with a question: “For how long can we continue with the extended regimen?” So further studies are essential to see the effects of the extended use of DRSP/EE in humans. The single-item Scott and White Mood Scale (0–10) was significantly correlated to all 17 elements of the Penn Daily Symptom Report with Spearman R correlation coefficient, a fact that has a beneficial effect on further studies in this arena.

The study subjects seem to have a poor representation, and this is evident from the fact that patients entering on a 19-nortestosterone–containing OC had been longer-term users of it than those entering on the newer DRSP/EE OC, and this may lead to type II error2 and may limit the generalizability of the study to a larger population. Further clarification on whether the study subjects were aware of the different treatment regimens they received is appreciated. Although drospirenone with its antimineralocorticoid effect was found to improve symptoms of premenstrual syndrome (PMS) and premenstrual dysphoric disorder,3 the new regimen and new drug itself might cause the study participants to respond in a different way favoring the new regimen.

A sympathetic approach based on counseling, practical advice, and reassurance have also to be considered in managing this cumbersome condition.4

In conclusion, the work of Coffee et al1 adds valuable information to our existing body of knowledge on the management of PMS. But we need to bear in mind the specific subset of patients to which the conclusions reported are related.

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REPLY

Thank you for your interest in our study. One of the study goals was to investigate symptoms encountered by women using oral contraceptives and document improvements that would positively affect quality of life. Study participants were variable for both the types of symptoms and the ranges of severity of scores within symptoms related to different points of the standard 21/7 pattern of oral contraceptive (OC) use. In this analysis of premenstrual symptoms, as with other analyses of different symptoms,1,2 we chose the division of high and low cyclic variability because that would allow us to relate potential improvements in quality of life to those most affected, compared with those least affected.

As discussed in the manuscript, some women did not show much cyclic variability and, overall, did not show much benefit. This supports the premise that continuous oral contraceptives prevent hormonal fluctuations and will have a benefit in women whose symptoms are related to cyclic hormonal changes. Those women who have high mood scores throughout the entire 21/7 cycle but do not have cyclic fluctuations may have underlying depressive symptoms and may require an antidepressant to treat their endogenous depression that is not associated with hormones. This does not preclude the utility of a continuous regimen, because these women may benefit from...
a reduction in bleeding, menstrually related headaches, and other symptoms.

Our study did show greater improvement over time. Whereas this study was limited to 24 weeks of continuous oral contraceptives, we are publishing the 1-year data set on continuous use, which shows the improvement seen at the end of 24 weeks is sustained for an entire year. In our clinical practice, we have had patients on continuous regimens for years, and a continuous OC without a placebo interval has recently received US approval.

Patients were self-selected with the requirement of use of OCs for a minimum of 3 months. This trial was not blinded, and a placebo response to some extent cannot be ruled out. Regardless of which pill patients used when they entered the study, there was an increase in symptoms before and during the hormone-free interval of the 21/7 phase of the cycle. This pattern continued as patients were converted to the 21/7 regimen of the study OC, and improvements in symptoms were seen with the conversion from 21/7 of study OC to the extended regimen of study OC. Further studies are needed to assess the effects of continuous regimens on common premenstrual and menstrual complaints that often lead to noncompliance and discontinuation.

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Repeate antenatal steroid trial methodology

TO THE EDITOR: The recent randomized controlled trial of antenatal corticosteroids raises 2 important methodological issues.

The pregnancy was used as the unit of analysis. A multiple pregnancy was counted as having an outcome if either or both twins had it, which is equivalent to taking the worst outcome from each pregnancy.

There are several problems with this approach. First, the denominator used is the number of women, not the number of babies, so the incidence of outcomes will be overestimated. This may affect the risk ratio estimate and its confidence interval. Second, it is not possible to tell from the information in Table 3 how many of the whole sample of 596 babies had any of the outcomes. For example, in the repeat corticosteroids group, 20 primary outcomes are reported among 250 pregnancies. If these all occurred in twin pregnancies, there could have been 40 babies who had the primary outcome. At the other extreme, these could have occurred in 20 single pregnancies. Systematic reviews will require accurate information on the numbers of infants with outcomes, which are not available in the paper. Finally, and most importantly, analysis by pregnancy can be misleading. Depending on the distribution of outcomes among single and multiple pregnancies, substantial bias can be introduced into the risk ratio estimate.

Analyzing outcomes among the whole sample of babies gives an unbiased risk ratio estimate, and its confidence interval can be adjusted to take account of nonindependence between babies from the same pregnancy by standard methods for cluster randomised trials. The second issue relates to the analysis of babies born at less than 32 weeks. This is a subgroup analysis classified by a variable (gestational age at birth) not known at randomization. Such subgroup analyses are known to be potentially highly misleading because there may be differences between the populations with and without an outcome (delivery before or after 32 weeks), which will confound the comparison of the subgroups. For example, there may be differences in gestational age at recruitment or underlying maternal pathology that could cause the apparent difference in outcome. The conclusion that effectiveness may be greater if delivery is before 32 weeks may therefore be unsound. A simple analytical strategy to avoid this bias has been suggested by Rochon and such an analysis should be performed before any conclusions about the effects of the timing of birth are drawn.

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Genetic epidemiologic studies of preterm birth: studies of disease or of “rescue by birth”? 

TO THE EDITORS: The PREBIC Genetics Working Group recently published guidelines for research into the genetic epidemiology of preterm birth (PTB) in the American Journal of Obstetrics & Gynecology. They emphasized the importance of selecting a suitable control group for such genetic association studies. Although we agree with many of the author’s assertions, their recommendation to use only uncomplicated term deliveries as controls is overly simplistic. Many cases of spontaneous PTB represent a healthy adaptive response of the fetus to escape a hostile intrauterine environment resulting, for example, from intrauterine infection/inflammation, hemorrhage, or placental vascular compromise with nutritional and oxygen deprivation. The molecular mechanisms responsible for this “rescue by birth” response are not completely understood, but likely involve the fetal inflammatory cascade leading to fetal maturation, degeneration of the fetal membranes, and uterine contractions.

This response would be evolutionarily beneficial to both the fetus and mother. Because the genes facilitating it are likely equally present among those rescued and healthy term deliveries, the comparison with such controls may confound genetic association studies. Other term deliveries may also be abnormal because of a failure to trigger the protective rescue-by-birth response in an appropriate setting. Even in the presence of a strong proinflammatory event, not all pregnancies initiate labor spontaneously. A failure of the fetoplacental unit to trigger the rescue-by-birth response in such a setting will likely lead to further fetal compromise or stillbirth unless such fetuses are salvaged by obstetric intervention.

A genetic comparison of pregnancies that survived an intrauterine insult by rescue by birth (spontaneous PTB) with those that failed to trigger this protective mechanism (antepartum stillbirths) may be more informative than the comparison with healthy term deliveries. Similarly, some fetuses fail to trigger parturition in a timely fashion leading to postterm pregnancy, which is associated with increased perinatal morbidity and mortality. The high recurrence rate of postterm pregnancy and the observation that fetuses born postterm (longer than 42 weeks) are at increased risk of unexplained death in the first year of life suggest that genetic factors are involved. Incorporation of an additional control group of postterm pregnancies is therefore likely to improve our understanding of the genetics of parturition, both at term and preterm.
REPLY

We would like to thank Drs Froen, Pinar, and Norwitz for their comments on the recently published guidelines for research into the genetic epidemiology of preterm birth (PTB). PTB represents a complex heterogeneous phenotype: its etiology is likely to be multifactorial and involve both genetic and environmental factors. We agree with the authors that at least some cases of PTB may represent an appropriate attempt to “rescue by birth” through activation of a normal, adaptive mechanism; however, the first essential requirement to conduct genetic association studies to decipher a complex disease is the development of a standardized phenotype. How do we classify the phenotype of those “rescued by birth”? How do we differentiate this from idiopathic spontaneous PTB? Are all cases of spontaneous PTB the “rescued by birth” phenotype, or is it only a subset of spontaneous PTB? We believe that it is only by accurate description of the phenotype, ideally utilizing the optimal data set described in our review, that these and many other important questions relating to the etiology of PTB will be able to be addressed in genetic epidemiologic studies of PTB.

The authors question the validity of our recommendation to use uncomplicated term deliveries (a heterogeneous control population) as controls for genetic epidemiologic studies of PTB. The use of unselected population-based controls as a reference to a disease cohort is a routine approach in the study of common complex diseases. Although the presence of individuals with the risk genotype in the control group (eg, those with a failure to trigger the protective rescue-by-birth response) will serve to dilute any potential genetic association, it is unlikely that this effect will be significant because the expected prevalence of these individuals in the control group is likely to be low.

The authors suggest that a series of antepartum stillbirths or postterm deliveries may be a better control population than uncomplicated term deliveries. Antepartum stillbirth is a hugely heterogeneous phenotype with little evidence of a genetic basis. Similarly, postterm delivery is a heterogeneous phenotype, and it is likely that a different suite of genes controls postterm delivery, compared with preterm delivery. Although comparing PTB to antepartum stillbirths or postterm deliveries may offer some insight into the genetic basis for PTB, the International PREterm BiRth Collaborative (PREBIC) Genetics Working Group believes that uncomplicated term deliveries represent the best compromise when defining the control group for genetic epidemiologic studies of PTB.

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Reliability of a preventability model in maternal death and morbidity needs further assessment

TO THE EDITOR: Today we witness a heightened awareness of the global problem of maternal morbidity and mortality. From multiple sources, efforts are being made to reduce this global toll. The work by Geller et al 1 merits appreciation because it seems to have successfully measured the reliability of a model that defines preventability in maternal morbidity and mortality with high agreement between the 2 independent groups of medical experts who identified potentially preventable system errors.

But certain issues beg emphasis and clarification. Personnel factors seem to be overlooked by the investigators. They are found to be significant and sometimes critical when considering issues of decreasing maternal morbidity and mortality. Domestic violence, homicide, road traffic accidents, neoplasia unaffected by the presence of pregnancy, and suicide are some of these factors that need deep inquiry. 2

Domestic violence (physical and mental) is now a major concern to the pregnant women younger than 18 years of age. This necessitates every woman to be interviewed at least once, on a one-on-one basis and inquiries about violence are included in the social history. 3

Some religious groups with good general health but refused all modern medical care exhibited elevated maternal mortality rates. 4 The importance of maternal age, parity, ethnicity, cultural norms, and socioeconomic status should not be overlooked. 2 The fact that only 4 factors of 10 potentially preventable system errors (diagnosis/recognition of high risk, referral, policies and procedures, and documentation) had high proportion of agreement and statistically significant kappa values between the 2 independent groups of medical experts merits further studies in this arena with a larger sample size. Nevertheless, this study adds valuable information to our knowledge on potentially preventable system errors that lead to maternal morbidity and mortality.

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We thank Marasinghe et al for their letter and positive comments regarding our analysis in the article “Reliability of a preventability model in maternal death and morbidity.” 1 As stated in the article, we found that patient factors played a relatively small role in the overall issue of preventability. Therefore, we chose to report on provider and system preventable factors, which had a much greater impact on the progression of illness. In a previously published analysis, we found that provider factors were present in 90% of the morbidities and deaths we studied, whereas patient factors occurred in 13-20%. 2 Furthermore, we found that these patient factors did not contribute to the progression of maternal morbidity after controlling for sociodemographic characteristics. Revisiting the data collected for the validation study, we did find 88% agreement between the 2 review groups on the presence of patient factors (kappa = 0.61; P < .0001), indicating substantial agreement. 3

In our sample, the most common patient factor identified was noncompliance with medical advice (7% of all cases). Failure to seek care (3%) and inadequate prenatal care (4%) were also noted. Substance abuse (5% of all cases) was also an issue in our population. We agree that violence against women deserves further attention, although in our sample there were no reported cases. More research is clearly needed to characterize all preventable causes of maternal morbidity and mortality. Studies performed among different populations and with larger sample sizes is the first step in reducing maternal illness and death.

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Elevated uterine activity increases the risk of fetal acidosis at birth

TO THE EDITOR: I commend the authors of the recent article “Elevated uterine activity increases the risk of fetal acidosis at birth” by Bakker et al,1 which looks at the intrauterine pressure recordings from the last 60 minutes of the first stage of labor and the entire length of the second stage of labor (33-37 minutes) comparing 77 fetuses with an umbilical arterial pH 7.11 or less to 1356 fetuses with pH greater than 7.12. They found that as uterine activity increases, the umbilical arterial pH decreases. They point out that neither the British2 nor American3 guidelines for interpretation of electronic fetal monitoring mention using uterine activity at all. The authors hope that adequate reading and interpretation of the uterine contraction curve can be a solution to the fetal heart rate (FHR) monitoring debate and reduce the number of unnecessary obstetric interventions.

The primary finding of this study is not surprising. As a contraction occurs, blood flow to the intervillous space is decreased, which is the basis of the contraction stress test (CST). A compromised fetus may not show evidence of hypoxia-ischemia, such as late decelerations, if there are too few contractions during the CST; and even a normally oxygenated fetus will show late decelerations if there are too many contractions, which is why, to have an interpretable CST, we aim to have 3 contractions within 10 minutes.4 In the interpretation of CSTs, the FHR response to the contraction is more clinically useful than an analysis of the contractions.

In the Comment section, the authors state that they did not perform an extensive analysis of the FHR pattern; however, a subanalysis of decelerations was performed. They report that they found no significant difference in decelerations between the 2 groups, although these data are not given in the paper. Without an analysis of the FHR changes, this paper does not prove that uterine contractions are an independent indicator of intrapartum hypoxia-ischemia. Longstanding evidence indicates that uterine activity is linked to fetal neurologic injury through FHR changes, not as a stand-alone sole predictor.5 Based on our experience with the CST, it seems that focusing solely on the uterine contraction pattern would be even less likely to identify intrapartum hypoxia-ischemia than analysis of the FHR patterns have been.6 Maybe by combining the contraction readings with FHR changes, we may be better able to identify intrapartum hypoxia-ischemia, but when used alone, I do not expect the uterine contraction readings to enhance the usefulness of electronic fetal monitoring.

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REPLY

In his comment, Dr Graham suggests that contraction readings together with fetal heart rate (FHR) changes are a better tool to identify intrapartum hypoxia-ischemia than the reading of the uterine contraction curve alone. We fully agree.

The purpose of our study was to determine how uterine activity affects fetal outcome. The ultimate goal of the paper was to draw attention to the identification of the fetus at risk for intrapartum hypoxia-ischemia on the basis of excessive...
uterine activity and certainly not to replace a tool that is already proven useful to assess fetal hypoxia-ischemia (ie, the FHR trace).

Our study results provide convincing evidence that next to adequate reading and interpretation of the FHR trace, contraction monitoring deserves full attention as well. There is no doubt that uterine activity is among the factors influencing the fetal condition and the FHR pattern. Awareness of the contraction pattern by the obstetrician and adequate reaction to the various patterns (eg, stop oxytocin infusion in case of tachystole/hypertonia) can prevent occurrence of unnecessary intrapartum fetal hypoxia-ischemia and in this way avoid the associated changes in the FHR pattern.

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Discussion: ‘Value of HPV testing in follow-up of treated high-risk CIN1’ by Alonso et al

In the roundtable that follows, clinicians discuss a study published in this issue of the Journal in light of its methodology, relevance to practice, and implications for future research. Article discussed:


DISCUSSION QUESTIONS

- Can you summarize the objectives of the study?
- How would you characterize the design of the study?
- Were the study objectives met?
- When is the receiver operating characteristic curve useful?
- What are the strengths and weaknesses of the stepwise method used in the multivariate models?
- Can you comment on choice of regression model?
- What are the strengths and weaknesses of the study?
- Do you think that study design and limitations influenced the results?
- Can you summarize the key findings in Tables 1 through 4?
- What are the conclusions of the study?

INTRODUCTION

Cervical cytologic screening is one of the greatest success stories in cancer screening. However, women continue to die from the disease, particularly in poor countries where testing and treatment are not easily obtained. In 2002, cervical cancer was the third leading cause of cancer death among women worldwide. This year, an estimated 11,150 women in the United States will be diagnosed with cervical cancer, and 3,670 women will succumb to the disease. While research has typically focused on treatment of cervical intraepithelial neoplasia (CIN) grade 2/3, Alonso and colleagues chose to study patients with a CIN1/low-grade squamous intraepithelial lesion (L-SIL).

George A. Macones, MD

STUDY OBJECTIVES AND DESIGN

Thaker: Preinvasive diseases of the cervix are extremely prevalent around the world. In many countries, successful screening and early treatment have allowed for a reduction in the incidence of invasive cervical cancer. Still, there are several unanswered questions about our care for women with preinvasive disease. One major question is how best to follow women who have had treatment for a preinvasive lesion. The answer clearly lies in understanding which women will have recurrent lesions or have progression of their disease. These questions are the focus of a new paper by Alonso and colleagues.

Thaker: Can you summarize the objectives of the study?

Kizer: The objective of this study was to evaluate the results of post-treatment follow-up in a cohort of women with CIN1/L-SIL. These women met the American Society for Colposcopy and Cervical Pathology (ASCCP) risk criteria for disease progression, based on a series of pre- and post treatment variables. Specifically, the usefulness of high-risk human papillomavirus (HR-HPV) testing as a follow-up tool was examined.

Thaker: How would you characterize the design of the study?

Kizer: The design of this study is best characterized as a prospective cohort study, which has several components. First, a specific group of subjects is identified. The participants are then followed over time. Potential predictor variables...
are measured at the beginning, and outcomes are subsequently ascertained.

In this study, the identified group consisted of 77 women with CIN1/L-SIL who were treated with a loop electrosurgical excision procedure (LEEP) in the Hospital Clinic of Barcelona’s Department of Obstetrics and Gynecology between March 1998 and February 2005. These women had unsatisfactory colposcopy; large lesions or positive endocervical curettage; persistent CIN1/L-SIL for longer than 2 years; or they were aged 40 years or older.

The authors then sought to identify factors (or exposures) within this group that might be associated with either residual/recurrent CIN1/L-SIL or progression to CIN2/3. The potential predictor variables (or exposures) were presence of HR-HPV and viral load pre- and post-treatment, cytology results, and involvement of surgical margins. Subjects were followed at 6, 12, 18, and 24 months.

This study is classified as prospective because all subjects and exposures were defined prior to any outcomes having occurred. This is not clearly stated within the paper, but inferences can be made by the strict methods described for performing colposcopy and HR-HPV testing, and for maintaining quality assurance of pathology reviews of cervical biopsies. In addition, all patients consented to participate prior to undergoing the LEEP. The prospective nature and the reasonable follow-up times are strengths of this study.

Thaker: Were the study objectives met?
Kizer: The objectives of this study do appear to have been met. Researchers successfully identified 52 women who fit the study criteria and had confirmed CIN1 on the LEEP specimen. All of these women were followed for an average of 12.6 months. In that time, 15 developed recurrent/residual disease and 2 progressed to CIN2/3.

Statistical Analyses
Thaker: The authors mentioned that they had generated receiver operating characteristic (ROC) curves for HR-HPV viral load and post-LEEP recurrence and residual disease. Can you describe the ROC curve and comment on when it is useful? What are the strengths and weaknesses of this approach?
Allsworth: ROC curves are important tools in the study of diagnostic accuracy. They plot the true positive rate (or sensitivity) on the vertical axis against the false positive rate (or 1-specificity) on the horizontal axis. This illustrates the tradeoff between increasing or decreasing sensitivity and specificity—and vice versa—as well as the accuracy of a test. The area under the curve or c-statistic is a measure of the discrimination of a test. That is, given randomly selected positive and negative test results, it is the probability that the positive test will have been assigned a higher score.

ROC curves are very appropriate for ascertaining a cutoffpoint for a continuously distributed predictor, such as HR-HPV viral load. The actual ROC curve was not presented in this paper, but the authors used a cutoffpoint of 100 relative light units. I assume this is based on the ROC curve findings, but since neither the ROC curve nor its findings were shown in the results, it is not possible to comment on it.

Thaker: Can you comment on the stepwise method used in the multivariate models? When is this useful? What are the strengths and weaknesses of this approach?
Allsworth: Stepwise regression models are those where the final covariate set is determined automatically through a series of tests; usually F-tests, but other tests are possible. Variables that meet preset criteria, such as a particular alpha level, are included or excluded from a model. Stepwise models can employ forward selection (adding variables one at a time), backward selection (the model starts with all variables, and these are deleted one by one), or a mixture of both forward and backward selection.

Stepwise regression techniques are considered controversial by many. While proponents of stepwise methods feel that they produce valid and parsimonious models, there has also been considerable criticism of them. Among the concerns about this method are that it excludes investigator judgment; it may have potential problems in the presence of collinear variables; and it might result in confidence intervals that are artificially narrow.

Careful model-building should consider statistical and data concerns, biologic plausibility, and clinical importance. Stepwise regression is basically an automated process, which takes some of the thinking out of model-building. I generally recommend that multivariate models be developed by someone with appropriate expertise.

If stepwise regression is used, it is generally helpful to include some details about this in the methods section. For example, was forward or backward selection used? How were variables chosen for inclusion and retention? Including some of this information can help readers to best judge the validity of the results.

Thaker: Can you comment on choice of regression model?
Allsworth: The authors used logistic regression to estimate associations between residual/recurrent disease and a positive cone margin, pre-treatment HR-HPV load, and post-treatment HR-HPV load. While using logistic regression to estimate an odds ratio as an approximation of the relative risk is common in cohort studies when the outcome is binary, it is important to check whether certain assumptions are met. Specifically, an odds ratio is a reasonable estimate of the relative risk when the prevalence of the outcome is rare (usually <10%). In a 1998 paper, Zhang and Yu illustrated that for common outcomes, the odds ratio is often an overestimate of the true relative risk.4

In the current study by Alonso et al, the prevalence of residual/recurrent disease is 28.6%—well above 10%. A number of approaches are available to researchers in this situation. First, they can apply a correction factor such as that described by Zhang and Yu. This correction is a simple calculation that takes into consideration the prevalence of the outcome. Using this correction for the odds ratios presented in Table 4, the odds ratios of 3.0, 1.27 and 1.72, represent relative risks of 1.92, 1.18, and 1.43 respectively. Second, researchers can use alternate regres-
sion approaches that directly estimate the relative risk, such as log binomial regression or Poisson regression with robust error variance.

**Thaker: What are the strengths and weaknesses of the study?**

**Nguyen:** This study’s strength is primarily based on the fact that it was a prospective study. The findings are interesting and certainly generate questions for future studies. I think this study demonstrates some of the difficulties with observational research. For example, not all of the patients were measured for pre- and post-LEEP viral loads. Likewise, it is not entirely clear whether all of the patients completed the appropriate amount of follow-up. I think these are the kinds of “real-life” issues that develop in clinical research—no study is going to have perfect follow-up, etc. These sorts of issues certainly make the analysis more difficult as well. If a patient does not have, for example, a pre-LEEP viral load done, then she should have been included in a multivariable model or excluded? Likewise, I think we need to be aware of whether or not there might have been some bias entered, based on which patients did and did not have pre- and post-LEEP viral loads. Even with some of these issues, I think the study adds to the body of literature on this subject and certainly sets the stage for future research.

There was some inconsistency in the numbers of subjects used for analysis in Table 2. For age, 52 subjects were included in the analysis, while 31 and 52 subjects were used in the analyses for pre-treatment HPV load and cone margins, respectively. I think this probably is related to the fact that not all subjects had all of the testing done, but it would be useful to know this for certain.

**Thaker: Do you think that study design and limitations impacted the results?**

**Nguyen:** We definitely need to consider limitations when interpreting a study. The numbers of subjects used in the analyses and their testing results can certainly skew the overall finding. However, despite these small flaws, this study demonstrated interesting findings that generate more questions for future studies.

**CONCLUSIONS**

**Thaker: Can you summarize the key findings in Tables 1 through 4?**

**Kizer:** Table 1 shows that only 1 of 8 cases with a negative LEEP specimen had residual or recurrent disease; 22% of specimens harbored a higher-grade CIN2/3. In Table 2, associations between patient age, pre-treatment HR-HPV viral load, surgical margins, and risk of recurrence/residual disease are illustrated. With a high viral load prior to treatment, the risk of recurrence rose from 15.4% to 50%. Likewise with positive surgical margins, the risk of recurrence increased from 25% to 50%.

Table 3 further analyzes the associations between risk of recurrent/residual disease and the cone margin result obtained after LEEP; results of the first cytology after obtaining a positive cone margin; and HR-HPV load, as determined by Hybrid Capture 2. Sensitivity, specificity, positive predictive value, and negative predictive value of each are presented. In this study, post-treatment HR-HPV presence was the most sensitive test (100%) for identifying risk of recurrence, as all patients with recurrence were positive for HPV. The authors also state that measurement of post-treatment HR-HPV can provide an excellent negative predictive value. In other words, the absence of HPV was a very good predictor that the CIN lesion would not recur/persist. Additionally, Table 4 shows a multivariate logistic regression analysis, where only post-treatment HR-HPV presence was significantly associated with CIN 1 persistence/recurrence. The odds ratio was 1.715.

**Thaker: What are the conclusions of the study?**

**Kizer:** The study has several notable conclusions. First, all patients with CIN/L-SIL and a high-risk profile (unsatisfactory colposcopy, positive endocervical curettage, age older than 40 years, persistence of lesion for more than 2 years, or large lesions) should be treated with LEEP, as more than 1/5 of the women in this study harbored a higher-grade lesion.

Second, of the 8 women with negative LEEP specimens, all but one of them had negative HR-HPV test results, suggesting that in women with CIN/L-SIL and a negative HR-HPV screen, a LEEP procedure should be avoided even if the patient is considered to be at high risk. Third, pretreatment HR-HPV testing may provide useful information for clinicians, helping to flag women who need to be more closely followed because they may be at higher risk for recurrence. Fourth, age, cytology, and involvement of surgical margins were not predictive of recurrence. Lastly, post-treatment HR-HPV testing shows excellent sensitivity while retaining good specificity in predicting chance of recurrence. As cytology still has better specificity, it is crucial to always obtain a Pap smear with follow-up HR-HPV testing.

**Thaker: How does this study help us clinically?**

**Zighelboim:** I believe the relevance of this study is somewhat limited. Transient L-SIL and CIN1 are believed to represent active HPV cervical infection. More than 1 million women are diagnosed with L-SIL and CIN1 in the U.S each year. In most cases, these patients will clear the infection and will not develop progressive cervical dysplasia. Only persistent infections will progress to CIN3 and potentially, to invasive disease. The probability of a patient with biopsy-proven CIN1 progressing into high-grade dysplasia is low; approximately 10%-15%. Furthermore, we know that in the absence of immunosuppression or other host-specific risk factors, further progression from severe dysplasia into invasive cancer takes several years.

In 2001, the ASCCP published consensus guidelines for the management of women with cervical dysplasia. These recommend cytologic follow-up and/or HPV testing without treatment as the preferred strategy for patients with biopsy-proven CIN1 and satisfactory colposcopy. The guidelines also stipulate that patients with low-grade dysplasia and an unsatisfactory colposcopy have endocervical curettage to assess for occult disease in the canal in preparation for an excisional or ablative procedure. Prior studies suggest that when LEEP is performed for patients with L-SIL and bi-
opsy-proven CIN1, the chance of discovering a high-grade histologic lesion varies from 10% to 55%. In this series of 77 patients with risk factors for persistent HPV infection plus evidence of low-grade dysplasia, the incidence of CIN2-3 was 22%. Among the members of this subgroup, 24% had persistent or progressive disease that was predicted by HR-HPV testing. Overall, the study helps us recognize patients who have high pretreatment HR-HPV loads and require more rigorous follow-up; their cancer biology is different from that of their counterparts who are at reduced risk.

**Thaker:** *Will the data change your practice management and if so, how?*

**Zighelboim:** At this time, I am not sure we can advocate HR-HPV testing for prognostic purposes in patients who have low-grade lesions and have not yet undergone invasive treatment. Only a relatively small number of patients with a low-grade, slowly progressive lesion would benefit. It will be helpful when the technology is economical for worldwide utilization, since cervical dysplasia and cervical cancer impact developing countries tremendously.

Even though I am confident that screening for specific HR-HPV subtypes with quantitative methods will eventually become the standard of care, this technology is not readily available for use in clinical practice. Women at high risk for poor compliance with follow-up may eventually become candidates for more aggressive approaches based on risk-screening strategies, such as the one proposed by Alonso et al. For these strategies to be effective, they would have to rely on high throughput analyses with rapid turnaround time, and these are not currently available.

**Thaker:** *What future studies would you suggest in the management of L-SIL?*

**Zighelboim:** Recent data suggest that rates of recurrence and progression after treatment for dysplasia vary with the specific HR-HPV subtypes involved. It would be interesting to know whether these data specifically apply to patients with early dysplasia. Additionally, our understanding of the genetic and immunologic factors that influence host response to HPV infection are continuously evolving. A good first step would be a longitudinal cohort study with molecular and immunologic characterization, as well as HPV subtype analysis. These data may help us develop clinical trials of surveillance programs and of interventions for women with mild cytologic abnormalities.

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Neoadjuvant chemotherapy plus radical surgery in locally advanced cervical cancer during pregnancy: a case report

Innocenza Palaia, MD; Milena Pernice, MD; Marialida Graziano, MD; Filippo Bellati, MD; Pierluigi Benedetti Panici, MD

CASE REPORT

A 30-year-old white woman presented at our institution complaining of vaginal bleeding. Gynecologic pelvic examination revealed a pelvic mass extending to the umbilicus and a cervical lesion 5 cm in diameter involving the right parametrium. A cervical biopsy was performed that revealed an invasive, poorly differentiated, squamous cell carcinoma. An ultrasonographic examination, performed to better define the large abdominopelvic mass revealed, unexpectedly, an enlarged uterus for a pregnancy at the 19th week. A pelvic magnetic resonance imaging (MRI) confirmed the presence of a cervical lesion of 4.5 × 5.5 cm in diameter and involvement of both lateral parametria. Because the patient strongly desired to continue the pregnancy, after complete and meticulous counseling, we decided for neoadjuvant chemotherapy with cisplatin 75 mg/m² plus paclitaxel 175 mg/m² every 3 weeks, followed by caesarean section and radical hysterectomy. At the first cycle, the patient presented a severe allergic reaction to paclitaxel despite premedication with antihistamines and steroids. We decided to continue the treatment with cisplatin alone. After the third cycle of chemotherapy, a gynecologic vaginorectal examination and a new abdominopelvic MRI revealed a partial response of the disease. At 35 weeks’ gestation, a caesarean section, followed by radical hysterectomy and pelvic lymphadenectomy, was performed (Figure). The infant was a female, with an Apgar score at 1 and 5 minutes of 7 and 9, weighing 2400 g. After placental expulsion, a fast hysterorrhaphy, followed by type III radical hysterectomy, according to Piver classification, plus pelvic lymphadenectomy were performed. The patient and infant were discharged on the seventh postoperative day in good general condition. Histologic report revealed a poorly differentiated cervical carcinoma of 2.5 cm and negative pelvic lymph nodes. No further adjuvant treatment was proposed. Pediatric follow-up of the newborn infant showed no sign of any metabolic or hematologic abnormality. The auditory brain stem evoked potential test was normal. At last follow-up (10 months post-surgery) both the mother and infant are in good general condition.

COMMENT

Cervical cancer is the most common malignancy diagnosed during pregnancy, with a reported incidence of from 1 in...
1200 to 1 in 2500. In a study from Smith et al, it was observed that more than 50% of cancers were found in the postpartum period, 30% at delivery, and only 20% in the prenatal period. The management of cervical cancer during pregnancy represents a challenge for the physician, and it is influenced mostly by gestational age at diagnosis, stage of disease, and patient’s desire of maintaining the pregnancy. Therapeutic options include surgery with or without adjuvant treatments, concurrent chemoradiation, and neoadjuvant chemotherapy plus radical surgery. Some authors have published cases of patients in whom the treatment was intentionally delayed to allow fetal maturity. Most of these suggest no adverse maternal outcomes when delays of 11-17 weeks were allowed, in case of the early stage of disease. As to chemotherapy during pregnancy, counseling should include the potential fetal effects of treatments. During the first trimester, the estimated teratogenic risk for the fetus ranges from 7.5-17% when a single agent is administered, and increase to 25% when combination chemotherapy is used. On the other hand, the risk of birth defects when cytotoxic drugs are administered during the second and third trimesters are similar to those of the general population (1-3%). The main fetal effects of chemotherapy during the second and third trimesters relate to intrauterine growth restriction, fetal death in utero, prematurity, and low birthweight. Furthermore, hematopoietic suppression, infertility, retarded development, carcinogenesis, and second-generation teratogenesis have been observed. As for cervical cancer, few cases are reported in literature on the use of neoadjuvant chemotherapy plus radical surgery during pregnancy (Table). The aim of this report is to add a new case of FIGO stage Ib cervical cancer in a pregnant patient who successfully submitted to neoadjuvant chemotherapy plus radical surgery. As a preliminary conclusion, reviewing the literature and considering our case, platinum-based chemotherapy in pregnant patients affected by cervical cancer appears to be feasible and safe for both the mother and infant. Longer follow-up and more studies are needed to define the safe outcome of both children and patients.

**REFERENCES**

Successful continuation of pregnancy after repair of a midgestational uterine rupture with the use of a fibrin-coated collagen fleece (TachoComb) in a primigravid woman with no known risk factors

Izumi Shirata, MD; Ritsuto Fujiwaki, MD; Kenji Takubo, MD; Toshihiko Shibukawa, MD; Kohji Sawada, MD

Uterine rupture is 1 of the most serious obstetric complications and may result in maternal and neonatal morbidity and mortality. It is generally believed that an unscarred primigravid uterus is immune to rupture, and spontaneous uterine rupture in a midgestational primigravid woman is exceedingly rare. Termination of the pregnancy with concurrent uterine repair or hysterectomy constitutes the usual approach to uterine rupture.1 However, if uterine rupture occurs at a very premature stage of fetal development, there is even a higher risk of neonatal morbidity and mortality compared with rupture at term.2 Uterine repair to allow continuation of the pregnancy potentially reduces these risks from prematurity. We report a case of spontaneous uterine rupture occurring at 24 weeks of gestation in a primigravid woman with no known risk factors. Utility of a fibrin-coated collagen fleece (TachoComb, Nycomed, Linz, Austria) for uterine repair and significance of close surveillance of preterm labor during continuation of pregnancy are discussed.

CASE REPORT
A 30-year-old Japanese primigravid woman presented at 24 2/7 weeks of gestation to an emergency department complaining of acute onset of severe abdominal pain since midnight. Her past medical history was noncontributory, and her antenatal care had been uneventful. No uterine contractions or history of recent abdominal trauma were reported. For 4 days she had experienced a vague sensation of discomfort in the upper abdomen. On physical examination, there was marked tenderness to palpation throughout the upper abdomen, with guarding and rebound tenderness. Blood pressure was 94/47 mm Hg; pulse, 100; respirations, 32; and temperature, 36.5°C. Laboratory values included hemoglobin, 10.4 g/dL; hematocrit, 32%; white blood cell count, 7000 cells/mm3; and platelets, 283,000/mm3. Ultrasonography and computed tomography suggested a hematoperitoneum, located primarily in the right iliac fossa with minimal free fluid in the cul-de-sac, but imaging scans could not identify the primary lesion. Pelvic examination found no vaginal bleeding, the cervical os was closed, and the size and position of the uterus were consistent with gestation with no detected uterine activity. Obstetric ultrasound examination showed a single uterine pregnancy with normal fetal heart rate. The site of placental implantation was the right fundus, and there was no evidence of abruption. The working diagnosis at this stage was intra-abdominal hemorrhage of uncertain cause, possibly secondary to rupture of an artery in the gastrointestinal organs or the abdominal wall. Three hours later, the patient’s condition had gradually deteriorated (blood pressure, 72/36 mm Hg; pulse, 125). The hemoglobin and hematocrit dropped to 8.9 g/dL and 25.7%, respectively. Thus, the transfusion of red blood cells was commenced.

Although we informed the patient and her families of the possibility of termination of the pregnancy before operation, they expressed a strong desire for the pregnancy to continue regardless the risks. Emergency laparotomy was performed by surgeons together with obstetricians and perinatal staff, and blood loss state consisting of 2200 mL of blood was found. Although abdominal examination detected no abnormality in the gastrointestinal organs, a partial uterine rupture of approximately 3 cm in the right posterior wall of the uterine fundus was identified as the source of bleeding. In addition, 2 surface varicose veins were torn. The site of placental implantation was located just beneath the partial rupture. The tear extended to about two thirds of the uterine wall, but the placental tissue was not protruding through the dehisced area. The patient’s condition, including blood pressure (105/60 mm Hg) and pulse (80), improved after the

We report the first case of successful continuation of pregnancy after repair of a midgestational uterine rupture with the use of a fibrin-coated collagen fleece (TachoComb, Nycomed, Linz, Austria). For midgestational uterine rupture, adequate uterine repair and close surveillance of preterm labor could improve perinatal outcome by permitting continuation of the pregnancy.

Key words: fibrin-coated collagen fleece, uterine rupture
administration of 3 units of red blood cells and 2000 mL of crystalloid. The early gestational age, patient's relatively stable condition, and desire to maintain the pregnancy led to the decision to attempt the repair of the uterine rupture. The uterine rupture and torn varicose veins were repaired by using interrupted stitches of polyglactin (Vicryl, Ethicon, Somerville, NJ); however, adequate hemostasis could not be obtained because of neovascularization of the uterine wall over the placental implantation site. Therefore, a 50 × 30 mm piece of TachoComb (Nycomed) was placed to cover the entire repaired dehiscence. Manual compression with the TachoComb (Nycomed) was applied for 10 minutes, to achieve complete hemostasis. Six units of red blood cells and 2500 mL of crystalloid were administered intraoperatively. Postoperatively, the patient was strictly monitored and received an additional 2 units of red blood cells (total dose: 8 units) and 2000 mL of volume resuscitation. Mild pulmonary edema occurred, but the patient's respiratory condition was stable on room air. Because frequent uterine contractions were noted immediately after the operation, ritodrine hydrochloride—the first choice for tocolysis in Japan—was carefully administered, which resulted in the resolution of uterine contractions. Repeated ultrasonography showed a normal fetal heart rate. Although few uterine contractions had occurred since the day after operation, transvaginal ultrasonography demonstrated that cervical length had gradually shortened. Twenty-three days after the primary surgery, the patient underwent emergency MacDonald cerclage because cervical dilatation of 2 cm with membranes was noted. The postoperative clinical course was uneventful. The ultrasound follow-up showed normal fetal growth and no abnormality of the placenta. At 32 weeks of gestation, magnetic resonance imaging revealed no evidence of placenta percreta through the dehisced area. However, after 35 weeks of gestation, the patient repeatedly experienced discomfort in the upper abdomen similar to the sensation she had experienced before uterine rupture. Therefore, a primary low transverse cesarean section was undertaken at 35 2/7 weeks of gestation, delivering a 2612 g male infant with consistent Apgar scores of 9 at 1 and 5 minutes. The placenta was easily removed. Examination of the uterus showed no abnormal findings with the exception of inflammatory changes in the posterior wall. The TachoComb (Nycomed), which had been fitted over the rupture site, was found to be intact. Again, the patient's postoperative clinical course was uneventful. At the 8-week postnatal visit, both the mother and infant were found to be doing well. This case report was conducted with the patient's informed consent and with the approval of the hospital ethics board.

**Comment**

Few cases of spontaneous uterine rupture in midgestational primigravid women have been reported, and most of these have been associated with risk factors such as uterine abnormality, history of gynecologic surgery, and placenta percreta. Our patient had no known risk factors for rupture. After uterine repair, she underwent emergency cervical cerclage because of painless cervical dilatation, similar to cervical incompetence. Uterine rupture and cervical incompetence each have been related to abnormality of the connective tissues, which results from collagen deficiency. Although the exact mechanism in our patient was unclear because of lack of pathologic or biologic examination, weakening of the connective tissue of the uterine wall induced by unidentified factors may have contributed to the pathogenesis of these complications. Uterine rupture must be considered in the differential diagnosis of patients with acute, severe abdominal pain accompanying hemodynamic instability even in midgestational primigravid women with no known risk factors.

To our knowledge, there have been only 3 reported cases of repair for partial uterine rupture with the continuation of pregnancy occurring at migestation (19–28 weeks of gestation). Two cases were repaired by using only stitches, but 1 case required a Gore-Tex soft tissue patch (W. L. Gore and Associates, Inc, Flagstaff, AZ) for repair of uterine dehiscence. All patients underwent cesarean delivery between 33–34 weeks of gestation, resulting in delivery of an intact infant. Our case is the first report of the successful continuation of pregnancy after repair for uterine rupture by using TachoComb (Nycomed), which consists of a sheet of collagen, coated on 1 side with human fibrinogen, bovine thrombin, bovine aprotinin, and riboflavin. An in vitro study previously showed that TachoComb (Nycomed) provided reliable sealing and high adhesive strength. Its hemostatic efficacy and safety have been clinically assessed in various operative procedures, such as cardiac, thoracic, and hepatic surgery. We used TachoComb (Nyomed) on an oozing uterine rupture, thereby achieving complete hemostasis. Persistent retention of this graft on the uterus and the lack of complications from the midterm trimester to the third trimester were apparent after the cesarean delivery. TachoComb (Nycomed) may be an effective hemostatic agent for the repair of uterine rupture and may support the ruptured lesion during gestation. For patients with otherwise favorable conditions, uterine repair, together with the use of artificial agents, should be considered to allow the pregnancy to continue, thus improving perinatal outcome in premature pregnancies. In addition, during the continuation of pregnancy after uterine repair, close surveillance of preterm labor, including painless cervical dilatation, is necessary. However, because the actual maternal risk associated with uterine repair during pregnancy may be much higher than that associated with such a repair after the termination of pregnancy and a hysterectomy, this essentially experimental approach should be followed only in carefully selected patients, after consideration of the patient's condition and desire to maintain pregnancy. Information about the possibility of increasing maternal risk and reducing neonatal morbidity is also helpful for patients and their families when making decisions regarding this approach.
REFERENCES