NOTES FROM THE ASSOCIATION OF MEDICAL SCHOOL PEDIATRIC DEPARTMENT CHAIRS, INC.

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50 Years Ago in The Journal of Pediatrics—Brain Abscess in Infants

Sarah S. Long, MD, Philadelphia, Pennsylvania

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Joel J. Liem MD, FRCPC, Shamima I. Huq, BSc, Okechukwu Ekuma, MSc, Allan B. Becker, MD, FRCPC, and Anita L. Kozyrskyj, PhD, Winnipeg, Manitoba, Canada

Palivizumab Prophylaxis, Respiratory Syncytial Virus, and Subsequent Recurrent Wheezing


Immunogenicity and Safety of a Combination Diphtheria, Tetanus Toxoid, Acellular Pertussis, Hepatitis B, and Inactivated Poliovirus Vaccine Coadministered with a 7-Valent Pneumococcal Conjugate Vaccine and a Haemophilus Influenzae Type b Conjugate Vaccine

Michael E. Pichichero, MD, Henry Bernstein, DO, Mark M. Blatter, MD, Lode Schuerman, MD, Brigitte Cheuvart, PhD, and Sandra J. Holmes, PhD, MHA, for the 085 Study Investigators, Rochester, New York, Lebanon, New Hampshire, Pittsburgh, Pennsylvania, and Rixensart, Belgium

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Alison E. Niebanck, MD, Avrum N. Pollock, MD, Kim Smith-Whitley, MD, Leslie J. Raffini, MD, Robert A. Zimmerman, MD, Kwaku Ohene-Frempong, MD, and Janet L. Kwiatkowski, MD, Philadelphia, Pennsylvania

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Margaret Ip, MD, FRCPath, FRCP(Glasg), Hong Kong

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Enrico Lopriore, MD, PhD, Frans J. Walther, MD, PhD, Dick Oepkes, MD, PhD, and Frank P. Vandenbussche, MD, PhD, Leiden, The Netherlands

**Reply**
Roland Broadbent, MB, ChB, Dunedin, New Zealand
Communicating with parents after a child’s death

In this issue of The Journal, Meert et al report on interviews with parents who recently lost a child in the pediatric intensive care unit (ICU) about their perspectives on physician-parent conferences. They surveyed 56 parents of 48 children who had died in the pediatric ICU of one of six children’s hospitals in the NICHD network. The results showed that the majority wanted to meet with their child’s intensive care physician, and 82% of these were willing to return to the hospital for a meeting. They wanted to be able to discuss the sequence of events that led to the ICU admission and death, cause of death, autopsy results, genetic risks, and many other issues. They also wanted the opportunity to seek reassurance, and the opportunity to voice any possible complaints, but also to express gratitude. It would seem that routinely establishing such meetings is going to be the standard of care.

—Robert W. Wilmott, MD
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Good news for combination and simultaneously administered vaccines

Pichichero et al report a carefully designed non-inferiority trial that confirms the immunogenicity and lack of important reactogenicity of the combination diphtheria and tetanus toxoids-, acellular pertussis-, hepatitis B- and inactivated poliovirus-containing vaccine with separately but simultaneously administered Haemophilus influenzae b vaccine (Hib) and pneumococcal 7-valent conjugate vaccine.

It is indeed remarkable that combination vaccines can be developed that retain the good part (ie, immunogenicity) and don’t add a bad part (ie, reactogenicity), and that can decrease the number of injections and increase the likelihood of an “on-schedule” immunization history.

—Sarah S. Long, MD
page 43
Cystic Fibrosis Foundation guidelines for diagnostic sweat testing

The Cystic Fibrosis Foundation (CFF) accredits cystic fibrosis centers in the United States, and part of the evaluation includes procedures for the performance of sweat tests. In 2006, the CFF developed new guidelines for sweat testing, which are presented and discussed in this issue of *The Journal* by LeGrys et al. The main changes relate to infants identified through newborn screening programs for cystic fibrosis.

—Robert W. Wilmott, MD

Neuropsychological performance in children after cardiac surgery

There have been tremendous advances in our ability to perform palliative and even corrective surgery in children with congenital heart disease. Because of this, children with congenital heart disease are living longer, and it is now estimated that there are more adults than children with congenital heart disease. An important question is whether such children (and adults) have normal neurocognitive function after their surgery. In this issue, Miatton et al examine this question in a cohort of post-surgical patients 6–12 years after surgery. They found that minor motor deficits and subtle difficulty with language were common. Executive function and memory were also affected. It will be important to follow these children over a longer term and ultimately determine new ways of protecting and maximizing neuropsychological performance in these patients.

—Stephen R. Daniels, MD, PhD

Palivizumab prophylaxis for respiratory syncytial virus infection

The report of Simoes et al on the effect of palivizumab prophylaxis on subsequent recurrent wheezing will be cited often. The study report deserves careful reading. The accompanying editorial attempts to put the investigation methodology and findings into context. Although substantial limitations of study design make it inappropriate to draw conclusions about the worth of palivizumab prophylaxis in reducing recurrent wheezing, or to support a broadening of recommendation for its use, the study fosters the hypothesis that a complex relationship exists between respiratory syncytial virus (RSV) infection and asthma, in which host genetics, developmental biology, and infection play variable roles. Prevention of premature birth and prevention of RSV through vaccination is where Willie Sutton would go.

—Sarah S. Long, MD

Transient tachypnea of the newborn and wheezing in childhood

An interesting idea is whether transient tachypnea of the newborn (TTN) is associated with the development of wheezing syndromes in early life. This question was investigated by Liem et al in the Sage study. Data were obtained from the Population Health Research Data Repository at the Manitoba Centre for Health Policy, and more than 12,000 children from the province of Manitoba were studied. Of these children, 2.4% developed TTN, and infants with TTN were found to be at significantly increased risk of a wheezing disorder in childhood.

Many of us believe that TTN is a self-limiting disease with no significant sequelae. This finding challenges that assumption. The authors discuss whether the association is due to maternal asthma, is a risk factor for TTN, whether genetic factors are at play, or whether environmental factors such as early exposure to antibiotics may be a factor.

—Robert W. Wilmott, MD
Lymphoma despite metabolic reconstitution in adenosine deaminase deficiency-severe combined immunodeficiency

Husain et al describe a child treated for adenosine deaminase (ADA) deficiency-severe combined immunodeficiency (SCID) with polyethylene glycol-adenosine deaminase (PEG-ADA) for more than 10 years. Although metabolic abnormalities and humoral immune responses were corrected, T-lymphocyte function never normalized, and declined over time. At 14 years of age, she developed Burkitt lymphoma.

Few patients with this rare disorder have been treated with PEG-ADA (because human leukocyte antigen [HLA]-identical sibling stem-cell transplant is preferred) for so long and have had immunologic function so carefully assessed longitudinally.

The development of Burkitt lymphoma following decline in T-lymphocyte function over time in this child provides the rare glimpse of an outcome that enlightens understanding of immunologic mechanisms in healthy hosts.

—Sarah S. Long, MD  
page 93

Iron for preterm infants

Preterm infants are born with very low iron stores, and rapid growth further stresses their limited iron stores. Although it is standard practice to supplement preterms with iron, there is no consensus about when to begin oral iron supplement or how much iron can be safely given. The major concern has been that iron-induced oxidant stress may result in tissue injury.

Brække et al report an observational study from Norway of the initiation of supplemental oral iron therapy with 18 mg iron daily beginning at 6 weeks of age. The daily dose ranged from 8 to 16 mg/kg in the very low birth weight infants. This dose is much higher than is generally used. Nevertheless, there was no indication of increased oxidant products or injury one week after the therapy was started. Very low birth weight infants seem to tolerate high dose iron therapy well.

—Alan H. Jobe, MD, PhD  
page 23 (article)  
page 3 (editorial)

NO use in the preterm

In this issue of The Journal, Hintz et al report that very ill preterm infants randomized to nitric oxide (NO) had no improvement in neurodevelopment outcomes relative to controls. This result differs from the improvements in short-term indicators of brain injury or neurodevelopment for less ill cohorts of infants as reviewed by Kinsella and Abman in this issue. This comprehensive review highlights the different populations and study designs used to evaluate NO therapy for preterms. The effect of NO on oxygenation, the surrogate clinical indicator of pulmonary hypertension, has not been consistent or impressive in the preterm. Thus, any benefits from NO therapy may result from other effects of NO. The long-term neurodevelopmental outcomes for the infants enrolled in the recently completed trials will be important to a decision about the indications for NO in preterms.

—Alan H. Jobe, MD, PhD  
page 16 (Hintz et al)  
page 10 (Kinsella and Abman)

Population-based incidence of necrotizing fasciitis in Canadian children

Eneli and Davies report the population-based incidence of two relatively uncommon diseases in children—group A streptococcal (GAS) and non-GAS necrotizing fasciitis. In addition to reporting an unexpected finding of highest disease burden of necrotizing fasciitis in infant males <12 months of age, investigators show that a comprehensive, active surveillance system can be implemented across a country and can provide incidence data for uncommon diseases.

—Sarah S. Long, MD  
page 79
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Women remain underrepresented in medical leadership positions in the United States in spite of gender equity in medical school enrollment. This underrepresentation exists even in the field of pediatrics, where women constitute more than half of all pediatricians and where the trend toward female predominance is increasing. Women represent 66% of pediatric trainees and 63% of pediatricians taking the American Board of Pediatrics certifying examination for the first time. In spite of their numbers in pediatrics, women are underrepresented in leadership positions both in academic and community pediatric organizations. Women direct only 21% of full professors in pediatrics. The American Academy of Pediatrics (AAP) also suffers from gender inequity. Currently, 6 of the 14 (43%) of the officers and directors of the AAP are women. But only 6 of 24 (25%) of the 2006 AAP National Conference Planning Group were women, and only one third of faculty listed for that conference were women (33 out of a sample of 103 or every fifth person listed in the conference faculty directory). Within Washington State, the current state chapter of the AAP has only 1 woman officer out of 5, and only 2 of the 9 trustees are women. There has only been 1 woman president in the history of the state chapter. Lack of conditions that promote gender equity in pediatric leadership is found in both academics and community pediatrics. The experience of 1 local center working to change these conditions and lessons learned from this process are shared below. We envision that there will be many such local efforts at the grass roots level, before we see a wave of institutional support to bring about national and societal changes that will eliminate underrepresentation of women in pediatric leadership positions. These culture changes will occur if the younger generation of pediatricians are supported in their expectations for family-friendly careers and see real models of institutional support in their training and in the jobs available to them in both academics and community pediatrics.

Lesson 1: Start With Sharing, Networking, Skill Building, and Inspiration and Include All Women Pediatricians in the Region

In an attempt to address these gender disparities in pediatric leadership at a local level, a group of women pediatricians, with strong support from the Chair of Pediatrics at the University of Washington, established the Puget Sound Women’s Pediatric Society (PSWPS) in 2001. An invitation was sent to all women pediatricians, residents, and fellows in the local region inviting them to participate with a questionnaire about their interests, issues, and ideas for programs and speakers. A steering committee was formed from interested pediatricians, with a balance between those in community and academic physicians and included fellow and resident trainees. A mission statement was developed: “To foster and encourage leadership among women in pediatrics in the Puget Sound area and to support their professional and personal development.”

The steering committee created a statement of guiding principles that emphasized the importance of uniting women in academics and in community practices, organizational inclusiveness and transparency, promotion of leadership models and roles that fostered a balance between personal and professional lives, and commitment to use of leadership skills to advocate for the health and well-being of children and their families.

Activities have included 1 to 2 dinners yearly, with each attended by 40 to 80 women/session and half-day skill-building workshops. The dinners have included time for socializing and networking. Prominent national physician leaders have spoken to the group at the dinners including Dr Joycelyn Elders, former U.S. Surgeon General; Dr Barbara Barlow, former head of pediatric surgery at Harlem Hospital; Dr Iris Litt, Director of the Robert Wood Johnson Fellowship program; Dr Judith Hall, former Chair of Pediatrics at Vancouver BC Children’s Hospital; and Dr Jane Schaller, former Chair of Pediatrics at Tufts and Executive Director of the International Pediatrics Association, the first African American woman Washington State Health Officer, and the first woman athletic director at the University of Washington. These dinner events have focused on the life trajectories of these amazing women leaders and have been both inspirational and intimate, with opportunities for participants to speak from both their hearts and minds. Other events have included panel discussions on child health advocacy and work/life balance with local pediatric leaders. Each year, skill-building and personal development workshops have been held, usually as half-day workshops on Saturday mornings, and each has been...
attended by 10 to 20 participants. Topics have included negotiating skills for physicians, communication styles, applied mediation, child health advocacy, mastering information overload, and efficient retrieval of electronic information. Participants have affirmed the usefulness of the skills taught in these workshops in both their personal and work lives.

Lesson 2: This Is not Solely a Women’s Issue

Men have been invited to participate, as well as women, and come both as pediatricians interested in the topics and as partners of women physicians. The organization has come to view itself similar to a group such as the League of Women Voters, an organization of women that sponsors events and forums open to both sexes to benefit the entire community. In the case of PSWPS, the goal is to bring more balance into the lives of pediatrics and to develop skills to make them more effective leaders in pediatrics and advocates for child health. Work/life balance and having an opportunity to share equally in child rearing is an issue that is important to men, as well as women. As we have moved into addressing institutional change, male pediatricians, especially those in the academic setting have increasingly lent their support, ideas, and passion to this effort.

Lesson 3: You Don’t Need a Lot of Money to Start the Process Rolling or to Keep It Moving

Funding for activities of the organization was obtained from an initial grant of $10,000 from the Children’s Hospital and Regional Medical Center (CHRMC) and continued by donations from women pediatricians and small grants from supporting individuals and companies. Donated funds go into a “Women’s Leadership Fund” at CHRMC. Events are priced to cover costs and to subsidize participation by residents and fellows. PSWPS operates on a budget of $3000–5000 per year. Over the last year, the Chief Operating Officer of CHRMC agreed to support a part time administrative support person to coordinate this process.

Lesson 4: Opening Pathways for Women to Assume Leadership Will Require Profound Institutional, Cultural, and Societal Change

Two years ago, our steering committee felt that it was time to move from inspirational talks and skill-building workshops to initiate institutional change that would promote work/life balance in pediatric careers. Important issues, such as the impact of pregnancies during pediatric residency and the continuing lack of on-site quality child care, maternity, or elder care leave, and flexible work settings in both community and academic settings, among others, were focal points for our discussions. Similar issues were raised by a national task force addressing challenges to women pediatricians.

Successful solutions to these barriers will benefit both men and women and will foster movement of women into positions of leadership.

Lesson 5: Develop a Proactive and Inclusive Process to Address These Issues at the Institutional Level

We recognized that changes were needed in both academics and community pediatrics but decided to address the academic setting initially. The leadership of both CHRMC and the Department of Pediatrics in the School of Medicine were concerned about these issues and agreed to approach the issues of family support structures, flexible training and work settings, academic advancement, and midlife career transitions in a proactive and investigative manner. Over the past year, work groups were established with the charge to investigate best institutional practices to support work/life balance in pediatric careers. The work groups addressed (1) family support structures such as on-site child care and parenting and elder care leave, (2) flexible training and work settings to accommodate family and personal needs, (3) academic advancement and promotion issues, and (4) facilitating midlife career transitions into academics by experienced clinicians and researchers at a time in their lives when family responsibilities may be less demanding.

The work group investigations culminated in a symposium where these issues were discussed in small interactive groups, and recommendations for institutional change were presented to administrators of the Department of Pediatrics and CHRMC. Dr. Bonita Stanton, a major champion of developing women leaders and Chair of Pediatrics at Wayne State University, was invited to be the keynote speaker for this symposium. Her moving presentation promoted a lively discussion with the pediatric community and an open exchange with administrators. The process has unfolded a road map for institutional change to support work/life balance for pediatricians. The leadership of the Department and the hospital are now working with an implementation committee that will actualize and monitor the recommendations that came out of the symposium work groups.

See Table for a summary of selected recommendations and measurable action outcomes (available at www.jpeds.com).

Community pediatricians have now developed a task force that is looking at similar issues as they relate to their own practice settings. They are developing a score card of “family-friendly” practice features that residents and fellows can use as they seek jobs in the community sector. Changing expectations of job-seeking pediatricians will help change what is available in community practices. The community task force also plans to take discussion and sharing of information about “family-friendly” practice features to the annual meeting of the Washington State Academy of Pediatrics to promote statewide awareness of this issue.

With the help of the PSWPS and the many committed people who joined this process, institutional change that supports physicians who want and need more balance between their work and personal lives will occur. These changes will allow women to move more easily into positions of leadership in pediatrics both in academics and in national pediatric associations. If change happens at the local level, more rapid changes at the national level will follow. It is anticipated that this process can serve as a model for other institutions and organizations faced with the similar challenges.

The authors want to dedicate this commentary to the memory of Gail G. Shapiro, MD, an outstanding pediatric allergist and nationally recognized woman leader in her field who helped establish PSWPS and was an active member of its steering committee until her untimely death during heart surgery in August 2006.

### Table. Selected recommendations and measurable action outcomes

<table>
<thead>
<tr>
<th>Work group</th>
<th>Recommendation</th>
<th>Measurable action outcomes</th>
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<tbody>
<tr>
<td>Family support structures</td>
<td>On-site child care</td>
<td>Addition of on-site child care to new research facility and all future new buildings</td>
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<tr>
<td>Flexible training and work</td>
<td>Clear policies on part-time work and parental leave</td>
<td>Policies written, discussed, and posted on faculty and resident web sites</td>
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<tr>
<td>Academic advancement</td>
<td>Increase promotion recognition for mentoring</td>
<td>Establish a department of pediatrics faculty mentoring award</td>
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<tr>
<td>Facilitating midlife career</td>
<td>Faculty making this transition need extra orientation and support</td>
<td>Peer counseling group set up to mentor/ provide guidance to these faculty</td>
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REFERENCES


Developing Women Leaders in Medicine at the Grass Roots Level: Evolution from Skills Training to Institutional Change 2.e1
Iron Supplementation in Prematurity: How Much is Too Much?

The body must control cellular iron concentrations within a narrow range to prevent deficiency or toxicity. In prematurity, iron deficiency may critically disrupt normal development, including brain development. At the other extreme, iron overload is problematic due to the relatively poor antioxidant capabilities of premature infants. In prematurity, recognizing iron deficiency or excess is also difficult, due to lack of clinically available indices of deficiency or toxicity. Thus, defining the precise therapeutic target for iron administration in premature infants has been a challenge. In this issue of The Journal, Brække et al1 present work that helps to address the question: How much iron is too much? The authors give relatively high-dose oral iron supplements to stable premature infants and evaluate antioxidant status, as well as oxidative stress. In conjunction with iron, the authors also supplemented vitamin E. As has been practiced in Norway for years,2 the authors treat stable premature infants with supplemented vitamin E. As has been practiced in Norway oxidative stress. In conjunction with iron, the authors also supplemented vitamin E. As has been practiced in Norway for years,2 the authors treat stable premature infants with 18 mg of ferrous fumarate daily, which can deliver as much as 18 mg/kg of elemental iron to very low birth weight infants. This dose, which is not adjusted for weight, is severalfold higher than the American Academy of Pediatrics recommendation of 2 to 4 mg/kg per day elemental for premature infants3 and 3 to 6 mg/kg per day in premature infants treated with erythropoietin (rhEpo).4

Historically, neonatologists in the United States have used caution when supplementing growing premature infants with iron. In the 1970s, vitamin E–deficient oxidative hemolysis occurred in premature infants fed iron–fortified formulas with a high unsaturated fatty acid content.5 Although biochemical deficiency may be seen, clinical vitamin E deficiency is rare in premature infants because of the low polyunsaturated fatty acid content relative to vitamin E content of fortified human milk and current premature infant formulas.

On the other extreme, recent changes in clinical practice place premature infants in potential jeopardy for insufficient tissue iron. In 1989, premature infants of birth weight less than 1500 g received between 8 and 10 transfusions before hospital discharge, but they currently receive fewer than 2 transfusions.6–8 Erythrocyte transfusions are rich in iron. Advances in perinatal care that improve infant stability, lower phlebotomy losses, and lower hematocrit targets for transfusions have decreased mean erythrocyte transfusion numbers.7,8 Currently it is unclear which premature infants are candidates for rhEpo, but treating with rhEpo may increase the risk for tissue iron depletion. In term infants, iron deficiency anemia in early life may have long-term consequences, including neurocognitive disturbances that remain after therapeutic iron replacement.9 Compared with outcomes after the diagnosis of iron deficiency anemia, early iron supplementation of at-risk term infants may improve long-term neurologic outcome.9 Because of improved outcomes in iron-supplemented, at-risk term infants, the positive and negative consequences of early supplements in at-risk premature infants should be further studied.

The strength of the current work by Brække et al1 is the thorough interrogation of high-dose iron supplementation using multiple indices of iron status, measures of oxidative stress, and indicators of antioxidant status. The current work complements earlier work in rhEpo-treated premature infants given oral or intravenous iron.10,11 These studies used sensitive and specific indicators of hydroxyl radicals and found no evidence of oxidative stress.10,11 Similar to the current study, Miller et al12 examined iron therapy in the absence of rhEpo treatment and showed stable blood and urine isoprostanes in premature infants given a sliding-scale ferrous sulfate dose that reached as high as 12 mg/kg per day. Neither Brække et al1 nor Miller et al12 evaluated the prooxidant effects of erythrocyte transfusions, a potential oxidative stress. However, Dani et al13 showed that erythrocyte transfusion neither increased oxidative stress nor impaired antioxidant status despite increased non–transferrin-bound iron levels.

There are some limitations to the study. The study is small and short term in nature. Because all infants in this study were fed human milk, it is unclear whether findings would be replicated in infants fed premature formula. Although ferrous fumarate is not a universally available product, there are some conclusions to be gained from this study. First, iron
dosage that appears excessive to many in neonatology may not be excessive. Second, because developmental implications favor the strategy of prevention compared with treatment of iron deficiency in at-risk infants, this topic is worthy of further scientific interrogation.

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REFERENCES


For Whom The Bell Tolls . . . .

Although the concept of “family-centered care” is nearly a half a century old, its impact on changing the “hospital culture” is most apparent over the last two decades.1 Listening to and understanding the parental perspective regarding death follow-up meetings is an implementation of family-centered care values within our hospitals and medical practice. Many books and medical journals have reported the emotions and opinions of parents during the days, months, and years after the death of their child.2-5 One of the recurring themes is the parents’ need for information about their children’s hospital care and death. In addition, parents reflect on the quality of care for a scheduled meeting, and who would attend the meeting. Limitations of the study are 1) a relatively small sample size, 2) the interviews were conducted within a time span of 4 to 15 months after the child’s death, and 3) parental responses were obtained from only English- and Spanish-speaking participants, thus potentially limiting data concerning cultural variation. Despite these limitations, valuable insights are provided about parents’ perspectives of medical practice after the death of patients, and these insights should encourage us to examine our death follow-up meeting practices.

Not surprisingly, 59% of parents desired to meet with the physician, timing and location of the meeting, and who would attend the meeting. Limitations of the study are 1) a relatively small sample size, 2) the interviews were conducted within a time span of 4 to 15 months after the child’s death, and 3) parental responses were obtained from only English- and Spanish-speaking participants, thus potentially limiting data concerning cultural variation. Despite these limitations, valuable insights are provided about parents’ perspectives of medical practice after the death of patients, and these insights should encourage us to examine our death follow-up meeting practices.

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meeting to discuss events leading to pediatric ICU admission and death, cause of death, treatment, autopsy results, and seeking advice about how to communicate about the death with other family members. Clearly, parents desire to participate in death follow-up meetings. Some that chose not to follow up commented that they were satisfied with the information already provided. The median time of request for interview was 8 months after the child’s death. Such postponement may have influenced some parents not to participate, especially if the parents felt there was no new information to be shared. Slightly more than one third of those parents who did not wish to return for a meeting stated that they were dissatisfied with the physician’s availability and communication skills. Parents requested an appointment that would include both parents; some preferred the inclusion of a grandparent and one of the child’s ICU nurses.

Who should be invited to death follow-up meetings? Parents in this study chose their closest support person—usually a spouse. Some requested the child’s grandparents or another family member who might help the parents facilitate communication with other family relatives. Not only is the death follow-up meeting an informative and healing service to the family, but it is also an educational opportunity for physicians and the entire health care team. Therefore, fellows and residents, who may have developed a close rapport with the family, should be included as well. How else is their education and communication skills provided? The complexities and pitfalls of communication between physicians and parents are well described in the article by Fox et al.9 Parents who cited dissatisfaction with the physician’s availability and communication skills provide us with an opportunity to improve our communication practices in intensive care medicine and to avoid parental feelings of mistrust.

How we communicate with families about palliative care and end-of-life issues during the hospitalization of their children significantly affects the success of future communications with the families.4,9,10 Quill et al10 have examined our responses during end-of-life situations and have provided instructive advice on empathic communications with families.

It is encouraging to see a study that examines the parental perspective of death follow-up meetings. The Institute of Medicine Committee on Palliative and End-Of-Life Care for Children and Their Families has endorsed further scientific research regarding this subject and recommended that the NIH establish priorities to fund studies in this area.11 Further studies involving family death follow-up meetings will provide insights into the communication practices used in our pediatric and newborn intensive care units. These insights should provide direction for future investigation to improve the quality of our practice. Evaluating the parental perspective on our death follow-up practices is a challenge for all health care providers to “look into the mirror” and see the reflection of our communication skills and humanity. Will we like what we see?

Who benefits by participating in death follow-up meetings? We all do. Perhaps the seventeenth century writer John Donne had death follow-up meetings in mind when he wrote, “[A]ny man’s death diminishes me, because I am involved in mankind, and therefore never send to know for whom the bell tolls; it tolls for thee.”12

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REFERENCES
An association between viral lower respiratory tract infection in the first 2 years of life and episodes of recurrent wheezing, reactive airway disease, and pulmonary function abnormalities later in childhood has been established.1 A number of respiratory viruses have been implicated in this association, although respiratory syncytial virus (RSV) appears to be the virus most strongly linked with recurrent wheezing.2,3 It is unclear whether subsequent wheezing occurs more commonly after RSV infection or whether this complication occurs with equal frequency after other viral respiratory tract infections (such as those caused by rhinovirus, influenza virus, parainfluenza virus, or human metapneumovirus).4–6 Whether the pathogenesis of subsequent airway obstruction is different after infection with different viruses, as suggested by the variable association with atopy, is not clear.7,8 Variations on 3 basic theories have been proposed to explain the link between early RSV infection and subsequent asthma. One possibility, a causal relationship, is that changes induced by viral replication early in life alter the pattern of normal lung development in such a way that the infant is predisposed to subsequent episodes of wheezing. A second possibility, a triggering relationship, is that certain infants have a pre-existing aberration of either airway function or the immune response, and early viral infection serves as an initiating or triggering event to acute airway obstruction. Because of certain inconsistencies in these 2 proposals and clinical observations, Martinez proposed a third hypothesis, a more complex relationship.9 In this model, the response to a viral infection is a function of several factors: the genetic makeup of the infant, concomitant exposure to other environmental antigens, and the degree of maturation of the infant’s immune system and airway at the time of the infection. This theory proposes no single response to a viral infection, but rather the outcome is a function of several intrinsic and extrinsic factors.

If viral lower respiratory tract infections can be a sufficient cause of asthma, prevention of infection should reduce the incidence of asthma. Alternatively, if viral lower respiratory tract infection acts to initiate the events leading to subsequent episodes of airway obstruction in a predisposed child, prevention of infection can be expected to reduce the wheezing associated with the acute illness, but will have only a modest or no impact on the incidence of asthma. Simes et al report in this issue of The Journal about an industry-sponsored study addressing the effect of avoidance of RSV lower respiratory tract infection on recurrent wheezing. Infants who received prophylaxis with palivizumab were compared with a retrospectively selected cohort matched by gestational and postnatal age that did not receive prophylaxis.10 Children born prematurely at 1 of 27 sites in Europe or Canada were observed for 24 months starting at a mean age of 19 months and assessed for episodes of wheezing. Physician-diagnosed recurrent wheezing during the 24-month period of follow-up (from approximately 19 months until 43 months of age) was 8% in prophylaxis recipients and 16% in the comparator group. These data suggest that in this group of premature infants without chronic lung disease, a reduced rate of RSV infection correlated with a lower incidence of recurrent wheezing. If the findings are reproducible, these results will support the theory that avoidance of early RSV infection can reduce the risk of long-term pulmonary complications.

The prevalence of hospitalization attributable both to bronchiolitis and to asthma have increased in recent years.11,12 Thus, the results of this study have important implications about the usefulness of passive immunoprophylaxis against long-term complications of RSV infection, and its design requires careful scrutiny. As discussed by the authors, there are substantial limitations to the study design and selection of subjects for both groups. No description is provided of the criteria used by physicians at the 27 sites to determine which infants were chosen to receive prophylaxis. Were the criteria the same for all sites? For both the prophylaxis and the comparator groups, little information is provided about the total number approached or who was not included for what reason. Although prophylaxis recipients had slightly lower mean birth weight (1.36 ± 0.44 kg versus 1.62 ± 0.51 kg) and were slightly more premature (29.9 ± 2.2 weeks versus 31.4 ± 2.5 weeks) than infants in the comparator groups, infants who did not receive prophylaxis had more risk factors for RSV infection (number of siblings, day care attendance, siblings in day care). Gestational age <32 weeks is a common threshold for administration of prophylaxis for most infants at most centers, and the presence of more risk factors in the comparator group that did not receive prophylaxis suggest a lack of consistency. In addition, families were aware of whether their child had received prophylaxis early in life both during the telephone contact and during the 6-month visits. This may have introduced bias when responding to questions about wheezing, although the investigator asking the questions was blinded.

The validity of any comparison between groups depends on the proper selection of control subjects. The authors correctly point out that a randomized, placebo controlled trial would not be ethically permissible because of the well-documented efficacy of palivizumab in reduc-

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immunoprophylaxis. This is particularly true when the number of subjects enrolled is small. The authors of this study are senior investigators who have contributed numerous important insights toward our understanding of disease caused by RSV. However, the unavoidable questions about selection of infants with this study design mean that the conclusions are uncertain.

Approximately 3% of all infants in the first 12 months of life will be hospitalized because of RSV infection (>100,000 hospitalizations per year) and most of these infants will be previously healthy, term infants. Development of a live attenuated RSV vaccine with reverse genetics or a vectored vaccine holds the greatest promise for future control of disease. In the absence of a broadly useful antiviral agent for treatment or chemoprophylaxis, passive immunoprophylaxis remains the most important means of lowering the hospitalization rate and reducing the burden of RSV disease in infants who are at high risk. On the basis of preliminary results from a phase III trial, a second generation monoclonal antibody (motavizumab) appears to be as efficacious as palivizumab in reducing the risk of RSV hospitalization, but the cost of this intervention is likely to continue to restrict prophylaxis to those infants at greatest risk. The results reported here suggesting a possible reduction in recurrent wheezing after palivizumab can be considered only as exploratory and do not justify any broadening of the existing recommendations for immunoprophylaxis.

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REFERENCES


Children with Cerebral Palsy Assess Their Parents’ Influence on the Quality of Their Lives: Implications for Intervention

A planned pregnancy is usually a time of joyous anticipation; parents expect the birth of a healthy, cute, vigorous infant who will give little anxiety while growing up to become an independent, capable adult who will provide grandchildren to cherish and spoil. The premature delivery of their child, or an infant born with an obvious medical problem or an illness that becomes evident later in infancy, provides a brutal awakening from these rosy dreams. Most parents react with anger at this injustice and become depressed and often overwhelmed by this unanticipated and unwelcome blow. How they learn to cope with the challenge will have life-long consequences for them, their affected offspring and other children, and even more distant family members.

Cerebral palsy (CP) refers to a static motor deficit of brain origin present since...
birth or early childhood. It is not a specific disease with a predictable course or cure; rather, it results in a broad range of types and severities of motor handicap, can affect any part of the body, and is often associated with epilepsy or deficits in other skills, notably language and cognition, which may have as much or more to do with quality of life than the motor deficits per se. Most of the time, CP’s full impact is not manifested for several years.

Counterintuitively, this impact on the parents and family may not be all negative, and it may even turn out to have some positive consequences, at least for those with adequate resilience. Yes, parents will have unanticipated time commitments and expenses. To increase their availability, mothers (usually) may have to curtail their personal interests or ambitions, quit their jobs, and give up the family’s second paycheck. Or, if they are in a position to command sufficient income to continue working, they may be forced to import expensive outside help to cope with CP’s multifold demands. Attention to siblings almost inevitably diminishes. The risk of fathers (usually) leaving and family breakup increases. In a crunch, grandparents, other relatives, or friends may reluctantly have to be asked for help, which is not necessarily an easy option.

On the positive side, however, rising to the challenge of coping may actually raise some parents’ morale. To their surprise, they discover in themselves unsuspected organizational strengths and parenting skills. They will inevitably meet a number of well-educated professionals and therapists and may discover hitherto unknown community resources and stimulating fields. They may enlarge their circle of friends by bonding with parents of children with CP or other handicaps. To become effective advocates for their children, they may turn themselves into experts by reading or scouring the internet to keep abreast of the latest discoveries and entitlements. A few mothers even return to school to start a new career related to their child’s handicap. Siblings are likely to learn about the realities of life and mutual obligations of family members earlier than their peers. Being called on to help a needy sibling is likely to enhance their maturity, generosity, responsibility, and awareness and tolerance of human differences. In several of my cases, disastrous brain diseases have seriously and communicated to parents, teachers, and therapists the quality of life of their children with CP needs to be taken

A limitation of the study is the small sample size, given the variability of the manifestations of CP and broad age range of the children. A number of factors contributed to smallness of the sample. One was requiring an IQ of at least 75 in the children with CP, to enable them to respond to the questionnaire. Consequently, the conclusions from this study do not apply to more severely handicapped youngsters, whose care is likely to be much more burdensome. Other restrictions were the need for each child to have at least 1 school-age sibling of normal intelligence and for the family to be Hebrew-speaking, thus excluding recent immigrants to Israel and limiting generalization of the findings to other cultural groups.

Quality of life of affected subjects and their families is increasingly considered crucial to the evaluation of the multitude of interventions offered for chronic neurologic disorders like CP or epilepsy. A number of standardized questionnaires assess the impact of illness (particularly CP) on various aspects of the life of affected individuals, parents, and families, but not the impact on the affected subjects of their parents’ coping and affect. Quality of life is a subjective dimension that is more difficult to capture quantitatively. Responses to standardized questionnaires, despite their inherent limitations, yield quite reliable data, especially if averaged over representative groups of subjects.

The novel information from the study by Aran et al on the effects of parents’ mood, outlook, and coping strategies on the quality of life of their children with CP needs to be taken seriously and communicated to parents, teachers, and therapists of handicapped children. It indicates that, rather than focusing resources entirely on the needs of the affected child, resources allocated to the emotional support and education of parents, helping them learn to curtail their understandable impulse to be overprotective and overcontrolling of their handicapped child, are likely to pay important dividends in the future. Having a suitable job (fully self-supporting or not) and a social circle of understanding coworkers and friends outside the family are effective safeguards against unwarranted life-long dependence on society and a quality of life that no healthy person would find acceptable. The monetary cost to society over the life span of entirely dependent individuals with CP is considerably higher than the added cost of services to parents to help them cope with their undeserved burden, discover some of its positive consequences, and teach them how to foster self-reliance and independence in their children.
REFERENCES

Inhaled Nitric Oxide in the Premature Newborn

JOHN P. KINSELLA, MD, AND STEVEN H. ABMAN, MD

Early reports of inhaled nitric oxide (iNO) therapy in near-term and term newborns with hypoxemic respiratory failure and persistent pulmonary hypertension described marked improvements in gas exchange.1,2 Subsequent randomized trials demonstrated that iNO decreased the need for extracorporeal life support in this population.3,4 iNO is uniquely suited to the treatment of persistent pulmonary hypertension of the newborn because of its selectivity for the pulmonary circulation and the absence of apparent short-term toxicities when used at low doses.

Laboratory and clinical studies have also shown that in addition to its effects in the reduction of pulmonary artery pressure, other beneficial effects of iNO may include improvements in ventilation/perfusion matching, decreased lung inflammation and oxidant stress, and favorable modulation of angiogenesis and growth in the immature lung. Thus, there is also considerable interest in the potential role of iNO in premature newborns with respiratory failure. However, persistent concerns about potential toxicity have limited the use of iNO in premature newborns to controlled, clinical trials. The results of recent clinical trials have helped to more clearly define the potential role of iNO in the premature newborn with respiratory failure, particularly as it relates to the prevention of bronchopulmonary dysplasia (BPD) and its effects on brain injury. In this report, we review the rationale for the use of iNO in premature infants and summarize the results of recent clinical trials.

Background: Rationale for iNO Therapy in the Premature Newborn

The effects of iNO on the pulmonary circulation in infants with severe pulmonary hypertension were evident in early clinical studies where iNO caused marked improvement in oxygenation by decreasing extrapulmonary right-to-left shunting in both term and premature newborns.1,2,6 Laboratory studies in term-gestation animal models confirmed that low-dose iNO caused selective and sustained pulmonary vasodilation in the transitional and neonatal pulmonary circulation.7,8 Parallel studies showed that endogenous NO modulates pulmonary vascular tone and contributes to pulmonary vasodilation at birth in extremely preterm sheep, and that low-dose iNO (5 ppm) causes sustained improvements in gas exchange and reduces pulmonary vascular resistance during mechanical ventilation of premature lambs with respiratory distress syndrome (RDS).9,10

In addition to its effects on pulmonary hemodynamics and gas exchange during inhalation, endogenous NO may regulate vascular permeability and neutrophil adhesion in the microcirculation.11 In premature lambs, low-dose iNO increases pulmonary blood flow and improves gas exchange without increasing pulmonary edema, and it decreases lung neutrophil accumulation.12 The effects of low-dose iNO to reduce early neutrophil accumulation in the lung may have important clinical implications because neutrophil inlux plays an important role in the inflammatory cascade that contributes to acute lung injury and the development of BPD.13-16 Therapies that reduce neutrophil accumulation in the lung in RDS could potentially modify the early inflammatory process that amplifies acute lung injury and contributes to the development of BPD.

iNO may also reduce oxidant stress in the premature newborn exposed to high inspired oxygen concentrations. Lambs delivered at 130 days gestation and mechanically ventilated for 5 hours with iNO showed no evidence of lung oxidative stress injury (lung malondialdehyde, reduced glutathione, glutathione reductase) compared with controls,17 consistent with other studies on the role of iNO in reducing oxidant stress.18-20

In addition to the acute effects of iNO on pulmonary vasodilation, lung inflammation, and oxidant stress, there is increasing evidence that impaired endogenous NO production contributes to the pathogenesis of BPD. For example, lung endothelial nitric oxide synthase (eNOS) expression is decreased in ovine and primate models of BPD,21,22
and mice genetically deficient for eNOS have abnormal distal lung architecture.25,26 The potential for iNO to modulate the evolution of lung injury in animal models of BPD has been the focus of recent studies, providing further experimental rationale for the role of iNO in premature subjects.25 Lin et al found that hyperoxia inhibited lung vascular growth and impaired alveolarization in neonatal rats, and that treatment with iNO after neonatal hyperoxia enhanced late lung growth and improved alveolarization in this model of BPD.26 McCurnin et al studied the effects of iNO in a baboon model of BPD over the first 14 days of life.27 They found that iNO partially improved early pulmonary function, lung structure, and extracellular matrix deposition in mechanically ventilated premature baboons with evolving BPD. In chronically ventilated premature lambs, Bland et al found that iNO administration preserved structure and function of airway smooth muscle and enhanced alveolar development.28 Thus, compelling evidence suggests that endogenous NO plays a vital role in pulmonary vascular and alveolar development in the immature lung, and that low-dose iNO may have beneficial effects on both the acute and chronic perturbations that are associated with the pathogenesis of BPD in the premature newborn.

Early Clinical Reports of iNO Use in the Premature Newborn

Early reports of iNO therapy in a premature newborn with pulmonary hypertension demonstrated marked improvement in oxygenation caused by effective treatment of severe pulmonary hypertension and resolution of extrapulmonary right-to-left shunting,6 and similar findings have been reported as well in preterm infants with severe respiratory failure.29,30 Subsequently, several randomized controlled trials (RCTs) have confirmed the acute improvement in oxygenation caused by iNO treatment. However, in contrast to the direct pulmonary vasodilator effects of iNO, the focus of the most recently published studies has been on the potential beneficial effects of prolonged iNO administration on lung parenchymal and vascular development.31 Early case reports also raised concern about potential adverse effects, including intracranial hemorrhage (ICH).29,30 In one of these retrospective reports, the rate of ICH appeared high in premature newborns treated with iNO, but this was not different from a group matched for severity of respiratory distress.30 Concern for a possible increased risk for ICH with iNO therapy was based, in part, on laboratory and clinical studies suggesting that high doses of iNO prolong bleeding.32-34 Although there is substantial evidence that low-dose iNO may protect the immature lung through various mechanisms described above, RCTs have reported conflicting results on its safety and efficacy. However, interpreting the results of these studies is complicated by design difference (eg, masked/unmasked), the diverse nature of the study populations, and the timing, dose, and duration of iNO therapy in the various trials. We briefly review the results of these RCTs below.

RCTs of iNO Use in the Premature Newborn

In a small, unmasked, randomized trial of iNO (20 ppm) and dexamethasone treatment, Subbedar et al reported no differences in survival, chronic lung disease, or ICH between iNO-treated infants and controls.35 In a randomized, masked, multicenter clinical trial of low dose iNO therapy (5 ppm) in severely ill premature newborns with RDS who had marked hypoxemia despite surfactant therapy (aA O2 ratio \( \leq 0.10 \)), iNO acutely improved \( \text{PaO}_2 \), but it did not reduce the incidence of mortality or BPD.36 Notably, there was no increase in the incidence or severity of ICH in this trial, and the incidence of the most severe ICH (grade 4) was 19% for the iNO group and 29% for the control group. The Franco-Belgium study group reported the results of an acute iNO response study (2-hour oxygenation end point); however, the brief duration of therapy and a high rate of crossover before the 2-hour trial end point compromised the interpretation of late outcome measures.37 Hascoet et al reported the results of an unmasked, randomized trial of iNO in 145 premature newborns with hypoxic respiratory failure.38 They found no difference between the iNO and control groups in the primary outcome measure (intact survival at 28 days), and no differences in adverse events. As noted by Finer in an accompanying editorial, interpretation of the findings is limited by a relatively high rate of “open-label” iNO use and the lack of important outcomes such as death before discharge and BPD incidence at 36 weeks.39 These investigators also studied the effect of low-dose iNO on serum markers of oxidative stress, and they found that iNO treatment apparently reduced signs of oxidative stress in these patients.40 However, changes in these measurements during iNO therapy may have been biased by the higher baseline \( \text{FIO}_2 \) in the treatment group in comparison with controls.

Field et al described the findings of the UK INNOVO trial. In this unblinded study, 108 premature infants with severe hypoxic respiratory failure were randomized to receive or not receive iNO.41 There was no difference between the iNO and control groups in the main outcome measure (death or severe disability at 1 year corrected age) and no difference in adverse events. Limitations of the study included an 8% crossover to iNO treatment, and treatment with other pulmonary vasodilators in 30% of the control group. Moreover, Field et al describe a lack of equipoise among investigators demonstrated by the observation that 75 infants eligible for enrollment were treated with iNO outside of the trial, leaving only infants with very severe lung disease enrolled in the study.42

The largest trials of iNO therapy in premature newborns reported to date include the single center study of Schreiber et al,43 and the multicenter trials of Van Meurs et al,44 Ballard et al,45 and Kinsella et al.46 All of these studies were randomized, controlled, and masked, but they have key differences in patient population, disease severity, dose and duration of therapy, and other factors.

Schreiber et al randomized 207 infants to treatment with iNO or placebo. The main finding of the trial was a
reduction in the incidence of BPD and death by 24% in the iNO group. These benefits appeared to accrue predominantly in the subset of newborns with relatively mild respiratory failure (oxygenation index [OI] <6.94). However, in addition to apparent pulmonary benefit caused by low-dose iNO, these authors also reported a 47% decrease in the incidence of severe ICH and periventricular leukomalacia (PVL). Moreover, in a subsequent report, Mestan et al showed that the early decrease in ICH/PVL associated with iNO treatment resulted in improved neurodevelopmental outcome on follow-up examinations of this population. In this follow-up study, 138 children (82% of survivors of the RCT) were evaluated for neurodevelopmental outcome at 2 years of age. In the group treated with iNO in the newborn period, 24% had abnormal outcomes (defined as cerebral palsy, blindness, hearing loss, or one score <70 on the Bayley Scales of Infant Development II), in contrast to 46% in the control group.

Van Meurs et al enrolled 420 newborns (401-1500 g birth weight) in a multicenter RCT. Although the focus of this study was on premature newborns and the major outcome measure was BPD, the design of the trial was similar to the previous NINOS trial in which term newborns were enrolled and acute changes in oxygenation determined continued treatment with study gas. That is, an acute dose-response study was performed, and only patients who showed significant improvement in PaO2 were continued on study gas. In contrast to other studies, the average duration of iNO treatment was only 76 hours. Moreover, this duration was only calculated for the subset of infants deemed responders. Thus, the average duration of treatment in the iNO group was approximately 57 hours. Overall, they found no difference in the incidence of death/BPD between the iNO and control groups. Interestingly, in post hoc analyses, infants with birth weight >1000 g showed a reduction in death/BPD following treatment with iNO (50% iNO vs 69% control).

However, a worrisome outcome was suggested in a post hoc analysis of newborns weighing ≤1000 g. This analysis showed an increased risk of ICH/PVL (43% iNO vs 33% control). These findings were in striking contrast with those of the Schreiber trial, and the basis for these opposing results is unclear. As noted in an editorial by Martin and Walsh, baseline ultrasonography examinations were not performed, and it cannot be determined whether these very severely ill infants had ICH before iNO was initiated. In addition, the severity of illness in the Van Meurs trial was also markedly different from that in the study of Schreiber et al. In the Van Meurs trial, the mean oxygenation index (OI) at enrollment for the iNO group was 23, compared with the median OI of 7.3 in the Schreiber study. This suggests that the degree of illness based on the severity of respiratory failure may be related to iNO safety and efficacy in this population; however, an increased risk of ICH/PVL was not observed in a previous trial of iNO in premature newborns with the same severity of hypoxemic respiratory failure as observed in the Van Meurs study (OI = 30). Other differences between these two trials may offer insights into the disparate outcomes, including the duration of iNO treatment (<3 days vs 7 days), birth weight (839 g vs 992 g), and gestational age (26 weeks vs 27.4 weeks). Thus, Van Meurs et al enrolled smaller, more immature infants with severe respiratory failure who were treated relatively briefly with iNO, making direct comparisons between these two trials problematic.

The results of the two largest randomized, controlled and masked trials of iNO treatment in premature newborns were recently reported. Ballard et al randomized 582 premature newborns with birth weights of 500 to 1250 g who required ventilatory support between 7 and 21 days of age. Infants were treated with study gas for a minimum of 24 days, and they had an estimated OI of 7. The researchers found that the incidence of survival without BPD was increased in the iNO treatment group (43.9%) compared with controls (36.8%) (P = .042, number needed to treat [NNT] = 14). A major finding of this trial was that the benefit of BPD reduction derived almost entirely from the subset of patients enrolled between 7 and 14 days, suggesting that early treatment is important to prevent BPD (Figure 1). There were no differences between the iNO and control groups in adverse events, including medical or surgical treatment of PDA. There also were no differences between the groups in ICH incidence; however, infants were enrolled after the first week of life. Thus, this trial does not provide additional information on iNO effects on brain injury in the premature newborn.

In the Kinsella trial, 793 premature newborns with birth weights of 500 to 1250 g requiring mechanical ventilation in the first 48 hour of life were randomized to treatment with 5 ppm iNO or placebo gas and were treated for 21 days or until endotracheal tube was removed. Overall, there was no difference in the incidence of death or BPD between groups; however, iNO therapy reduced the incidence of BPD for infants with birth weight >1000 g by 50% (P = .001, NNT = 3) (Figure 2). Low-dose iNO therapy reduced the incidence of PVL (P = .048), as well as the combined end points of ICH, PVL, and ventriculomegaly for the entire study population (P = .032, NNT = 16) (Figure 3). iNO therapy did not increase the incidence of adverse events, including mortality, ICH, PVL, pulmonary hemorrhage, and PDA treatment in any subgroup. In this trial there was no relationship between OI and brain injury risk, in contrast to the findings of Van Meurs et al. Mechanisms through which iNO therapy might provide neuroprotection in the premature newborn are uncertain, and they warrant further study. Based on laboratory studies, several possibilities exist that include modulation of circulating cells (including neutrophils, monocytes, and platelets) that may occur during NO exposure as they transit the pulmonary circulation. Alternatively, iNO-induced down-regulation of lung-derived cytokines may also reduce distant organ injury. Another possible mechanism may relate to distal delivery of NO or NO-related metabolites through the systemic circulation through red blood cell or protein mediated pathways.
Although iNO is a safe and effective treatment for near-term and term newborns with pulmonary hypertension and hypoxemic respiratory failure, its role in the premature newborn has been more difficult to define. The effectiveness of iNO in the near-term and term newborn is largely because of its properties as a selective pulmonary vasodilator. However, other putative effects may be equally or more important in the premature newborn, such as decreasing inflammation, reducing oxidant stress, and enhancing alveolarization and lung growth. Further laboratory studies are needed to better define the exact mechanisms through which iNO may modulate lung and brain injury, growth, and function.

The effects of iNO in the premature newborn may be dependent on the timing, dose, and duration of therapy, and on the nature of the underlying disease. The available evidence from clinical trials suggests that low-dose iNO may be safe and effective in reducing the risk of death/BPD for a subset of premature newborns, in particular infants with birth weights >1000 g. A neuroprotective effect of iNO has been demonstrated in large RCTs, but the relationship of disease severity and ICH/PVL risk is uncertain. Treatment of premature newborns with respiratory failure between 7 and 14 days after birth appears to be safe and effective in reducing the incidence of BPD. Currently, an industry-sponsored (INO Therapeutics) trial of low-dose iNO in premature newborns is underway in Europe. The results of this trial will provide more information about the safety and efficacy of iNO in this population. Indeed, if neuroprotection and/or BPD reduction...
with early iNO treatment are confirmed in this study, and long-term follow-up studies describe lasting neurodevelopmental benefit, then routine use of iNO in premature newborns should gain regulatory approval.

Meta-analysis of these clinical trials will follow, but it should be limited to studies that were properly masked and designed to effectively measure relevant outcomes (Table). Finally, early concerns about the potential adverse effects of iNO on surfactant function and PDA risk have been effectively eliminated with the cumulative results of clinical trials; however, routine use of iNO in premature newborns should await the results of follow-up studies from the largest clinical trials.

REFERENCES


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<td>5 ppm</td>
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*G.A. Gestational age.

*P < .05.

†Infants enrolled after 7 days of age.
Neurodevelopmental Outcomes of Premature Infants with Severe Respiratory Failure Enrolled in a Randomized Controlled Trial of Inhaled Nitric Oxide

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Objectives  We hypothesized that inhaled nitric oxide (iNO) would not decrease death or neurodevelopmental impairment (NDI) in infants enrolled in the National Institute of Child Health and Human Development Preemie iNO Trial (PiNO) trial, nor improve neurodevelopmental outcomes in the follow-up group.

Study design  Infants <34 weeks of age, weighing <1500 g, with severe respiratory failure were enrolled in the multicenter, randomized, controlled trial. NDI at 18 to 22 months corrected age was defined as: moderate to severe cerebral palsy (CP; Mental Developmental Index or Psychomotor score Developmental Index <70), blindness, or deafness.

Results  Of 420 patients enrolled, 109 who received iNO (52%) and 98 who received placebo (47%) died. The follow-up rate in survivors was 90%. iNO did not reduce death or NDI (78% versus 73%; relative risk [RR], 1.07; 95% CI, 0.95-1.19), or NDI or Mental Developmental Index <70 in the follow-up group. Moderate-severe CP was slightly higher with iNO (RR, 2.41; 95% CI, 1.01-5.75), as was death or CP in infants weighing <1000 g (RR, 1.22; 95% CI, 1.05-1.43).

Conclusions  In this extremely ill cohort, iNO did not reduce death or NDI or improve neurodevelopmental outcomes. Routine iNO use in premature infants should be limited to research settings until further data are available. (J Pediatr 2007;151:16-22)

The prognosis for survival of the most vulnerable preterm infants has improved dramatically because of advances in perinatal and neonatal care,1,2 but their neurodevelopmental outcomes do not appear to have benefited similarly. Cerebral palsy (CP) rates in extremely preterm and extremely low birth weight (ELBW) infants have been unchanged with time.3,4 Cognitive outcomes at 18 to 22 months corrected age have been variably reported to be worsening,5-7 unchanged,8 or mildly improved.4 Therefore, there is a substantial impetus to find interventions that improve neurodevelopmental outcomes.

Results of a single-center, randomized controlled trial in premature infants found that treatment with inhaled nitric oxide (iNO) significantly reduced death or chronic lung disease9 and disability or developmental delay at 2 years of age.10 A recent multicenter

See related article, p 10

BPD  Bronchopulmonary dysplasia  NICHD  National Institute of Child Health and Human Development
BSID  Bayley Scales of Infant Development  OI  Oxygenation index
CP  Cerebral palsy  PINO  Preemie Inhaled Nitric Oxide trial
ELBW  Extremely low birth weight  PDI  Psychomotor Developmental Index
iNO  Inhaled nitric oxide  PVL  Periventricular leukomalacia
IVH  Intraventricular hemorrhage  RR  Relative risk
MDI  Mental Developmental Index  *List of members of the NICHD Neonatal Research Network is available at www.jpeds.com.
NDI  Neurodevelopmental impairment

From the NICHD Neonatal Research Network.
INO Therapeutics provided the study gas, gas delivery systems, and site monitoring for all hospitals, and capitation funding for the hospital outside the NICHD Neonatal Research Network. The company was otherwise not involved in the study design, data analysis, data interpretation, or preparation of manuscripts. Dr Ehrenkranz reported having served as consultant to INO Therapeutics. Dr Konduri reports having received grant support from INO Therapeutics. Dr Van Meurs reports having received lecture fees from INO Therapeutics. See funding information available at www.jpeds.com.

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study of moderately ill preterm infants treated with iNO beginning at <48 hours showed a reduced rate of death or bronchopulmonary dysplasia (BPD) only in subgroup analysis of infants with a birth weight of 1000 to 1250 grams. Another multicenter study of preterm infants with more established lung disease treated with iNO beginning after 7 days of age showed an increased rate of survival without BPD. In the National Institute of Child Health and Human Development Neonatal Research Network, we conducted a multicenter trial (PiNO trial) of a more severely ill preterm cohort and found no overall reduction in death or BPD with iNO, but post-hoc analysis demonstrated benefit for infants with a birth weight >1000 g. Before the trial, we hypothesized that iNO would not reduce death or neurodevelopmental impairment at 18 to 22 months adjusted age; we now report those results and the overall neurodevelopmental outcomes of the PiNO trial cohort.

METHODS

NICHD PiNO Trial and Definitions

Enrollment in the multicenter, randomized, double-masked, placebo-controlled trial occurred Jan 4, 2001 to Sep 26, 2003, as previously published by Van Meurs et al. The primary outcome for that analysis was death or BPD, with BPD defined as treatment with oxygen at 36 weeks gestation. The institutional review boards of all participating centers approved the study, including neurodevelopmental follow-up. Informed consent was obtained from parents or legal guardians. Infants were eligible when they were <34 weeks of gestation, had a birth weight of 401 to 1500 grams, were mechanically ventilated, and had severe respiratory failure defined with specific criteria. Eligible infants must have received at least 1 dose of surfactant at least 4 hours before meeting the oxygenation index (OI) criterion for entry, which was defined as an OI ≥10 on 2 consecutive measurements of arterial blood gases between 30 minutes and 12 hours apart. OI entry criteria were modified at the request of the Data Safety and Monitoring Committee during the trial because of higher than expected mortality rates in both treatment groups. The respiratory criteria for entry were revised to an OI of at least 5 followed by an OI of at least 7.5 within 30 minutes to 24 hours. Thus, for the purposes of analysis, the study design was considered to have 2 strata on the basis of the OI entry criterion. Randomization to receive iNO (INO Therapeutics) or placebo was stratified according to center and birth weight category (401-750 g, 751-1000 g, 1001-1500 g). Research nurses collected demographic, perinatal, and infant data, including morbidities and treatments at each center, using common definitions developed by investigators of the PiNO Study, and as previously described. The mode of ventilation was not dictated by the study protocol, nor was it a randomization factor. Study gas was initiated at 5 ppm and could be increased to 10 ppm, with escalation and weaning criteria defined by means of the study protocol. The maximum study gas exposure was 14 days; the duration of study gas exposure for the iNO group was 76 + 73 hours (on the basis of N = 210 iNO subjects), and for the placebo group was 39 + 65 hours (on the basis of N = 208 placebo subjects). After a planned interim analysis by the data safety and monitoring committee indicated that the incidence of grade 3 or 4 intraventricular hemorrhage (IVH) or periventricular leukomalacia (PVL) was higher in the iNO group, recruitment was terminated with 95% of the targeted enrollment completed, after 210 infants had been randomized to the iNO group and 210 to the placebo group. Final data analysis demonstrated no significant overall difference in the rate of severe IVH or PVL or in the rate of death or BPD between the iNO and placebo groups. However, post-hoc subgroup analyses suggested that iNO was associated with a reduced rate of death or BPD for infants with a birth weight >1000 grams and an increased rate of severe IVH or PVL for infants with a birth weight ≤1000 grams.

Neurodevelopmental Follow-up

Our primary outcome for the follow-up component of the PiNO trial was death or neurodevelopmental impairment (NDI) at 18 to 22 months of age corrected for prematurity; we believed it was crucial to encompass patient outcomes from the time of randomization rather than focus solely on those patients who survived to hospital discharge. NDI was defined as moderate to severe CP, bilateral blindness (no useful vision in either eye), or deafness (requiring hearing aids in both ears), Bayley Scales of Infant Development (BSID) II Mental Developmental Index (MDI) or Psychomotor Developmental Index (PDI) <70. Death was defined as death during or after initial hospitalization at the time of the 18- to 22-month neurodevelopmental follow-up visit. Secondary outcomes included: death or moderate to severe CP, NDI and its components in the follow-up group; “isolated delay” in the follow-up group defined as MDI or PDI <70 in the absence of moderate to severe CP, blindness or deafness; and “unimpaired status” in the follow-up group defined as MDI and PDI ≥85, and no moderate to severe CP, blindness, or deafness.

The elements of the follow-up visit were based on the NICHD NRN Follow-up Study of ELBW infants, previously described in detail. All neurologic assessments were performed by certified, masked examiners who had been trained in the assessment procedure in an annual 2-day workshop. The neurologic examination was based on those of Amiel-Tison, Russell, and Palisano. CP was defined as a non-progressive central nervous system disorder characterized by abnormal muscle tone in at least 1 extremity and abnormal control of movement and posture, which interfered with or prevented age-appropriate motor activities. CP was classified as “moderate” when the child could sit independently or with support, but not independently ambulate, and “severe” when the child was unable to sit or walk even with support. Certified examiners administered the BSID-II MDI and PDI. Scores were adjusted for prematurity. Scores of <70 are 2 SDs less than the mean. Scores of 49 were assigned.
to infants whose extremely severe neurologic or neurodevelopmental impairment prevented examination.

Head circumference and weight percentiles were based on age corrected for prematurity. Visual and hearing impairment were assessed by parent interview, physical examination, and best available medical record. Research personnel administered standardized questionnaires about socioeconomic status information, including highest level of education attained by primary caregivers, and other factors.

Statistical Analysis

Clinical characteristics and unadjusted outcomes of iNO and placebo groups were compared by using the chi-square test or Fisher Exact test for categorical variables, t tests for means of continuous variables, and Wilcoxon tests for medians and interquartile ranges. Differences in treatment groups for primary and secondary outcomes were analyzed with relative risk (RR) and 95% CIs. Adjusted RRs and 95% CI were calculated with 2 Poisson regression models. "Model #1" included birth weight category, center, OI entry criterion stratum, sex, and treatment group as covariates. "Model #2" included those covariates and length of iNO exposure, IVH grade 3 or 4 or PVL, BPD, and postnatal steroids. Only model #1 could be applied to composite outcomes that included death because patients may have died before factors such as BPD, postnatal steroids, or even IVH/

Table I. Perinatal and early neonatal characteristics of trial cohort and follow-up group*

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<td>Birth weight</td>
<td>835 ± 265</td>
<td>830 ± 261</td>
<td></td>
</tr>
<tr>
<td>Gestational age</td>
<td>25.9 ± 2.3</td>
<td>25.9 ± 2.2</td>
<td>.67</td>
</tr>
<tr>
<td>Male</td>
<td>123 (62%)</td>
<td>122 (61%)</td>
<td>.90</td>
</tr>
<tr>
<td>Race‡</td>
<td></td>
<td>.77</td>
<td></td>
</tr>
<tr>
<td>White</td>
<td>91 (46%)</td>
<td>93 (47%)</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>64 (32%)</td>
<td>71 (36%)</td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>35 (18%)</td>
<td>32 (16%)</td>
<td></td>
</tr>
<tr>
<td>Asian/Pacific</td>
<td>4 (2%)</td>
<td>2 (1%)</td>
<td></td>
</tr>
<tr>
<td>Other</td>
<td>4 (2%)</td>
<td>2 (1%)</td>
<td></td>
</tr>
<tr>
<td>Antenatal steroids§</td>
<td>116/169 (69%)</td>
<td>114/167 (68%)</td>
<td>1.00</td>
</tr>
<tr>
<td>Born at study hospital§</td>
<td>156 (79%)</td>
<td>153/199 (77%)</td>
<td>.74</td>
</tr>
<tr>
<td>Cesarean section</td>
<td>138 (70%)</td>
<td>133 (67%)</td>
<td>.54</td>
</tr>
<tr>
<td>Apgar &lt;4 at 5 minutes§</td>
<td>27/166 (16%)</td>
<td>22/164 (13%)</td>
<td>.57</td>
</tr>
<tr>
<td>OI at randomization (median, interquartile range)</td>
<td>17.2 (12.0-27.4)</td>
<td>16.1 (11.0-27.4)</td>
<td>.29</td>
</tr>
<tr>
<td>OI at randomization</td>
<td>23.2 ± 17.0</td>
<td>22.1 ± 16.8</td>
<td>.54</td>
</tr>
<tr>
<td>HFV at randomization</td>
<td>115 (58%)</td>
<td>119 (60%)</td>
<td>.85</td>
</tr>
<tr>
<td>Number surfactant doses at randomization</td>
<td>2.1 ± 0.8</td>
<td>2.2 ± 0.9</td>
<td>.23</td>
</tr>
<tr>
<td>Hours of gas exposure (median, interquartile range)</td>
<td>65 (19-98)</td>
<td>2 (1-71)</td>
<td>&lt;.0001</td>
</tr>
</tbody>
</table>

Data presented as N (%) or mean ± SD unless otherwise noted.
†Trial cohort includes all patients for whom a primary outcome was determined; follow-up group includes patients surviving to neurodevelopmental follow-up.
‡Race or ethnic group was self-reported by the mother of the infant.
§Data were not available for all infants.

Table II. Later clinical characteristics of the follow-up cohort*

<table>
<thead>
<tr>
<th></th>
<th>iNO</th>
<th>Placebo</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>91</td>
<td>102</td>
</tr>
<tr>
<td>Sepsis/pneumonia†</td>
<td>45 (49%)</td>
<td>47 (46%)</td>
<td></td>
</tr>
<tr>
<td>Proven necrotizing enterocolitis‡</td>
<td>8/90 (9%)</td>
<td>8/101 (8%)</td>
<td>1.0</td>
</tr>
<tr>
<td>BPD§</td>
<td>52 (57%)</td>
<td>67/101 (66%)</td>
<td>.19</td>
</tr>
<tr>
<td>Postnatal steroids</td>
<td>46 (51%)</td>
<td>59 (58%)</td>
<td>.38</td>
</tr>
<tr>
<td>Grade 3 or 4 IVH or PVL§</td>
<td>25 (27%)</td>
<td>22/99 (22%)</td>
<td>.29</td>
</tr>
<tr>
<td>Threshold ROP§</td>
<td>27/88 (31%)</td>
<td>29/98 (30%)</td>
<td>1.0</td>
</tr>
<tr>
<td>Corrected age at follow-up (mo)</td>
<td>20.4 ± 3.5</td>
<td>20.6 ± 3.9</td>
<td>.68</td>
</tr>
<tr>
<td>Maternal education less than high school§</td>
<td>21/87 (24%)</td>
<td>24/96 (25%)</td>
<td>1.0</td>
</tr>
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</table>

Data presented as N (%) or mean ± SD unless otherwise noted.
†Sepsis/pneumonia after 72 hours of age.
‡Necrotizing enterocolitis stage IIA or greater.
§BPD defined as treatment with oxygen at 36 weeks gestation.
§Data were not available for all infants.
Table III. Primary and secondary outcomes: Unadjusted and adjusted relative risks (RR) and 95% confidence intervals (95% CI)

<table>
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<tr>
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<th>iNO n (%)</th>
<th>Placebo n (%)</th>
<th>RR (95%CI) Unadjusted</th>
<th>P value</th>
<th>Model #1*</th>
<th>P value</th>
<th>Model #2*</th>
<th>P value</th>
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<td>N died or had follow-up</td>
<td>200</td>
<td>200</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Death or NDI‡</td>
<td>154/198 (78%)</td>
<td>146 (73%)</td>
<td>1.07 (0.95-1.19)</td>
<td>.32</td>
<td>1.06 (0.95-1.17)</td>
<td>.30</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Death or moderate to severe CP</td>
<td>127/199 (64%)</td>
<td>109 (54%)</td>
<td>1.17 (0.99-1.38)</td>
<td>.07</td>
<td>1.15 (0.99-1.34)</td>
<td>.07</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>N follow-up group only</td>
<td>91</td>
<td>102</td>
<td></td>
<td></td>
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<td></td>
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<tr>
<td>NDI</td>
<td>45/89 (51%)</td>
<td>48 (47%)</td>
<td>1.07 (0.80-1.44)</td>
<td>.74</td>
<td>1.04 (0.79-1.36)</td>
<td>.78</td>
<td>1.19 (0.81-1.37)</td>
<td>.37</td>
</tr>
<tr>
<td>Moderate to severe CP</td>
<td>18/90 (20%)</td>
<td>11 (11%)</td>
<td>1.85 (0.99-3.71)</td>
<td>.11</td>
<td>2.01 (1.01-3.98)</td>
<td>.0453</td>
<td>2.41 (1.01-5.75)</td>
<td>.048</td>
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<tr>
<td>MDI &lt;70</td>
<td>37/86 (43%)</td>
<td>35 (36%)</td>
<td>1.20 (0.84-1.73)</td>
<td>.39</td>
<td>1.15 (0.83-1.60)</td>
<td>.40</td>
<td>1.38 (0.86-2.22)</td>
<td>.19</td>
</tr>
<tr>
<td>PDI &lt;70</td>
<td>29/85 (34%)</td>
<td>32 (32%)</td>
<td>1.06 (0.70-1.59)</td>
<td>.92</td>
<td>1.07 (0.73-1.56)</td>
<td>.74</td>
<td>1.04 (0.60-1.79)</td>
<td>.89</td>
</tr>
<tr>
<td>Deaf</td>
<td>5/90 (6%)</td>
<td>5 (5%)</td>
<td>1.13 (0.34-3.79)</td>
<td>1.00</td>
<td>Too few to model</td>
<td>Too few to model</td>
<td>Too few to model</td>
<td>.43</td>
</tr>
<tr>
<td>Blind</td>
<td>2/90 (2%)</td>
<td>1 (1%)</td>
<td>2.27 (0.21-24.58)</td>
<td>.60</td>
<td>Too few to model</td>
<td>Too few to model</td>
<td>Too few to model</td>
<td>.79</td>
</tr>
<tr>
<td>Isolated delay†</td>
<td>24/88 (27%)</td>
<td>35 (34%)</td>
<td>0.79 (0.51-1.23)</td>
<td>.37</td>
<td>0.72 (0.48-1.07)</td>
<td>.10</td>
<td>0.79 (0.44-1.42)</td>
<td>.43</td>
</tr>
<tr>
<td>Unimpaired‡</td>
<td>21/90 (23%)</td>
<td>26 (25%)</td>
<td>0.92 (0.56-1.51)</td>
<td>.86</td>
<td>0.85 (0.54-1.35)</td>
<td>.50</td>
<td>0.91 (0.46-1.81)</td>
<td>.79</td>
</tr>
<tr>
<td>Head circumference (cm)</td>
<td>46.8 ± 1.7</td>
<td>46.7 ± 1.9</td>
<td></td>
<td>.64</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>N (%) &lt;5th</td>
<td>14/86 (16%)</td>
<td>20/97 (21%)</td>
<td>0.79 (0.43-1.47)</td>
<td>.57</td>
<td>0.84 (0.48-1.47)</td>
<td>.55</td>
<td>0.99 (0.47-2.08)</td>
<td>.97</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>10.6 ± 1.4</td>
<td>10.6 ± 1.7</td>
<td></td>
<td>.72</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>N (%) &lt;5th</td>
<td>31/86 (36%)</td>
<td>41/98 (42%)</td>
<td>0.86 (0.60-1.24)</td>
<td>.51</td>
<td>0.93 (0.65-1.32)</td>
<td>.68</td>
<td>0.97 (0.60-1.57)</td>
<td>.92</td>
</tr>
</tbody>
</table>

*Adjusted RR—Model #1: adjusted for birth weight, center, OI entry criterion strata, sex; model #2: adjusted for birth weight, center, OI entry criterion strata, sex, BPD, IVH 3 or 4 or PVL, length of iNO exposure, postnatal steroids.

‡NDI—any of the following: moderate-severe CP, blind, deaf, MDI <70, or PDI <70.

†Isolated delay: MDI <70 or PDI <70 in the absence of moderate-severe CP, deafness, or blindness.

§Unimpaired designates all the following: MDI and PDI.

**Table III. Primary and secondary outcomes: Unadjusted and adjusted relative risks (RR) and 95% confidence intervals (95% CI)**

PVL could be determined or diagnosed. Interaction terms were tested when relevant. Modeling was not possible for some outcomes because of small numbers. For post-hoc analyses of outcomes stratified by birth weight category, Cochran-Mantel-Haenszel test controlling for OI strata was used.

**RESULTS**

**Patient Disposition**

Of the 420 patients enrolled, 109 receiving iNO (52%) and 98 receiving placebo (47%) died before 18 to 22 months adjusted age (adjusted P = .27; Figure; available at www.jpeds.com). In each treatment group, 10 patients were lost to follow-up; 5 declined to return, one moved out of state, 6 could not be contacted, and the reason for loss to follow-up was not reported for 8. The follow-up rate for survivors was 90% (91/101) for the iNO group and 91% (102/112) for the placebo group. The primary outcome for this analysis was able to be determined for 198 patients receiving iNO (95%) and 200 receiving placebo (95%); 2 patients in the iNO group could not be assigned a NDI status because of incomplete follow-up (1 missing PDI, another missing MDI and PDI). The lost to follow-up group was more likely to be black than the group that returned for neurodevelopmental follow-up (11/20 [55%] versus 62/193 [32%]; P = .0109).

**Characteristics of the Overall Cohort and Follow-up Group**

Among all patients for whom the primary outcome of death or neurodevelopmental outcome could be determined (“trial cohort”), there were no significant differences between treatment groups in major demographic and perinatal characteristics (Table I). There were also no significant differences in treatment groups in important later clinical characteristics known to be associated with adverse neurodevelopmental outcome in the follow-up cohort (Table II).

**Primary and Secondary Outcomes**

The rates of death or NDI were high for both iNO and placebo groups (78% versus 73%, respectively), and there was no significant difference between them (adjusted RR, 1.06; 95% CI, 0.95-1.17; P = .30; Table III). The rates of death or moderate to severe CP were also not significantly different in the treatment groups. In the follow-up group, there were no significant differences in treatment groups in the risk for NDI, MDI <70, PDI <70, deafness, blindness, isolated delay, or unimpaired status (Table III). The adjusted RR (95% CI) for moderate to severe CP was 2.41 (95% CI, 1.01-5.75; P = .048). Somatic measures did not differ between treatment groups (Table III).

We performed posthoc analyses to explore the potential effects of birth weight and mode of ventilation at randomization (HFV versus conventional) on outcomes (Table IV). Infants with a birth weight <1000 g who were given...
iNO had a significantly higher risk for death and for death or moderate to severe cerebral palsy. The interaction between birth weight and treatment group on death was significant ($P = .02$), but on death or moderate to severe cerebral palsy was not significant ($P = .12$). Infants who were given iNO by means of conventional ventilation had a significantly higher risk for death ($P = .02$), and for death or moderate to severe CP than infants who were given placebo ($P = .02$). The interaction between mode of ventilation and treatment group on death was significant ($P = .04$), but on death or moderate to severe CP or on moderate to severe CP in the follow-up cohort were not significant ($P = .22$ and $P = .35$, respectively). There was no significant association of birth weight group with mode of ventilation for the entire cohort ($P = .79$) or for the follow-up group only ($P = .39$). We further stratified results on the basis of birth weight category (Table V). In infants with a birth weight of 401 to 750 g, 73% (69/94) of the iNO group died, compared with 56% (55/99) of the placebo group ($P = .01$); 81% of the iNO group died or had moderate-severe CP, compared with 62% of the placebo group ($P = .0039$).

### DISCUSSION

In this follow-up analysis of the multicenter, randomized, controlled trial of iNO for premature infants with severe respiratory failure, we found that iNO was not associated with a reduction in death or NDI or with improved neurodevelopmental outcomes in survivors at 18 to 22 months of age corrected for prematurity. Indeed, we found a slightly increased risk of death or moderate to severe CP in survivors who were given iNO, and post hoc analyses suggested a higher risk of death or CP with iNO treatment in infants with a birth weight <1000 g.

In a follow-up to the single-center, double-masked, randomized, placebo-controlled trial by Schreiber et al, Messtian et al found a significant reduction in neurodevelopmental impairment in the iNO group (24%) compared with the placebo group (46%). Although our findings may appear to be inconsistent with these results, the NICHD trial differed substantially from the earlier study. Eligibility for the Schreiber trial was open to infants <34 weeks gestation and <72 hours of age who had received surfactant and were mechanically ventilated; there were no OI eligibility criteria. This likely contributed not only to the earlier age at study entry for patients in the Schreiber trial, but also to a significantly lower median OI at randomization in the follow-up cohort (6.94 in the Schreiber trial versus 17 in the PiNO trial). The early, more "routine" approach to intervention with iNO for preterm infants who were mechanically ventilated and a potentially longer period of exposure in a less severely ill population may have played roles in the observed reduction in severe morbidities and adverse neurodevelopmental outcomes. Subgroup analysis in the Schreiber trial demonstrated that only those infants with OI <6.94 (median) benefited from the treatment. These observations underscore the differences in the trials: in this analysis, only 11 of 193 survivors to follow-up had an OI at randomization <7. The Mestian study demonstrated that iNO was significantly associated with improved neurodevelopmental outcomes, even after adjustment for treatment group differences in intermediate variables such as severe IVH and BPD. This finding may imply previously reported nitric oxide mechanisms involving tolerance to cerebral ischemia and inhibition of cytokines. Such protective processes could attenuate subtle brain injury not easily demonstrated with cranial ultrasound scanning, suggesting the need for more advanced neuroimaging such as...
magnetic resonance imaging to be included in future trial designs.

The short-term results of 2 further multicenter, double-masked, randomized, placebo-controlled trials have also been published.11,12 Long-term neurodevelopmental outcome results from these trials are yet to come, but these study designs should be contrasted to the NICHD trial. The trial by Kinsella et al11 had no OI entry criteria and subsequently enrolled mildly to moderately ill preterm infants (iNO mean OI = 5.4; placebo mean OI = 5.8). Similar to the NICHD trial, there was no significant difference in death or BPD between treatment groups, but subgroup analysis showed that iNO was beneficial in infants with a birth weight of 1000 to 1250 g. The Ballard trial12 targeted preterm infants with more established lung disease. In both the Ballard and Kinsella trials, exposure to iNO was longer than that in the PiNO trial; this prolonged exposure may be needed for NO-associated lung growth and functional changes to occur.28,29 Overall, the NICHD PiNO trial sought to “rescue” a vastly more ill cohort than any of the other recent trials, and iNO exposure was relatively brief. Thus, it is not surprising that our cohort did not appear to benefit from the purported pulmonary or neuroprotective mechanisms of iNO treatment.

Our finding of an increased adjusted RR for moderate to severe CP with iNO is concerning, although it is only 1 of several secondary outcomes examined, and patient numbers are quite small. But our findings indicate that iNO use, as administered to this critically ill study cohort, certainly does not reduce the risk for moderate to severe CP. The results of our post-hoc analysis are also intriguing, but should be approached with considerable caution. Infants with a birth weight <1000 g who are given iNO appear to be at increased risk for death and death or CP. The results of our stratified birth weight analysis (Table V) suggest that this finding may be primarily explained by the outcomes of the very smallest preterm infants (401-750 g birth weight), which could prompt the need for circumspection in future trial designs. Infants who were given iNO on conventional ventilation at randomization also appear to be at increased risk. However, it is important to remember that the mode of ventilation was not randomized in this trial, and indications for conventional ventilation or HFV may have varied substantially in the centers. Therefore, discussion about the potential mechanism for this post-hoc finding would be purely speculative.

In conclusion, our findings demonstrate no benefit from iNO exposure on death or NDI or neurodevelopmental outcomes in early childhood in the severely ill premature infants in this trial. In light of previous analyses10 suggesting a reduction in adverse neurodevelopmental outcomes when iNO is administered earlier to a less severely ill preterm patient cohort, we await the follow-up studies of recently completed trials11,12 to determine the appropriate premature population, optimal timing for initiation of iNO, and length of treatment exposure. Until further data are available, routine iNO use among premature infants should be limited to research settings.

REFERENCES

These investigators participated in the Preemie Inhaled Nitric Oxide Study: Brown University Women & Infant's Hospital: William Oh, MD, Angelita Hensman, BSN, RNC, Daniel Gingras, RRT, Betty R. Vohr, MD, Lucy Noel, AS, Victoria Watson, MS, CAS, Theresa Leach, MEd, CAES; Emory University: Barbara J. Stoll, MD, Lucky Jain, MD, Ellen Hale, RN, BS, Irma Seabrook, BS, RRT-NPS, Ira Adams-Chapman, MD, Sheena Carter, PhD, Maureen Mulligan LaRossa, RN; Indiana University Riley Hospital for Children and Methodist Hospital: Greg Sokol, MD, Dianne Lorant, MD, Diana Dawn Appel, RN, BSN, Lucy Miller, RN, BSN, Dale Chrisscineks, BS, RRT, NPS, Jeff Artwood, RRT, Anna Dusick, MD, Leslie Richard, RN; Northwestern University: Robin Steinhorn, MD, Michael Sautel, RRT, Marissa de Ungria, MD, Marie Weissbourd, PhD; Stanford University Lucile Packard Children's Hospital: Krisa Van Meurs, MD, Bethany Ball, BS, CCRC, Dan Proud, RCP, Susan Hintz, MD, Julie Lee-Ancajas, PhD, Ginger Brudos, PhD; University of Alabama at Birmingham University Hospital-UAB: Waldemar A. Carlo, MD, Monica Collins, RN, BSN, Shirley S. Cosby, RN, BSN, Robert B. Johnson, RRT, Myriam Peralta-Carcelen, MD, Vivien Phillips, RN, BSN, Fred Biasini PhD, Kirstin Bailey, PhD; University of California-San Diego UCSD Medical Center and Sharp Mary Birch Hospital for Women: Neil N. Finer, MD, Maynard R. Rasmussen, MD, Chris Henderson, CRTT, Clarence Demetron, RN, Wade Rich, RRT-NPS, Christine Joseph, RRT-NPS, Kathy Arnell, RN, Yvonne Vaucher, RN, MPH, Martha Fuller, RN, MSN, Donna Posin, OTR, MPA; University of Cincinnati Hospital, Cincinnati Children's Hospital Medical Center and Good Samaritan: Jon Fridriksen, MD, Barb Warner, MD, Marcia Mersmann, RN, Barb Alexander, RN, Jody Shively, RN, Holly Mincey, RN, Mary Hoover, RRT, Sharon Sapienetz, RRT, Eric Stephenson, RRT, Jean Steichen, MD, Teresa Gratton, PA; University of Florida Wolfson Children's Hospital at Baptist Medical Center and Shands Jacksonville Medical Center: Mark Hudak, MD, Shannon Osbeck, RN, BSN, Elizabeth Case, RN, BSN, CCRC, Amanda Kellum, RRT, Lamont Hogans, RRT, David Childers, MD, Shawna Kemp, Joann Camacho, University of Rochester: Carl T. D’Angio, MD, Linda Reubens, RN, Greg Hutton, RRT, Gary Myers, MD, Diane Hust, RN, J. Kelley Yost, PhD; University of Texas Southwestern Medical Center at Dallas, Children's Medical Center Dallas, and Parkland Health and Hospital System: Abbot Laptook, MD, Susie Madison, RN, Gay Hensley, RN, Nancy Miller, RN, Glenn Metoyer, RRT, Roy Heyne, MD, Sue Broyles, MD, Jackie Hickman, RN, Janet Morgan, RN, Cristin Dooley, MS, Cathy Boatman, MS; University of Texas – Houston Memorial Hermann Children's Hospital: Kathleen Kennedy, MD, MPH, Georgia McDavid, RN, Danny Emerson, RRT, RCP, Brenda Morris, MD, Anna Lis, RN, Susan Dieterich, PhD; Medical College of Wisconsin: Ganesh Koduri, MD, Mike Paquette, RCP/CRT, Laurel Bear, MD, Sara Scott, RN, Chris Casey, Paula Jasperson; Wake Forest University Wake Forest University Baptist Medical Center, Forsyth Medical Center and Brenner Children's Hospital: Judy Aschner, MD, T. Michael O'Shea, MD, MPH, Nancy Peters, RN, B. J. Hansell, RRT, CCRC, Jennifer Griffin, RRT, RCP, Clay Adams, RRT, RCP, Robert Dillard, MD, Barbara Jackson, RN, Gail Hounshell, PhD, Ellen Waldrep, MS; Wayne State University Hutzel Women's Hospital & Children's Hospital of Michigan: Seetha Shankaran, MD, Rebecca Bara, RN, BSN, Geraldine Muran, RN, BSN, Wonder Weekfall, RRT, Yvette Johnson, MD, Deborah Kennedy, RN, Laura Goldston, MS; Yale University: Richard A. Ehrenkranz, MD, Patricia Gettner, RN, Art Caldwell, AS, RRT, Elaine Romano, MSN, Nancy L. Close, PhD, Walter S. Gilliam, PhD.

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The members of National Institute of Child Health and Human Development were: Rosemary D. Higgins, MD, Linda L. Wright, MD, Elizabeth McClure, MEd.

Central reading of head ultrasound scans was performed by: Children's National Medical Center: Dorothy Bulas, MD; University of North Carolina–Chapel Hill: David Mertens, MD; Wayne State University: Thomas Slovis, MD.

The members of the Data Safety and Monitoring Committee were: Children’s National Medical Center: Gordon Avery, MD (chair); Columbia University: Mary D’Alton, MD; RTI International: W. Kenneth Poole, PhD (ex officio); University of Virginia: John C. Fletcher, PhD (deceased); University of Washington: Christine A. Gleason, MD; University of Pittsburgh: Carol Redmond, PhD.
Figure. Patient disposition and attrition to neurodevelopmental follow-up at 18 to 22 months of age corrected for prematurity.
Funding information: NICHDI Neonatal Research Network grants 1996 to 2006

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Neurodevelopmental Outcomes of Premature Infants with Severe Respiratory Failure Enrolled in a Randomized Controlled Trial of Inhaled Nitric Oxide
Objective To evaluate whether our current practice of giving iron 18 mg daily to 6-week-old infants with very low birth weight (VLBW) was associated with increased oxidative stress markers or decreased antioxidant status.

Study design The study was a prospective observational study of 21 healthy VLBW infants (born at gestational age <32 weeks, birth weight <1500 g). Blood and urine were sampled twice before starting iron supplementation at 6 weeks postnatal age and after 1 week of iron supplementation at age 7 weeks. Urine 8-isoprostane was analyzed by gas chromatography–mass spectrometry and plasma total hydroperoxides were measured. Antioxidant status was assessed by ascorbic acid (vitamin C), α-tocopherol (vitamin E), ferric-reducing ability of plasma, and plasma glutathione.

Results After 1 week of iron supplementation, no significant changes in urine 8-isoprostane or plasma total hydroperoxides were seen, and plasma antioxidants were largely unchanged.

Conclusions Markers of oxidative stress in urine and plasma antioxidant status in healthy VLBW infants fed human milk remained unchanged after high-dose oral iron supplementation. (J Pediatr 2007;151:23-8)

Preterm infants are at risk for development of an iron deficiency anemia during their first year of life, caused by the low iron stores at birth compared with the large weight gain. This anemia is prevented by oral iron supplementation.

Oxidative stress is defined as an imbalance between prooxidant damage and antioxidant protection. Although iron supplementation may theoretically increase oxidative stress in vulnerable preterm infants, oxidative stress studies in the newborn period have often been done on animals or in vitro, with few studies in preterm infants. Human milk is the preferred nourishment for both term and preterm infants. The iron content of human milk is low but usually adequate for term infants during the first 6 months of life. All infants in Norway with birth weight less than 2500 g, however, are given a standard dose of oral ferrous fumarate 9 mg twice daily from 6 weeks until 1 year of age to prevent depleted iron stores and iron deficiency in accordance with suggestions from long-standing studies by Gairdner et al. Iron supplementation to preterm infants is standard treatment in most countries, but the dose and iron preparation vary. The ferrous iron dose used in Norway is relatively large (18 mg/d). A consequence of this fixed dose, irrespective of weight and age, is that the smallest infants will get the highest doses per weight unit. In the United States, the iron dose is given according to body weight, usually ferrous sulfate (3 to 6 mg/kg/d). The aim of this study was to evaluate whether the standard practice of giving 18 mg/d enteral iron supplementation to very low–birth weight (VLBW) infants from 6 weeks of age is associated with increased markers of oxidative stress or decreased antioxidant status.

METHODS

Study Subjects

The study was performed from March 2003 to December 2004 in the neonatal intensive care unit at Ulleval University Hospital in Oslo. Healthy, fully enterally fed VLBW infants (birth weight <1500 g and born at a gestational age <32 weeks) were...
studied, beginning at postnatal age 5 weeks. The 2 sampling times before the iron therapy (samples taken at weeks 5 and 6) were chosen to evaluate any alterations possibly induced by age and growth, before introduction of iron.

Infants were not eligible if they had serious diseases or malformations, if they needed more than 30% inspired oxygen or ventilator assistance at 5 weeks of age, or received a red blood cell (RBC) transfusion within 4 days before study start. Infants were withdrawn from the study if infections or the need for RBC transfusion occurred. The study was approved by the Regional Committee of Medical Research Ethics in Eastern Norway, and all parents gave written informed consent.

**Nutrition**

The standard procedure in our unit was followed. All infants were fed human milk (either own mother’s milk, donor milk, or both), orally or by feeding tube. Human milk was fortified with bovine milk protein fortification for infants weighing less than 2000 g, with 0.8 g PreSemp (Semper AB, Sweden) used per 100 mL human milk. PreSemp does not contain iron. Vitamin D (10 µg) and folic acid (0.1 mg) was given daily from day 5. Oral vitamin E (15 mg daily) was given to infants weighing less than 1500 g and to those receiving oxygen therapy. Oral iron supplementation was begun at 6 weeks of age as ferrous fumarate NeoFer (Nycomed receiving oxygen therapy. Oral iron supplementation was begun daily from day 5. Oral vitamin E (15 mg daily) was taken for determination of total hydroperoxides, vitamin C, total antioxidants, and total glutathione. These analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, analyses were performed at the Department of Nutrition, University of Oslo.

**Biologic Samples**

Baseline blood and urine samples were taken at age 5 weeks (ie, 1 week before commencing oral iron supplementation), again at week 6 (immediately before start of iron supplementation), and after 1 week of iron supplementation (week 7). Routine capillary blood testing at these 3 times included analyses of whole blood (hemoglobin, mean cellular volume, reticulocyte count) performed on a Sysmex XE2100 (Sysmex, Germany). Serum concentrations of iron, transferrin, C-reactive protein, bilirubin, and gamma-glutamyl transferase (GGT) were measured with the Cobas Integra 800 (Roche Diagnostics AS, Basel, Switzerland). Ferritin was measured with Centaur (Bayer Diagnostica AS, Leverkusen, Germany).

In addition to routine blood samples, samples were taken for determination of total hydroperoxides, vitamin C, vitamin E, total antioxidants, and total glutathione. These analyses were performed at the Department of Nutrition, University of Oslo. For the analysis of total glutathione, 25 µL 2 mol/L serine borate/mL whole blood was added to 1 of the 2 blood sampling tubes before the blood sampling to inhibit GGT, which degrades glutathione. The heparinized blood samples were kept on ice for a maximum of 30 minutes and spun in a centrifuge at 4°C and 2000 g for 10 minutes. For vitamin C determination, plasma 50 µL was mixed with 50 µL 10% meta-phosphoric acid in an Eppendorf tube and immediately frozen with the other samples at −76°C and stored until analysis. Measurements were done in duplicates except for vitamin C, vitamin E, and glutathione.

On the 3 sampling time points of the study, urine was also collected in urinary sample bags for 3 to 4 hours. Urine creatinine concentration was analyzed in 0.5 mL urine from each urine sample (Cobas Integra 800). Urine was kept at 4° to 8°C for up to 4 hours until frozen at −76°C.

**Markers of Oxidative Stress**

Urinary concentration of 8-iso-prostaglandin F_2α (8-isoprostanate) and its endogenous beta-oxidation metabolite, 2,3-dinor-8-isoprostaglandin-F(2-α) (2,3-dinor) was analyzed by gas chromatography–mass spectrometry at a collaborating laboratory in the United Kingdom, as described by Bessard et al, after shipment on dry ice. Urine concentration of 8-isoprostanate and 2,3-dinor (pg/mL) was related to urine creatinine concentration (µmol/mL), and the results were given in ng/µmol creatinine. The samples were analyzed in triplicate. Total hydroperoxides was measured in plasma with a diacron-reactive oxygen metabolite test from Diacron s.r.l. (Grosseto, Italy) as described previously, and the results are expressed in arbitrary units called “Carratelli units.”

**Antioxidant Status**

The determination of vitamin C (ascorbic acid) in 50 µL plasma was done with high-performance liquid chromatography (HPLC). Vitamin E (α-tocopherol) in 100 µL plasma was analyzed by an HPLC system as described by Richenheimer et al., except that a fluorescence detector with excitation and emission wavelength set to 295 and 330 nm, respectively, was used instead of diode array detector. The ferric-reducing ability of plasma (FRAP) assay was used to measure the concentration of antioxidants in plasma with a Technicon RA 1000 system (Technicon Instruments Corporation, New York, NY) as described by Benzie et al, with the exception that the samples were not diluted and the photometric measurements were done at 600 nm. Glutathione one determination by HPLC in plasma was done with a homocysteine kit from Bio-Rad (Bio-Rad Laboratories, Munich, Germany) as described by the manufacturer, except that all volumes were scaled down. The kit is validated by Bohn et al for also measuring glutathione concentrations.

**Statistics**

As all test results were not normally distributed, medians are reported (and 95% confidence intervals for the median), and Wilcoxon test for paired data was used for comparison between results from each study week. Mann-Whitney U test was used for comparison between groups. Spearman’s correlation was used to calculate correlation coefficients.

Statistical analyses were performed with Statistical Package for the Social Sciences (version 12.0; SPSS Inc, Chicago, Ill.). P < .05 was considered statistically significant.
**RESULTS**

During the study period, 105 VLBW infants were born in the hospital. By 5 weeks of age, 6 infants had died, 14 did not fulfill the inclusion criteria (6 were too ill to enter the study, 6 required ventilator support, 1 had been given iron, and 1 needed a transfusion just before entering the study), 24 had been transferred to a local hospital, 26 were discharged home, 5 infants were participants in another study, and the parents of 4 declined participation. Of the remaining 26 infants originally recruited for the study, 4 dropped out before the last urine or blood sample, and 1 was excluded because of receiving a blood transfusion after study inclusion. None of the included infants had infection develop during the study period and were assessed clinically and by C-reactive protein values (all within normal range, data not shown). Clinical characteristics of the 21 participating infants (7 males, 14 females) are shown in Table I.

The hematologic findings and iron, ferritin, transferrin saturation, and bilirubin concentrations are presented in Table II. Between weeks 5 and 6, serum iron concentrations remained unaltered, whereas ferritin concentration decreased significantly. After 1 week of iron supplementation, a statistically significant increase was observed in serum iron concentration and transferrin saturation (Table II), whereas the increase in ferritin concentration between weeks 6 and 7 did not reach statistical significance.

RBC transfusion had been given to 10 of the 21 infants 4 days before the start of the study. In the group of infants with birth weight below 1000 g, 7 of 10 infants had been given transfusion, whereas 3 of 11 larger infants (1000-1499 g) had been given transfusion before study start. In the group that had received RBC transfusion, median ferritin concentration was higher than in the group that had not received transfusion before the start of the study (week 6: 90 μg/L vs 38 μg/L, P = .01). Ferritin concentration at week 6 (and 5) correlated positively with the number of RBC transfusions the infant had received (Spearman’s correlation 0.61, P = .005).

The urine and plasma concentrations of oxidative stress markers, as well as plasma antioxidant concentrations, are presented in Table III. A significant decrease in plasma vitamin E was observed from week 5 to 6, the 2 time points before iron treatment. After 1 week of iron supplementation, median concentrations of urine 8-isoprostane and 2,3-dinor remained unchanged. The Figure demonstrates the individual 8-isoprostane urine concentrations at each time point measured. There was a significant positive correlation between 8-isoprostane and 2,3-dinor urinary concentrations analyzed at all time points (week 6: r = 0.90, week 6: r = .077, week 7: 0.85; P < .001). The 8-isoprostane or 2,3-dinor urinary concentrations did not correlate with infant weight at any time point, nor with gestational age, birth weight, or weight gain (data not shown).

Also for the other marker of oxidative stress, plasma concentration of total hydroperoxides, no significant increase was found after 1 week of iron supplementation (Table III). No correlation was found between 8-isoprostane or 2,3-dinor urinary concentrations and total hydroperoxides at any time point (data not shown).

Median plasma concentrations of most antioxidants remained largely unaltered through the 2 study weeks (from week 5 to 7), as shown in Table III. A small decrease was observed in the plasma vitamin E, FRAP, and total glutathione concentrations from week 6 to week 7.

Because the infants in the lowest weight group received a relatively higher iron dose per weight unit, we compared the group of the very smallest infants (birth weight <1000 g, n = 10) with the group of larger infants (birth weight 1000-1499 g, n = 11). We found no significant differences between these groups regarding plasma or urine concentrations of oxidative stress markers. There was no evidence for increased oxidative stress in the smallest infants as compared with the subgroup of larger infants, also when relating oxidative stress markers to their actual weight at start of iron supplementation (data not shown).

At study start (week 5), 9 of the infants received vitamin E supplementation (5 weighed less than 1500 g, 2 had just reached 1500 g, and 2 received a low dose of oxygen on a nasal catheter); by the end of the study 5 infants received vitamin E (4 infants were still under 1500 g and one received oxygen). Vitamin E plasma concentration was higher in the smallest infants (birth weight <1000 g), compared with the group of larger infants (birth weight 1000-1499 g) at week 6 (28.5 vs 17.0 μmol/L, P < .001). However, at week 7, when fewer of the smallest infants received vitamin E, the difference between the 2 subgroups was not significant (22.0 vs 17.4 μmol/L, P = .11).

**DISCUSSION**

Oxidative stress markers and plasma antioxidant status did not change significantly during the second month of life in healthy VLBW infants treated for 1 week with high doses of enteral iron supplementation. Additionally, there were no major changes in the measured variables before the introduction of iron, that is, between weeks 5 and 6, indicating no significant age-induced differences in oxidative stress markers or antioxidant status in this time period.

The 8-isoprostane is a highly reliable and stable marker of oxidative stress and is found in increased concentrations...
in conditions associated with oxidative stress. Urinary 8-isoprostan e measurement has the advantage of excluding ex vivo production of 8-isoprostane, because urine lipid concentration is minimal, and storage at 4°C before freezing is a sufficient handling procedure. Urine from preterm infants has a low creatinine concentration, and the isoprostane concentrations are best expressed relative to creatinine concentration. Urinary 8-isoprostane measurement is a noninvasive method that has been used in adults to monitor oxidative stress after 1 week of oral iron supplementation and in preterm infants to evaluate whether polyunsaturated fatty acid supplementation in preterm formulas increases oxidative stress.

Vitamin C (ascorbic acid) is a powerful antioxidant often consumed first in situations of oxidative stress, and it has been found to prevent lipid peroxidation in situations of excess iron. The ratio of oxidized to total ascorbic acid (DHAA/TAA) has been suggested as a more sensitive measure of oxidative stress. The concentrations of ascorbic acid, dehydroascorbic acid, as well as their ratio remained stable throughout our study period supporting the results of our other analyses, concluding that oxidative stress did not increase after oral iron supplementation.

In this study, the median vitamin E concentrations showed a gradual and significant decrease throughout the study period, from week 5 to 6 and week 6 to 7. This is most likely explained by the cessation of vitamin E supplementation, which is only given until the infant reaches 1500 g or no longer needs supplemental oxygen, which for many of the infants occurred around the latter part of the study period.

Plasma concentration of GGT is part of a routine biochemical blood analysis and has been proposed as a reliable, easy, and inexpensive measure of oxidative stress. GGT is involved in recycling of glutathione, and GGT will increase in a situation of oxidative stress. In our study, we found no increase in plasma concentrations from weeks 5 to 7; the concentrations were higher than in adults; reference values for infants are elevated and decrease to adult levels around 5 months of age.

### Table II. Hematologic, bilirubin, and iron values for the 21 included infants

<table>
<thead>
<tr>
<th>Marker</th>
<th>Week 5</th>
<th>Week 6</th>
<th>P*</th>
<th>Week 7</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hemoglobin (g/100mL)</td>
<td>9.7 (9.0-11.0)</td>
<td>9.1 (8.3-10.0)</td>
<td>.004‡</td>
<td>8.9 (8.6-9.5)</td>
<td>.07</td>
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<tr>
<td>Mean cellular volume (fl)</td>
<td>95 (93-98)</td>
<td>94 (91-95)</td>
<td>.006‡</td>
<td>90 (88-92)</td>
<td>.001‡</td>
</tr>
<tr>
<td>Reticulocytes (10⁹/L)</td>
<td>140 (114-158)</td>
<td>125 (114-146)</td>
<td>.07</td>
<td>125 (90-185)</td>
<td>.8</td>
</tr>
<tr>
<td>Iron (µmol/L)</td>
<td>11.6 (9.9-13.2)</td>
<td>12.2 (10.0-13.8)</td>
<td>.5</td>
<td>14.4 (12.5-17.0)</td>
<td>.005‡</td>
</tr>
<tr>
<td>Ferritin (µg/L)</td>
<td>84 (46-132)</td>
<td>59 (30-90)</td>
<td>.002‡</td>
<td>63 (42-111)</td>
<td>.7</td>
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<tr>
<td>Transferrin saturation (%)</td>
<td>27.1 (20-30)</td>
<td>26.3 (17-34)</td>
<td>.8</td>
<td>33.8 (27-43)</td>
<td>.001‡</td>
</tr>
<tr>
<td>Bilirubin (µmol/L)</td>
<td>65.0 (37-179)</td>
<td>76.0 (39-163)</td>
<td>.4</td>
<td>75.5 (25-98)</td>
<td>.4</td>
</tr>
</tbody>
</table>

The results are given as medians and 95% confidence intervals of the median.
*P* value for comparison of week 5 and 6.
†P value for comparison of week 6 and 7.
‡P value < .05.

### Table III. Results of the measurements of markers of oxidative stress and antioxidants

<table>
<thead>
<tr>
<th>Marker</th>
<th>Week 5</th>
<th>Week 6</th>
<th>P*</th>
<th>Week 7</th>
<th>P†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Urine 8-isoprostane (ng/µmol creatinine)</td>
<td>0.27 (0.15-0.45)</td>
<td>0.29 (0.22-0.46)</td>
<td>.8</td>
<td>0.29 (0.20-0.48)</td>
<td>.8</td>
</tr>
<tr>
<td>Urine 2,3 dinor (ng/µmol creatinine)</td>
<td>1.01 (0.72-1.64)</td>
<td>1.09 (0.86-1.34)</td>
<td>.7</td>
<td>1.00 (0.82-1.63)</td>
<td>.8</td>
</tr>
<tr>
<td>Plasma total hydroperoxides (Carr U)</td>
<td>77.0 (60-97)</td>
<td>78.0 (68-108)</td>
<td>.1</td>
<td>81 (72-110)</td>
<td>.5</td>
</tr>
<tr>
<td>Vitamin C (ascorbic acid, AA) (µmol/L)</td>
<td>33.9 (23.8-41.6)</td>
<td>36.6 (28.4-46.1)</td>
<td>.8</td>
<td>29.7 (21.3-48.7)</td>
<td>.5</td>
</tr>
<tr>
<td>Total ascorbic acid (TAA) (µmol/L)</td>
<td>42.7 (28.2-52.9)</td>
<td>43.5 (35.4-58.6)</td>
<td>.7</td>
<td>37.8 (25.7-58.6)</td>
<td>.6</td>
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<tr>
<td>Dehydroascorbic acid (DHAA) (µmol/L)</td>
<td>7.00 (4.4-10.1)</td>
<td>6.00 (3.4-10.6)</td>
<td>.4</td>
<td>4.60 (2.9-6.7)</td>
<td>.6</td>
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<tr>
<td>Ratio DHAA/TAA</td>
<td>0.15 (0.09-0.21)</td>
<td>0.17 (0.08-0.2)</td>
<td>.9</td>
<td>0.13 (0.10-0.17)</td>
<td>.4</td>
</tr>
<tr>
<td>Vitamin E (alpha tocopherol) (µmol/L)</td>
<td>29.2 (19.3-36.3)</td>
<td>22.9 (17.3-28.3)</td>
<td>.001‡</td>
<td>20.5 (16.4-22.9)</td>
<td>.002‡</td>
</tr>
<tr>
<td>FRAP (µmol/L)</td>
<td>1137 (822-1500)</td>
<td>1056 (940-1340)</td>
<td>.6</td>
<td>1040 (866-1301)</td>
<td>.04‡</td>
</tr>
<tr>
<td>GGT (U/L)</td>
<td>93 (53-176)</td>
<td>84 (44-198)</td>
<td>.3</td>
<td>92 (47-148)</td>
<td>.1</td>
</tr>
<tr>
<td>Total glutathione (µmol/L)</td>
<td>3.9 (2.7-7.3)</td>
<td>4.4 (3.2-6.4)</td>
<td>.9</td>
<td>4.05 (2.9-5.6)</td>
<td>.03‡</td>
</tr>
</tbody>
</table>

AA, Ascorbic acid; TAA, total ascorbic acid; DHAA, dehydroascorbic acid.
The results are given as medians and 95% confidence intervals of the median.
*P* value for comparison of week 5 and 6.
†P value for comparison of week 6 and 7.
‡P value < .05.
Glutathione is a major intracellular antioxidant. Plasma concentrations are much lower than the intracellular levels, but increased oxidative stress is to some extent reflected as decreased plasma glutathione concentrations. The normal plasma concentration for adults, using the same method as in our study, is 1 to 5 μmol/L, which is of similar magnitude to the infant concentrations found in our study. In this study, we found a slight decrease in infant plasma glutathione concentration after 1 week of iron supplementation, possibly indicating some degree of oxidative burden, which was compensated because the other plasma and urine markers of oxidative stress were unaltered in our study.

Because our indicators did not reveal increased oxidative stress after oral iron supplementation and the sample size was limited, we estimated the detectable difference for our main outcome, urine isoprostane. Before commencing the study, we did not have the standard deviation of the difference before and after iron supplementation, which is necessary for a power calculation of a paired study. We therefore aimed at recruiting 20 infants total in this pilot study. Retrospectively, in our study, we found that the mean urine concentration of 8-isoprostane/creatinine was 0.35 ng/μmol (median 0.29 ng/μmol), and the standard deviation of difference between before and after iron supplementation was 0.24 ng/μmol. Thus, with a power of 80% and a significance level of 0.05, the detectable difference was 0.156 ng/μmol, that is, we could have detected a 50% increase in our patient group after iron supplementation.

This study was not aimed at evaluating whether our standard high dose of iron is necessary or what proportion is absorbed. Iron status in preterm infants is complex to interpret because of developmental changes, but these iron variables indicate that the enteral iron supplementation is being absorbed. At this age, with a large weight gain (median weight gain 170 g/wk), a decrease in both iron and ferritin concentrations would have been expected if no iron supplementation had been provided.

Both ferrous sulfate and ferrous fumarate can be used as iron supplementation, and we are not aware of studies comparing these compounds in preterm infants. In older children, a better absorption for ferrous sulfate than ferrous fumarate was found in one study, whereas another study found equivalent absorption. One in vitro study found that ferrous fumarate induced less oxidative stress than ferrous sulfate when added to human milk. Thus in preterm infants there could be differences between ferrous sulfate and ferrous fumarate, both in absorption and tendency to induce oxidative stress.

The use of several methods for evaluating oxidative stress strengthens the conclusion that iron supplementation does not seem to increase oxidative stress in healthy preterm infants of this age. In support of our conclusion that oral iron supplementation does not induce oxidative stress, there is 1 report on iron- and erythropoietin-treated infants finding no sign of oxidative stress. The utility of zinc protoporphyrin to heme ratio, as an indicator of iron status in preterm infants, and also oxidative stress was evaluated, finding that serum and urine isoprostanes were not altered during iron sulfate supplementation, using doses up to 12 mg/kg.

The results from our group of healthy infants could be used as a reference for future studies of oxidative stress or antioxidant intervention. The oxidative status of younger infants or infants on other diets may differ from our group of infants fed human milk. Studies of oxidative stress in even smaller and sicker infants after iron supplementation should be undertaken, rather than extrapolating these results to other groups of preterm infants.

Wenche Sollien is acknowledged for her assistance in blood sampling and data collection.

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50 Years Ago in The Journal of Pediatrics

BRAIN ABCESS IN INFANTS


Munslow et al reported 4 cases of brain abscess in infants <6 weeks old. They noted 4 remarkable aspects of the cases: 1) young age; 2) lack of congenital predisposing condition or clinical source of infection outside the central nervous system; 3) survival of 3 of 4 infants, all treated with surgical drainage procedures and newly available “miraculous” antibiotics (eg, chloramphenical, tetracycline, streptomycin); and 4) the new problem of long-term management of hydrocephalus. We would note 5 remarkable aspects of the cases or state-of-the-art medical care in the 1950s: 1) unusual spontaneous pathogens (eg, Pseudomonas aeruginosa, Enterobacter aerogenes, Proteus species); 2) lack of any imaging capacity; 3) lack of bactericidal antibiotics with ability to cross the blood-brain barrier; 4) seeming medical misadventures (eg, intraparenchymal streptokinase-streptodornase); and 5) rudimentary techniques to manage hydrocephalus (eg, trephination, ventriculomastoidotomy).

Remarkable advances have been made in all aspects of management of brain abscess and in prevention in many instances with early treatment of meningitis by using highly effective agents. However, brain abscesses still occur in infants in the 21st century, predominantly as a complication of therapies to save or sustain lives of neonates who would certainly not have survived 50 years ago. The pathogens once again are unusual gram-negative bacilli, and frequently are resistant to the “miraculous” antibiotics developed in the interim.

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Transient Tachypnea of the Newborn May Be an Early Clinical Manifestation of Wheezing Symptoms

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Objective  To determine risk factors associated with transient tachypnea of the newborn (TTN) and whether TTN is associated with development of wheezing syndromes in early life.

Study design  The Population Health Research Data Repository at the Manitoba Centre for Health Policy is a healthcare administrative and prescription database. Data for children diagnosed with a wheezing syndrome (defined as bronchiolitis, acute bronchitis, chronic bronchitis, asthma, or prescription for asthma medication) were obtained. Term children diagnosed with TTN at birth were selected. Cox proportional hazards regression analysis for time to first event of hospitalizations, physician visits, or prescription for an asthma medication up to 7 years of age were calculated. The hazard ratios for wheezing in a child with TTN were compared with healthy newborns.

Results  Twelve thousand seven hundred sixty-three children were born at term in 1995 and currently live in the province of Manitoba. Of these children, 308 (2.4%) developed TTN. Maternal asthma, birth weight >4500 g, male sex, and urban location were risk factors for development of TTN. Infants with TTN at birth were at significantly increased risk of a wheezing disorder in childhood (adjusted hazard ratio [HR] = 1.17, 95% CI 1.02-1.34).

Conclusion  TTN is associated with development of wheezing syndromes in childhood.

(J Pediatr 2007;151:29-33)

Transient tachypnea of the newborn (TTN) was initially described by Avery et al1 in 1966. Historically, TTN has been viewed as a "transitory respiratory disturbance resulting from a delay in alveolar fluid resorption."1-3 It is characterized chiefly by tachypnea shortly after birth, which clears quickly within 2 to 5 days. Although associated with some morbidity, it is generally believed that once TTN resolves, there is no further increased risk for respiratory disease or other long-term sequelae.1-8

TTN has been reported to occur more frequently in preterm birth, cesarean delivery, and birth of a male infant.9-10 However, the etiology and pathogenesis of this condition are largely unknown. The fetal lung fluid is normally absorbed before and during delivery, and any delay in this physiological event may cause TTN.1,3 The primary mechanism of clearance involves active Na+ transport by the distal pulmonary epithelium.11-13 As well, activation of β- and β2-receptors has been shown to convert the in utero fetal lung from a fluid-secreting to a fluid-absorbing organ.14,15 The surge in fetal catecholamine secretion at birth provides a critical link between β-adrenergic stimulation and lung liquid clearance.11,13,16 It is thought that the suboptimal clearance of lung fluid in the newborn is a cause of TTN. However, once the fluid is resorbed, TTN is a self-limiting disease, with no future adverse sequelae.

Using a population-based database, the aim of this study was to determine if TTN is truly transient and innocuous as believed, or if it is associated with an increased risk of developing a wheezing syndrome early in life.

METHODS

This research is from the SAGE (Study of Asthma, Genes and the Environment) project, which is a study of the origins of asthma in a 1995 Manitoba birth cohort. One
of the research platforms for SAGE is the analysis of records from the Population Health Research Data Repository at the Manitoba Centre for Health Policy (a population-based, linked healthcare administrative and prescription database). This repository contains anonymized encounter-based records of individuals’ interactions with the provincial healthcare system. It is derived from information received by Manitoba Health, as part of the provision of the universal healthcare insurance program in the province. The complete healthcare records of the 1995 birth cohort, which includes physician visits, hospitalizations, and prescription records, were available for analysis. Records of physician reimbursement for medical care provided are submitted under a fee-for-service arrangement, and they contain information on patient diagnosis at the three-digit level of the ICD-9-CM classification system. Discharge abstracts for hospital services include information on up to 16 ICD-9-CM diagnostic codes, of which the first diagnosis is the primary diagnosis responsible for the hospital stay. Prescriptions records are submitted by retail pharmacies for reimbursement by provincial drug insurance plans and for drug utilization review purposes, and they contain data on the date of prescription dispensing, drug name and identification number, dosage form, and quantity dispensed. These databases are reliable and valid data sources for describing healthcare contact for specific conditions and prescriptions dispensed.17-18 Record linkages among databases are achieved by the use of anonymized personal identifiers. A family registration number present in the registry permits linkage of healthcare data of children with that of their parents and siblings.

Term children (gestational age 37-41 6/7 weeks) diagnosed with TTN (ICD-9 code of 770.6) at birth were selected. Analyses were performed using this group (TTN), and a subgroup in which other pulmonary insults (PI) at birth were excluded (TTN-PI) (ie, a diagnosis of ICD-9 code of 770.6, removing any child who also had an ICD-9 code of 769 [respiratory distress syndrome], 770.1 [massive aspiration syndrome], 770.2 [interstitial emphysema and related conditions, including pneumothorax], 770.3 [pulmonary hemorrhage], 770.4 [primary atelectasis], 770.5 [other and unspecified atelectasis], 770.7 [chronic respiratory disease arising in the perinatal period], 770.8 [other respiratory problems after birth], 770.9 [unspecified respiratory condition of fetus and newborn], 9609 [Nonoperative intubation and irrigation], or 9605 [other intubation]).

The number of children diagnosed with a wheezing syndrome (defined as hospital/physician visit ICD-9 code of 466 [acute bronchitis and bronchiolitis], 490 [bronchitis not specified], 491 [chronic bronchitis], 493[asthma], or a prescription for an asthma medication) was obtained. The risks of wheezing up to age 7 years (reported as hazards ratios, HRs) subsequent to TTN or TTN-PI were determined in healthy term newborns. Stratification by presence or absence of maternal asthma was also performed (defined as hospital/physician visit ICD-9 code of 493 from 1990 to 1995 or a prescription of an asthma medication in 1995) to determine the effect of genetics on TTN and future wheeze.

The study was approved by the Health Research Ethics Board at the University of Manitoba and the Health Information Privacy Committee of Manitoba.

Statistical Analysis

Logistic regression was performed to determine the risk factors (odds ratio, 95% CI) associated with TTN. The goodness of fit for the multivariate model was tested with the likelihood ratio test and the Hosmer and Lemeshow statistic. In this study, time-to-event was defined as the number of days from the child’s birth date to the first reporting of index diagnosis. Proportional hazards assumption was tested by examining the interaction of TTN or TTN-PI covariate with the log (time) and also by the examination of the weighted Schoenfeld residuals.19,20 Examination of the residuals revealed no outliers. To assess the effect of maternal asthma on TTN, Cox proportional hazards regression was conducted on models stratified by maternal asthma. All statistical analyses were performed with Statistical Analysis Systems software.21

RESULTS

In 1995, 16,320 children were born in the province of Manitoba, of which 13,980 (85.7%) continue to live in the province in 2002. Of the 13,980, 12,763 children were born at term. Three hundred eighty-two (2.4%) children developed TTN at birth. Two hundred forty-eight (1.9%) had TTN-PI (A sole diagnosis of TTN at birth excluding other PIs) (Table I).

Risk factors associated with the development of TTN include birth weight ≥4500 g, maternal asthma, male sex, urban location, and cesarean section (Table II).

Table III shows the unadjusted and the adjusted HRs for TTN and TTN-PI. The reference categories for the TTN

| Table I. Demographics of all children and children with TTN |
|------------------|------------------|
|                  | Number (%) in children with TTN |
| TTN              | 12,763           |
| Maternal asthma  | 1315             |
| Male sex         | 6459             |
| Cesarean section | 1723             |
| Low income at birth | 5841           |
| Birthweight <2500 g | 215              |
| Birthweight ≥4500 g | 337              |
| Urban location   | 7344             |
| TTN-PI           | 12,703           |
| Maternal asthma  | 1309             |
| Male sex         | 6421             |
| Cesarean section | 1696             |
| Low income at birth | 5818           |
| Birthweight <2500 g | 214              |
| Birthweight ≥4500 g | 332              |
| Urban location   | 7308             |

| Table II. Demographics of all children and children with TTN |
|------------------|------------------|
|                  | Number (%) in children with TTN |
| TTN              | 12,763           |
| Maternal asthma  | 1315             |
| Male sex         | 6459             |
| Cesarean section | 1723             |
| Low income at birth | 5841           |
| Birthweight <2500 g | 215              |
| Birthweight ≥4500 g | 337              |
| Urban location   | 7344             |
| TTN-PI           | 12,703           |
| Maternal asthma  | 1309             |
| Male sex         | 6421             |
| Cesarean section | 1696             |
| Low income at birth | 5818           |
| Birthweight <2500 g | 214              |
| Birthweight ≥4500 g | 332              |
| Urban location   | 7308             |
Table II. Factors associated with the development of TTN

<table>
<thead>
<tr>
<th>Factor</th>
<th>Odds ratio</th>
<th>95% confidence interval</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weights</td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;2500 g</td>
<td>0.98</td>
<td>0.40-2.43</td>
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<tr>
<td>2500-4499 g</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>≥4500 g</td>
<td>1.85</td>
<td>1.11-3.09</td>
</tr>
<tr>
<td>Maternal asthma (1)</td>
<td>1.48</td>
<td>1.07-2.05</td>
</tr>
<tr>
<td>No maternal asthma</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Cesarean section</td>
<td>3.63</td>
<td>2.85-4.61</td>
</tr>
<tr>
<td>No cesarean section</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>1.68</td>
<td>1.33-2.13</td>
</tr>
<tr>
<td>Female</td>
<td>1.00</td>
<td></td>
</tr>
<tr>
<td>Urban (2)</td>
<td>1.27</td>
<td>1.01-1.62</td>
</tr>
<tr>
<td>Rural</td>
<td>1.00</td>
<td></td>
</tr>
</tbody>
</table>

The likelihood ratio test for the above model has a P value < .0001 and the Hosmer and Lemeshow the statistic of P value = .43, which suggest that the model is a good fit to the data. Birth weight, cesarean section, and male sex were coded from hospital birth record. Cesarean section, male sex, and urban location were entered in the model as dummy variables. A dummy variable for income was initially included in the model but was later removed because it was not significant. Income variable used was determined by postal code regions and associated socioeconomic status.

*Reference category.
(1) Maternal asthma was defined by ICD-9 code 493 from 1990-1995 or at least one prescription of an asthma medication in 1995.
(2) Urban locations include the cities of Brandon and Winnipeg.

Numbers in parentheses are 95% confidence interval.

DISCUSSION

Using a population-based healthcare administrative and prescription database, we have found that TTN is a risk factor for a future wheezing syndrome, and it may not be as transient as previously thought. Several studies have shown an association between TTN and the development of asthma. Schaubel et al studied 16,588 children born in the province of Manitoba from 1984 to 1985 and found that TTN had an odds ratio of 1.38 (CI 1.00-1.92) for development of physician-diagnosed preschool asthma (up to 4 years of age). The odds ratio increased to 2.08 (CI 1.12-3.86) for hospital admissions for asthma. Shohat et al followed 58 newborns with TTN and 58 controls up to the age of 5 years. They found that children with TTN had a significantly higher incidence of recurrent episodes of wheezy breathlessness, symptoms consistent with asthma, and signs consistent with atopy (hay fever and atopic dermatitis). Smith et al performed a retrospective cohort study using Scottish morbidity record data of maternity, neonatal, and acute hospital discharges. They found that children who were diagnosed with TTN or respiratory distress syndrome were at increased risk for being admitted to a hospital with a diagnosis of asthma. Their conclusion was that neonates who experience respiratory distress syndrome or TTN at term are at increased risk for asthma in childhood. The relationship was unaltered by adjusting for hospital of birth, maternal age, height, deprivation category, parity and smoking, induction of labor, week of gestation at delivery, and the children's birth weight, sex, and Apgar score.

Independent risk factors for the development of TTN include large birth weight, maternal asthma, cesarean section, sex, and urban location (population > 50,000). Cesarean section has been well described as being a risk factor for TTN because of the absence of surge in catecholamines normally released in a vaginal delivery. This surge results in a β-adrenergically mediated response and subsequent Na⁺ pump absorption of fluid in the distal airways. Male sex and macrosomia have also been associated with increased TTN. Finally, the fact that our urban children had more
TTN does correlate with the increased prevalence of asthma found in urban centers compared with rural areas.

Several studies have found that maternal asthma may be a risk factor for TTN. Demissie et al. used a historical cohort analysis and found that maternal asthma is a risk factor for TTN. The presence of maternal asthma could be one explanation for our finding of an increased risk of wheeze in children with TTN. If more of these children with TTN have mothers with asthma, it would explain why the children would subsequently have more asthma. This leads us to question whether there are physiological or structural differences present at birth that result in TTN. One potential mechanism for the association between TTN and asthma has been the possible genetic predisposition for $\beta$-adrenergic hyporesponsiveness in these infants and mothers with asthma. The critical link may be the association of $\beta$-adrenergic response and activation of Na transport in fetal alveolar epithelium required to help clear neonatal lung fluid. Thus, TTN may be the first manifestation of asthma in these children.

Although genetics may play a role in the development of TTN, and in subsequent wheeze, environmental forces could also be a contributing factor. In 1995, almost all children admitted to our neonatal intensive care unit, and eventually diagnosed with TTN, were treated with intravenous antibiotics for at least 48 hours before blood cultures were reported as negative. This may have modified the gastrointestinal flora, which then would no longer provide flora-protective influence on the development of allergy and asthma. The composition of intestinal microbial flora in the neonatal period may be an important factor that affects the immune system maturation and the development of tolerance to allergens. Differences in the intestinal microbial flora between allergic and nonallergic infants have been reported. In a prospective study of 44 infants, Bjorksten et al. showed that the prevalence of bifidobacteria in feces was lower between 1 week and 12 months of age in atopic children compared with controls. Kalliomaki et al. prospectively followed up 76 infants at risk for atopy and found that the ratio of fecal counts of bifidobacteria to clostridia was reduced at 3 weeks of age in children who subsequently developed atopy by 12 months of age. We hypothesize that early life antibiotic treatment may modify the gut flora, and this may predispose the child toward development of allergy and asthma as suggested by the Hygiene Hypothesis.

We stratified our cohort into those children whose mothers have asthma and those who do not in an attempt to differentiate the genetic versus environmental influence. We found that those children with a maternal asthma history had an increased risk for wheeze, particularly those with TTN, supporting the genetics theory. It is probable that the combination of a genetic predisposition and the modification of the environmental exposure from normal gut flora by antibiotic treatment may provide a synergistic risk factor for future asthma.

We used two definitions of TTN in our study. The first included all children with TTN, whereas the second focused on those babies who were diagnosed with TTN without other PIs at birth (such as a pneumothorax or meconium aspiration). These complications may result in insults to the lung, which could skew our results for an increased risk to wheeze. However, even in our TTN without respiratory complications (TTN-PI) group, the HRs were statistically significant. Also, we decided to only analyze children who were born at term (37-41 6/7 weeks gestational age) to remove the risk of respiratory distress syndrome or bronchopulmonary dysplasia affecting our analyses.

Our definition of a wheezing syndrome was quite inclusive. As the diagnosis of asthma is difficult in the preschool...
years, we wanted to ensure that we captured every child who had a respiratory condition that could possibly be, or mimic, asthma. One advantage of our study is the fact that we have access to a prescription database that records whether a child has received a β2-agonist, inhaled corticosteroid, theophylline, leukotriene receptor antagonist, or mast cell stabilizer via prescription.

In summary, we found that TTN is associated with subsequent respiratory morbidity and may be an early manifestation of “asthma.” We hypothesize that the genetic and environmental interactions synergistically predispose these children for future wheeze. Prospective studies are required to better define these factors.

A special thanks to the SAGE (Study of Asthma, Genes and the Environment) team including Rishma Chooniedass, Miriam Clement, Donna Everette, Brenda Gerwing, Marilyn Lilley, Tanya Lilley-Chan, Ingrid Loewen, Melissa Moyen, Rasheda Rabhani, Michelle Tillett and John Weslake. We would like to acknowledge the computer analysis support of Matthew Dahl in creating the 1995 birth cohort.

REFERENCES


Palivizumab Prophylaxis, Respiratory Syncytial Virus, and Subsequent Recurrent Wheezing

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Objective Children who experience respiratory syncytial virus (RSV) lower respiratory tract infections (LRTIs) early in life have high rates of subsequent recurrent wheezing. Palivizumab, an anti-RSV monoclonal antibody, has 78% to 80% efficacy in preventing RSV hospitalization in premature infants without chronic lung disease. We hypothesized that palivizumab, by ameliorating or preventing early RSV LRTI in preterm infants, might decrease later recurrent wheezing.

Study design A cohort of preterm infants who had received palivizumab and were not hospitalized for RSV (n = 191) or who never received palivizumab (n = 230; 76 who were hospitalized for RSV and 154 who were not), were prospectively followed for 24 months beginning at a mean age of 19 months. The subjects were assessed for recurrent wheezing by caretaker or physician report.

Results The incidences of recurrent wheezing and physician-diagnosed recurrent wheezing were significantly lower in the 191 palivizumab-treated subjects (13% and 8%, respectively) compared with all 230 untreated subjects (26%, \( P = .001 \) and 16%, \( P = .011 \), respectively) and with the 154 patients in the subgroup not hospitalized for RSV LRTI (23%, \( P = .022 \) and 16%, \( P = .027 \), respectively). The effect of palivizumab treatment remained significant after adjustment for potential confounding variables.

Conclusions Our study suggests that preventing RSV LRTI with palivizumab may reduce subsequent recurrent wheezing in premature infants. (J Pediatr 2007;151:34-42)

Respiratory syncytial virus (RSV) is the most important respiratory viral pathogen in childhood. Although the immediate effects of severe disease are well known, RSV lower respiratory tract infection (LRTI; pneumonia and/or bronchiolitis) in early life has been associated epidemiologically with subsequent recurrent wheezing and asthma later in childhood. Prospective studies have demonstrated rates of subsequent airway reactivity 50% to 100% greater in children who developed RSV LRTI in early life than in uninfected controls. Recurrent wheezing has been observed up to 11 years later and may extend into early adulthood.

Preterm infants, even those without chronic lung disease (CLD), develop particularly serious RSV infections in the first year of life, are at higher risk for developing recurrent wheezing or asthma, and have persistent abnormal lung function. It has been demonstrated that the use of a polyclonal immunoglobulin, RSVIG-IV (RespiGam), can prevent serious RSV LRTI in children with CLD. In another small study, infants with CLD who had received RSVIG-IV also had less severe chronic asthma, as defined by improved pulmonary function tests, decreased hospital visits, and decreased medication use, compared with control children.

Palivizumab (Synagis), a humanized monoclonal antibody against the RSV fusion protein, has been demonstrated to substantially reduce hospitalization for severe RSV infections.
LRTI in large clinical trials involving preterm infants.\textsuperscript{14,15} We hypothesized that the use of palivizumab in preterm infants, by ameliorating or preventing early RSV LRTI, might decrease later recurrent wheezing. We report here on respiratory outcomes in an international cohort of previously preterm infants age 36 months and younger without CLD who received palivizumab in the 1999-2000 respiratory season and were followed up prospectively for 2 years, compared with a matched cohort that had never received palivizumab.

**METHODS**

**Patient Enrollment**

Because it is unethical to conduct a randomized placebo-controlled trial in premature infants (in whom palivizumab has been shown to reduce RSV hospitalization), we took advantage of incomplete uptake of palivizumab use and conducted a prospective multicenter, double-cohort, follow-up study in 27 centers in Spain, Germany, The Netherlands, Canada, Poland, and Sweden. The 27 sites were invited to participate in the study based on their use of palivizumab in some premature infants on a compassionate basis in the preceding respiratory season and ability to recruit and retain subjects for the 2-year duration of the study. Investigators reviewed medical records for preterm births and approached all who had received at least 3 doses of palivizumab in the first 12 months of life and had not had an RSV hospitalization (designated the “treated group”). Using chronicologic age (±3 months) and gestational age (±4 weeks) for matching, subjects from the large group of premature infants who had not received palivizumab were matched to those in the palivizumab-treated group and were approached for participation in the study (designated the “untreated group”). An attempt was made to recruit equal numbers of RSV hospitalized and nonhospitalized subjects, each matched to 1 subject from the palivizumab-treated cohort. All study participants were born prematurely (≥35 weeks gestational age) and had no CLD. Other exclusion criteria were mechanical ventilation at enrollment; congenital heart disease; renal, hepatic, or seizure disorder; life expectancy of <6 months; known immunodeficiency; or receipt of other RSV investigational vaccines or therapies. Most of the children (94%) were enrolled between mid-July 2001 and mid-March 2002 and were followed prospectively for 2 years after enrollment. Reasons for use or nonuse of palivizumab were not explored.

Written informed consent was obtained from each subject’s parent or legal guardian before the performance of any study-related procedures, after ethical review and approval by the institutional review board at each study site. The study was conducted in accordance with ICH Good Clinical Practice Guidelines and was monitored by Abbott Laboratories, Inc. An independent international steering committee (including the authors) was involved in the study design and data collection, and stipulated and oversaw all analyses reported in this article. At enrollment, a medical and sociodemographic history was obtained, a physical examination was performed, and serum samples were obtained for RSV-neutralizing antibody\textsuperscript{16} and IgE levels.\textsuperscript{17} The medical history included a validated respiratory questionnaire\textsuperscript{2} and a questionnaire on family history, medical history, and underlying diagnosis/disease (RSV) and current medications, demographics (eg, number of people in home, number and ages of siblings, daycare, passive smoke), environmental factors (eg, pets, woodburning stoves), and parental smoking or history of atopy (eg, asthma, allergic dermatitis or allergic rhinitis) in family members.

**Patient Follow-Up**

Monthly contact with the parents/caregivers was scheduled over the 24 months after enrollment. Visits to the study site were conducted at 6-month intervals; all other monthly contacts were conducted by telephone. Subject illnesses and other medical events occurring during the past month were recorded at each monthly follow-up contact. At 6-month intervals, physician records were reviewed for all intercurrent doctor visits, emergency visits, and hospitalizations for respiratory symptoms.

**Respiratory Assessment**

Outcomes were assessed clinically using a system adapted from those used in other studies of the long-term respiratory outcome of RSV.\textsuperscript{2,3} Wheezing, defined as bronchial obstruction, was assessed at each visit. An episode of wheezing was defined in the protocol as 1 or more consecutive days of wheezing preceded and followed by a nonwheezing, healthy period of at least 1 week. A priori recurrent wheezing was defined as 3 or more episodes of wheezing in the last 12 months but not necessarily verified by a physician. Physician-diagnosed recurrent wheezing was defined as 3 or more episodes of wheezing in the last 12 months verified by a physician at a physician’s visit, emergency room visit, or hospitalization. At the study sites, the primary physicians making decisions regarding wheezing were not study physicians. The subjects’ parents or guardians were informed at enrollment that they should notify the investigating physician immediately if the child experienced any respiratory symptoms. During unscheduled (sick) visits, an interval medical history was obtained and a physical examination performed.

**Statistical Methods**

A sample size of 200 patients per group was estimated to provide approximately 80% power for a 2-sided, .05-level test to detect a statistically significant difference in physician-diagnosed recurrent wheezing rates when the true rates are 10% for the untreated cohort and 3% for the palivizumab-treated cohort. Demographics and baseline characteristics were compared using 1-way analysis of variance for quantitative variables and Fisher’s exact test for categorical variables. The palivizumab-treated group was compared with both the combined untreated groups and the untreated non-RSV hospitalized group.
The primary efficacy endpoint was the incidence of recurrent wheezing over the 2-year (730-day) duration of the study. The secondary efficacy endpoint was the incidence of physician-diagnosed recurrent wheezing. For both definitions, the first wheezing event had to have occurred after informed consent was signed and the first study visit was completed.

Because no children included in the palivizumab cohort were previously hospitalized for RSV LRTI, we compared all treated outcomes with the non-RSV hospitalized subgroup of the untreated cohort as well as with the entire untreated cohort. For both endpoints, we compared the palivizumab-treated group with both the combined untreated groups and the untreated non-RSV hospitalized group using Fisher’s exact test; we also calculated the relative risk (RR) and 95% confidence interval (CI) for the RR.

We compared the incidence rates of recurrent wheezing and physician-diagnosed recurrent wheezing per 100 child-years between the palivizumab–treated group and both the combined untreated group and the untreated non-RSV hospitalized group. For the gestational age categories of <29, 29 to 32, and >32 weeks, we calculated the incidence rates and the RR and 95% CI for the RR of palivizumab treatment compared with no treatment (comparisons with the combined untreated groups and with the untreated non-RSV hospitalized group) for both endpoints.

We used a multiple logistic regression model to assess the association of baseline characteristics with the incidence of respiratory outcomes. A forward stepwise selection procedure was used to identify statistically significant relationships. $P$ values to enter or stay in the model were set at .15; only variables significant at the .05 level in the final model are displayed. Variables assessed included palivizumab treatment, history of RSV hospitalization before enrollment, history of recurrent wheezing, sex, age at enrollment, baseline RSV-neutralizing antibody titers (log_{2}RSV antibody level), family history of asthma, gestational age at birth, birth weight, multiple birth status, number of adults in the home, number of siblings in the home, number of siblings in day care, and presence of a wood-burning stove in the home.

We compared the time to onset of recurrent wheezing or physician-diagnosed recurrent wheezing between the palivizumab–treated group and the combined untreated groups and the untreated non-RSV hospitalized group using both the Cox proportional hazards model and the log-rank test. Kaplan-Meier estimates of the time to onset of respiratory outcomes were computed and displayed graphically. We used a multivariable Cox proportional hazards model to assess the association of baseline characteristics on the time to onset of respiratory events, and a forward stepwise selection procedure to identify statistically significant relationships, as described earlier.

Role of the Funding Source

This study was funded by Abbott International. The sponsors of the study collaborated on study design, data collection, and data analysis. The corresponding author and all members of the steering committee had full access to all
data in the study and had the final responsibility of submitting the report for publication.

RESULTS

We studied 193 infants and children who had received palivizumab and 231 subjects who had not received palivizumab (76 with a history of RSV hospitalization and 155 without RSV hospitalization) at study onset. Three children (2 who received palivizumab and 1 untreated non-RSV hospitalized child) who did not meet entry criteria and were not followed for more than 1 day were excluded from analysis. The mean ages of the subjects at enrollment were comparable
(ie, 18.7 months in the palivizumab-treated cohort, 20.0 months in the combined untreated cohort, and 19.5 months in the untreated non-hospitalized subgroup), as were the mean durations of follow-up (23.4, 23.3, and 23.4 months, respectively). In the palivizumab-treated cohort, the mean age at the time of the last palivizumab dose was 7.5 months, and the mean age at time of last RSV hospitalization for subjects in the RSV hospitalized subgroup was 4.3 months. Differences at baseline between the 2 cohorts were observed in gestational age, birth weight, multiple birth status, mean number of siblings, siblings in daycare, and those with no RSV antibody. IgE levels were comparable between the groups, as were other demographic factors (Table I). Because there were no RSV hospitalizations before enrollment in the palivizumab-treated cohort (by exclusion criteria), we also compared this cohort with the subjects not treated with palivizumab and not hospitalized with RSV before enrollment. Table I shows 3 variables (multiple births, siblings in day care and RSV antibody) that differ significantly between the palivizumab-treated and untreated groups but not between the palivizumab-treated and palivizumab untreated non-RSV hospitalized groups.

Significantly smaller proportions of palivizumab-treated children had recurrent wheezing episodes or physician-diagnosed recurrent wheezing during the 24 months of follow-up (Figure 1A). There was a 49% relative reduction in the proportion of children with recurrent wheezing and a 51% relative reduction in the proportion of children with physician-diagnosed recurrent wheezing in the palivizumab-treated group (P ≤ .01). Statistically significant relative reductions were also observed when comparing the palivizumab-treated group with the untreated, non-RSV hospitalized group. Similar results were observed in the assessment of incidence of recurrent wheezing and physician-diagnosed recurrent wheezing per 100 child-years, with statistically significant differences for all comparisons (Figure 1A). The effect of gestational age on the reductions of recurrent and physician-diagnosed recurrent wheezing was examined by univariate analysis (Table II) but not by multivariate analysis, because the individual strata were small. For all strata in both the palivizumab-treated and untreated groups, the rate of recurrent wheezing (physician-diagnosed or not) varied inversely with gestational age. For recurrent wheezing, the RRs were similar in all 3 groups. For physician-diagnosed recurrent wheezing, the RRs varied inversely with gestational age, but only the 29- to 32-week age group had large numbers of subjects and showed significance.

Final logistic regression models demonstrated that palivizumab treatment was significantly associated with fewer events of recurrent wheezing and physician-diagnosed recurrent wheezing (55% to 65% reduction in the odds of an event) compared with either the combined untreated group or the untreated, non-RSV hospitalized group (Table III). Greater gestational age was associated with a reduced risk of a wheezing event in all models (11% to 21% reduction in the odds of an event per 1 week increase in gestational age). In 3 of the 4 comparisons, a family history of asthma was associated with an approximate 2-fold increase in risk for recurrent wheezing or physician-diagnosed recurrent wheezing. In one comparison, the presence of a wood-burning stove in the home was associated with a 2-fold increase in recurrent wheezing.

In multivariable analyses using a backward-elimination selection procedure, palivizumab treatment was significantly associated with lower risk of recurrent wheezing or physician-diagnosed recurrent wheezing in all models (data not shown). Enrollment at individual investigational sites generally was too small to enable evaluation of center effects. When sites were grouped by country, the hypothesis of homogeneity of odds ratios was not rejected.

Kaplan-Meier estimates of the time to onset of recurrent wheezing or physician-diagnosed recurrent wheezing are shown in Figure 1B and 1C. Palivizumab treatment was associated with a 47% to 54% reduction in the risk of adverse respiratory outcomes. Palivizumab-treated subjects demonstrated significantly longer times to onset of the first episode of recurrent wheezing (hazard ratio [HR] = 0.46, 95% CI = 0.29 to 0.74) and physician-diagnosed recurrent wheezing (HR = 0.46; 95% CI = 0.25 to 0.83) compared with the combined untreated group. The results were similar when palivizumab-treated subjects were compared with the untreated non-RSV hospitalized group for time to onset of recurrent wheezing (HR = 0.53; 95% CI = 0.32 to 0.88) and physician-diagnosed recurrent wheezing (HR = 0.47; 95% CI = 0.25 to 0.90).

The potential influence of baseline characteristics on the time to onset of adverse respiratory outcomes was explored using the Cox proportional hazards model. The final models demonstrated that palivizumab treatment remained associated with a significantly longer time to onset of both recurrent and physician-diagnosed recurrent wheezing (54% to 64% reduction in risk) compared with either the combined untreated group or the untreated, nonhospitalized group (Table IV). Greater gestational age was associated with a reduced risk of an adverse event in 3 of the 4 comparisons (13% to 21% reduction per 1 week increase in gestational age). In the model in which gestational age was not significant, higher birth weight was associated with a reduction in the risk of recurrent wheezing (39% reduction per kg increase in birth weight). In 3 of the 4 comparisons, a family history of asthma was associated with an approximate 2-fold increase in risk for recurrent wheezing or physician-diagnosed recurrent wheezing. In the assessment of recurrent wheezing compared with the combined untreated group, history of recurrent wheezing, history of RSV hospitalization, and presence of a wood-burning stove in the home were each associated with an increased risk of an event.

**DISCUSSION**

This study found that preterm infants without CLD who had received palivizumab before enrollment had a statistically significant lower incidence of recurrent wheezing and physician-diagnosed recurrent wheezing over a 2-year follow-up period compared with preterm infants who had not
received palivizumab. The association between palivizumab treatment and improved respiratory outcomes remained statistically significant after adjusting for potential confounding variables. In addition, subjects previously treated with palivizumab had significantly longer time to onset of recurrent wheezing and physician-diagnosed recurrent wheezing even after adjustment for potential confounders. Furthermore, when the palivizumab-treated cohort was compared only with the untreated subjects with no previous history of RSV hospitalization, statistically significant differences in respiratory outcomes were still observed in all analyses. The significant differences in outcomes between treated and untreated subjects who were not hospitalized with RSV LRTI suggest that palivizumab exerted a protective effect by preventing RSV LRTI, not just by preventing hospitalization.

By design, the palivizumab-treated cohort did not have subjects who were hospitalized because of RSV. At the time of study initiation (2000-2001), palivizumab had just been introduced in Europe and Canada. Because palivizumab was expected to be 78% to 80% protective in this population, it would have been challenging to recruit a substantial cohort of palivizumab-treated, RSV hospitalized children. Thus, given the exploratory nature of the study, we chose to exclude RSV hospitalized subjects who had received palivizumab prophylaxis. However, in a secondary analysis, we compared the rates of recurrent wheezing and physician-diagnosed recurrent wheezing in a cohort of children who were not hospitalized (who had or had not received palivizumab) and found similar results. Ours is the first study of palivizumab prophylaxis to demonstrate a potential effect on RSV LRTI (because both cohorts were not hospitalized). RSVIG-IV is known to

### Table II. Effect of palivizumab treatment on recurrent wheezing or physician-diagnosed recurrent wheezing within gestational age categories

<table>
<thead>
<tr>
<th></th>
<th>Palivizumab-treated</th>
<th>Palivizumab-untreated</th>
<th>RR (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Recurrent wheezing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined untreated groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;32 weeks gestational age</td>
<td>2/20 (10%)</td>
<td>20/85 (24%)</td>
<td>0.43 (0.11, 1.67)</td>
</tr>
<tr>
<td>29 to 32 weeks gestational age</td>
<td>13/117 (11%)</td>
<td>27/111 (24%)</td>
<td>0.46 (0.25, 0.84)</td>
</tr>
<tr>
<td>&lt;29 weeks gestational age</td>
<td>10/54 (19%)</td>
<td>12/34 (35%)</td>
<td>0.52 (0.26, 1.08)</td>
</tr>
<tr>
<td><strong>Untreated non-RSV hospitalized group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;32 weeks gestational age</td>
<td>2/20 (10%)</td>
<td>9/49 (18%)</td>
<td>0.54 (0.13, 2.30)</td>
</tr>
<tr>
<td>29 to 32 weeks gestational age</td>
<td>13/117 (11%)</td>
<td>16/80 (20%)</td>
<td>0.56 (0.28, 1.09)</td>
</tr>
<tr>
<td>&lt;29 weeks gestational age</td>
<td>10/54 (19%)</td>
<td>10/25 (40%)</td>
<td>0.46 (0.22, 0.97)</td>
</tr>
<tr>
<td><strong>Physician-documented recurrent wheezing</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Combined untreated groups</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;32 weeks gestational age</td>
<td>0/20 (0%)</td>
<td>11/85 (13%)</td>
<td>N/M</td>
</tr>
<tr>
<td>29 to 32 weeks gestational age</td>
<td>6/117 (5%)</td>
<td>18/111 (16%)</td>
<td>0.32 (0.13, 0.77)</td>
</tr>
<tr>
<td>&lt;29 weeks gestational age</td>
<td>9/54 (17%)</td>
<td>8/34 (24%)</td>
<td>0.71 (0.30, 1.66)</td>
</tr>
<tr>
<td><strong>Untreated non-RSV hospitalized group</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&gt;32 weeks gestational age</td>
<td>0/20 (0%)</td>
<td>5/49 (10%)</td>
<td>N/M</td>
</tr>
<tr>
<td>29 to 32 weeks gestational age</td>
<td>6/117 (5%)</td>
<td>12/80 (15%)</td>
<td>0.34 (0.13, 0.87)</td>
</tr>
<tr>
<td>&lt;29 weeks gestational age</td>
<td>9/54 (17%)</td>
<td>7/25 (28%)</td>
<td>0.60 (0.25, 1.42)</td>
</tr>
</tbody>
</table>

N/M, not meaningful; 0/20 palivizumab-treated subjects had physician-diagnosed recurrent wheezing, resulting in a noninformative relative risk calculation.

### Table III. Factors associated with occurrence of recurrent wheezing or physician-diagnosed recurrent wheezing by multiple logistic regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Versus combined untreated groups</th>
<th>Versus untreated non-RSV hospitalized group</th>
<th>Versus combined untreated groups</th>
<th>Versus untreated non-RSV hospitalized group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Palivizumab treatment</td>
<td>0.42 (0.23, 0.76)</td>
<td>0.45 (0.25, 0.82)</td>
<td>0.35 (0.18, 0.68)</td>
<td>0.35 (0.17, 0.72)</td>
</tr>
<tr>
<td>Gestational age (per 1-week increase)</td>
<td>0.89 (0.80, 0.99)</td>
<td>0.87 (0.77, 0.98)</td>
<td>0.84 (0.74, 0.95)</td>
<td>0.79 (0.68, 0.92)</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>1.83 (1.06, 3.16)</td>
<td>n/a</td>
<td>2.22 (1.19, 4.14)</td>
<td>2.27 (1.10, 4.68)</td>
</tr>
<tr>
<td>Wood-burning stove in home</td>
<td>2.08 (1.07, 4.04)</td>
<td>n/a</td>
<td>n/a</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Variables shown are statistically significant at the .05 level. “n/a” indicates that the variable was not included in the final model.
reduce RSV LRTI in high-risk infants, so it is not surprising that palivizumab, a more potent and highly specific molecule would also reduce RSV LRTI.

Many previous epidemiologic studies have compared long-term outcomes in children hospitalized for RSV with controls not hospitalized for RSV or compared cohorts with and without serious RSV LRTIs. Therefore, we analyzed subgroups with both RSV-hospitalized and non-RSV hospitalized subjects in this study. RSV antibody levels were measured at study onset to determine RSV exposure. There were significant differences in RSV antibody levels at age 18 to 19 months (57% palivizumab-treated subjects with detectable antibody vs 70% in the untreated cohort, respectively). This was not surprising, because in the untreated cohort, a subgroup of subjects (1/3 of the whole) were hospitalized for RSV. However, when we examined just the untreated non-RSV hospitalized group, the proportion of subjects with detectable RSV antibody levels (61%) was comparable with the palivizumab-treated cohort (57%). Because the last palivizumab dose was given at a mean age of 7.5 months, and RSV-neutralizing antibodies were measured in these subjects at a mean age of 18.7 months, it is unlikely (given the monoclonal antibody half life of 17 to 20 days) that these levels reflect residual neutralizing monoclonal antibody. Further, because the levels of RSV neutralizing antibody in the palivizumab treated cohort and the untreated, nonhospitalized subgroup were similar at enrollment, it is unlikely that even residual palivizumab would have had a direct effect on subsequent wheezing over the next 2 years.

This study has several limitations. First, the results of this study may not be generalizable to term infants, because there is evidence to suggest that the mechanisms determining recurrent wheezing are different in preterm and term babies. Therefore, our findings do not support widespread use of palivizumab. Second, the follow-up of 24 months (to a mean age of 43 months at study completion) does not address longer-term outcomes of RSV LRTI. Additional follow-up of these subjects is planned with lung function assessment as a primary outcome. Third, the fact that parents and healthcare providers knew treatment group assignment may have influenced the outcomes of this study. However, we think this is unlikely because physicians who diagnosed wheezing events were not the study investigators. Further, the similarity between physician-diagnosed versus parent-diagnosed wheezing events argues against potential bias.

Because palivizumab had already been demonstrated to prevent RSV LRTI in premature babies without CLD (our study population), it was impossible to conduct a randomized placebo controlled trial to examine our hypothesis in this population of premature infants. A case-control study would have been retrospective and data collection would not have been complete (for the outcomes we studied), hence we designed this trial as a matched double-cohort study. The main disadvantage of this design is that results may reflect differences in study groups and the absence of randomization allows the possibility of imbalance of confounding factors. In our study, palivizumab was more frequently administered to infants with lower birth weight and gestational ages. This is expected as guidelines for palivizumab use favor treatment for smaller, more premature infants.

Because palivizumab had just been introduced to the countries, mostly on a compassionate basis, its use in the first year was restricted mostly to children with CLD and very premature infants. Thus, though we matched infants by gestational age (±4 weeks) there was still a significantly lower mean gestational age in the palivizumab-treated group. These risk factors also would have increased the risk for recurrent wheezing in the palivizumab treated subjects compared with the untreated cohort. In fact, the converse was the case. In our study, palivizumab was more frequently administered to infants with fewer siblings at home and fewer siblings in daycare, which could potentially decrease the relative risk for RSV acquisition and wheezing in the treatment group.

Multivariable analysis was used to take these factors into account; both the logistic and Cox regression models still showed a significant protective effect of palivizumab.

### Table IV. Factors associated with time to onset of recurrent wheezing or physician-diagnosed recurrent wheezing by multivariable Cox proportional hazard regression

<table>
<thead>
<tr>
<th>Variable</th>
<th>Recurrent wheezing</th>
<th>Physician-diagnosed recurrent wheezing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Versus combined</td>
<td>Versus untreated</td>
</tr>
<tr>
<td></td>
<td>untreated groups</td>
<td>non-RSV hospitalized group</td>
</tr>
<tr>
<td>Palivizumab treatment</td>
<td>0.46 (0.27, 0.78)</td>
<td>0.47 (0.28, 0.81)</td>
</tr>
<tr>
<td>Gestational age (per 1-week increase)</td>
<td>n/a</td>
<td>0.87 (0.78, 0.97)</td>
</tr>
<tr>
<td>Family history of asthma</td>
<td>1.94 (1.23, 3.07)</td>
<td>n/a</td>
</tr>
<tr>
<td>History of recurrent wheezing</td>
<td>1.93 (1.01, 3.66)</td>
<td>n/a</td>
</tr>
<tr>
<td>Birth weight (per kg higher)</td>
<td>0.61 (0.38, 0.99)</td>
<td>n/a</td>
</tr>
<tr>
<td>History of RSV hospitalization</td>
<td>1.86 (1.07, 3.22)</td>
<td>n/a</td>
</tr>
<tr>
<td>Wood-burning stove in home</td>
<td>1.80 (1.04, 3.10)</td>
<td>n/a</td>
</tr>
</tbody>
</table>

Variables shown are statistically significant at the .05 level. "n/a" indicates that the variable was not included in the final model.
We specifically studied the prevention of RSV LRTI in young infants because the association between RSV LRTI and childhood recurrent wheezing disease appears to be greatest in those who develop RSV LRTI in infancy.3 Culley et al demonstrated in a newborn mouse model that early infection with RSV biases airway immune responsiveness to a Th-2 phenotype, resulting in more severe inflammatory changes on reinfection. Although it is unclear whether Th-2-type immune responses play a role in the pathogenesis of post-RSV recurrent wheezing in humans,26,27 it is known that the fetal Th-2 polarized state develops to the more balanced mature Th-1 dominant state throughout infancy and later childhood.28 A second potential explanation for the association of RSV LRTI and recurrent wheezing comes from the observation that airway tissue damage in early life from viral infections and/or inhalant allergens causes interference in ongoing differentiation of lung tissue. This results in subsequent altered lung function and airway hyperresponsiveness.29,30 Because palivizumab was administered only in the first year of life, we postulate that palivizumab substantially reduced subsequent recurrent wheezing by protecting susceptible infants against lower respiratory tract damage from RSV in their most vulnerable period. Finally, it has been demonstrated that preventing alterations in small airway neural networks caused by RSV in infancy is one mechanism by which palivizumab may prevent subsequent wheezing. This neural response to RSV also appears to be developmentally regulated.31 Piedimonte et al demonstrated that the administration of palivizumab to weaning rats resulted in reduced viral load and significantly decreased RSV-related neurogenic inflammation. They postulated that in this way, palivizumab use might prevent subsequent airway reactivity and recurrent wheezing.32 We demonstrated an approximately 50% lower incidence of recurrent wheezing in the infants who received palivizumab compared with controls. This could indicate that the attributable fraction of recurrent wheezing due to RSV could be as high as 50%. In the remainder, true atopic asthma may account for some proportion of cases.33 Recently, rhinovirus infection in infants has been found to be a significant cause of subsequent recurrent wheezing in early childhood.33 Other respiratory pathogens also have been implicated in the development of this recurrent wheezing.33

Clearly, the relationship between RSV and recurrent wheezing is complex. The interactions among developing immune and neural systems, as well as the genetic susceptibility to RSV and to subsequent recurrent wheezing after RSV, all contribute to the eventual phenotype. The results of our study suggest that palivizumab, by preventing RSV lower respiratory tract infection, may play a role in protecting against subsequent recurrent wheezing in premature infants without CLD.

REFERENCES


Palivizumab Prophylaxis, Respiratory Syncytial Virus, and Subsequent Recurrent Wheezing 41
APPENDIX

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The Netherlands: Prof. J. L. L. Kimpen, Wilhelmina Children’s Hospital, Utrecht.

Abbott International: Dr Jessie Groothuis, Dr. P. Polack, M. McCue, S. Williamson, M. S. King, D. Morris.

Colorado: Dr. E. A. F. Simoes, The Children’s Hospital, University of Colorado School of Medicine, Denver.
Immunogenicity and Safety of a Combination Diphtheria, Tetanus Toxoid, Acellular Pertussis, Hepatitis B, and Inactivated Poliovirus Vaccine Coadministered with a 7-Valent Pneumococcal Conjugate Vaccine and a Haemophilus Influenzae Type b Conjugate Vaccine

MICHAEL E. PICHICHERO, MD, HENRY BERNSTEIN, DO, MARK M. BLATTER, MD, LODIE SCHUERMAN, MD, BRIGITTE CHEUVART, PhD, AND SANDRA J. HOLMES, PhD, MHA
FOR THE 085 STUDY INVESTIGATORS*

Objective To evaluate the immunogenicity and safety of a diphtheria and tetanus toxoids, acellular pertussis, hepatitis B, and inactivated poliovirus-containing vaccine (DTaP-HepB-IPV) coadministered with pneumococcal 7-valent conjugate vaccine (PCV-7) and Haemophilus influenzae type b vaccine (Hib), with separate vaccines concurrently, or staggered (delayed) administration of PCV-7.

Study design At 2, 4, and 6 months of age, infants received either DTaP-HepB-IPV plus PCV-7 and Hib (n = 199), separate vaccines (n = 188), or DTaP-HepB-IPV plus Hib with PCV-7 administered 2 weeks later (n = 188). Blood was drawn before and after vaccination. Parents reported symptoms for 4 days after each dose and adverse events throughout the entire study.

Results Immunogenicity in the Combination Vaccine Group was noninferior to that of the Separate and Staggered Vaccine Groups with respect to seroprotective rates for diphtheria, tetanus, and poliovirus and to geometric mean concentrations for pertussis. Seroprotective rates for HepB and Hib were not different between groups. Seropositivity for PCV-7 was high in all groups. Administration of combination vaccine appeared to be associated with higher rates of irritability, fever ≥100.4°F (38.0°C) and some local symptoms compared with separate vaccines (exploratory P < .05). No group differences were observed in rates of symptoms for which parents sought medical advice.

Conclusions DTaP-HepB-IPV was highly immunogenic and well tolerated when coadministered with Hib and PCV-7 at 2, 4, and 6 months of age. (J Pediatr 2007;151:43-9)

During the past decade, additions to the recommended childhood immunization schedule in the United States have required administering as many as 5 injections at each of the 3 primary immunization visits at 2, 4, and 6 months of age. Use of combination vaccines can help to decrease the number of injections administered at these visits, increase parent compliance, and improve timely vaccination. The safety and immunogenicity of a combination vaccine containing diphtheria and tetanus toxoids, acellular pertussis, hepatitis B, and inactivated poliovirus vaccines (DTaP-HepB-IPV)

ANCova Analysis of covariance
CI Confidence interval
DT Diphtheria toxoid
DTaP Diphtheria–tetanus–acellular pertussis vaccine
ELISA Enzyme-linked immunosorbent assay
ELUSA ELUSA units
FHA Filamentous hemagglutinin
GMC Geometric mean concentration
GPT Geometric mean titer
HepB Hepatitis B vaccine
HBSAg Hepatitis B surface antigen
Hib Haemophilus influenzae type b vaccine
IPV Inactivated poliovirus vaccine
LF Limit of flocculation unit
PCV-7 Pneumococcal 7-valent conjugate vaccine
PRN Pertactin
PRP Polysaccharide
PT Pertussis toxoid
TT Tetanus toxoid

From the University of Rochester Medical Center, Rochester, New York (M.E.P.); the Dartmouth Medical School, Dartmouth-Hitchcock Medical Center, Lebanon, New Hampshire (H.B.); Primary Physicians Research, Pittsburgh, Pennsylvania (M.M.B.); GlaxoSmithKline Biologicals, Rixensart, Belgium (B.C.); and formerly of Clinical/Medical Affairs, Vaccines-NA, GlaxoSmithKline, King of Prussia, Pennsylvania (S.J.H.).

Supported by a grant from GlaxoSmithKline Biologicals, Rixensart, Belgium. Editorial support was provided by Scientific Therapeutics Information, Inc, Springfield, New Jersey.

This trial is registered on the GlaxoSmithKline Clinical Trial Register (Study ID number 217744/085), available at: http://ctr.gsk.co.uk/Summary/Vaccine_Pediatricsstudyfacts.asp.

The study design and collection of the data were performed by GlaxoSmithKline. All analyses were performed at the University of Rochester, Rochester, New York (MEP Laboratories), except for the analysis of the anti-Streptococcus pneumoniae antibody, which was performed at the GlaxoSmithKline Laboratory in Rixensart, Belgium. Interpretation of data, writing of the manuscript, and decision to submit the paper for publication was made by the authors with contribution from GlaxoSmithKline. No form of payment was provided to any of the authors in conjunction with development of this manuscript.

Michael E. Pichichero, MD, has received research grants and/or honoraria from GlaxoSmithKline, Sanofi Pasteur Inc, Wyeth, and Merck & Co, Inc, for vaccine-related research. Henry H. Bernstein, DO, has been a recipient of grant/research funding from GlaxoSmithKline. Mark M. Blatter, MD, serves on the Speakers’ Bureau for Sanofi Pasteur, Inc, and GlaxoSmithKline. He has received grant/research funding from GlaxoSmithKline, Sanofi Pasteur, Inc, Wyeth, Merck & Co, Inc, MedImmune, Inc, and Chiron Corporation. Lode Schuerman, MD, is an employee of GlaxoSmithKline and has stock options at the company. Brigitte Cheuvart, PhD, is an employee of GlaxoSmithKline, Sandra J. Holmes, PhD, MHA, is a former employee of and previous consultant for GlaxoSmithKline, and Michael E. Pichichero, MD, has received grant/research funding from GlaxoSmithKline.

*Other members of the 085 Study Group are listed in the Appendix.

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(PEDIARIX, GlaxoSmithKline Biologicals, Rixensart, Belgium) have been demonstrated to be comparable with that of separate coadministration of the component vaccines (DTaP, HepB, and poliovirus vaccines) when Haemophilus influenzae type b vaccine (Hib) was coadministered to both groups. However, a pneumococcal 7-valent conjugate vaccine (PCV-7, Prevnar, Lederle Laboratories, Philadelphia, Pa) for the prevention of invasive disease caused by Streptococcus pneumoniae was approved in 2000 shortly before the licensure of DTaP-HepB-IPV vaccine and became recommended for infants at 2, 4, and 6 months of age. Because some inconsistencies were observed in immunologic responses when routine vaccines were administered with PCV-7 and because increased rates of fever were reported when PCV-7 was coadministered with separate DTaP, Hib, HepB, and IPV, the investigation of the immunogenicity and safety of DTaP-HepB-IPV vaccine, when coadministered with Hib and PCV-7 vaccines, was conducted.

The primary objective of this study was to compare the immunogenicity of DTaP-HepB-IPV vaccine coadministered with PCV-7 and Hib vaccines (Combination Vaccine Group) with that of separately administered DTaP, HepB, IPV, Hib, and PCV-7 vaccines (Separate Vaccine Group), with respect to diphtheria, tetanus, pertussis, and poliovirus seroprotection and/or antibody responses. A secondary objective was to compare immune responses between the Combination Vaccine Group and a Staggered Vaccine Group in whom DTaP-HepB-IPV was coadministered with Hib vaccine, and PCV-7 vaccine was given 2 weeks later to evaluate the immunologic responses, with respect to hepatitis B, H influenzae type b, and S pneumoniae serotypes, and to compare safety assessments among the three study groups.

METHODS

Subjects

The study was conducted at 22 sites in the United States, all of which received approval by their institutional review board before initiation. Healthy infants 2 months (6 to 12 weeks) of age at the time of first vaccination were enrolled after written informed consent was obtained from their parents or guardians. Infants were excluded if any of the following was present: premature birth (<36 weeks’ gestation); previous vaccination against or history of diphtheria, tetanus, pertussis, poliomyelitis, H influenzae type b, or S pneumoniae disease, or hepatitis B disease or more than 1 previous dose of HepB; confirmed or suspected immune dysfunction; previous chronic administration of immunosuppressants or other immune-modifying drugs; history of hypersensitivity to any components of the vaccines; major congenital defects or serious chronic illness; neurologic or seizure disorder; or no telephone access.

Vaccines

Six different vaccines were used in this study. Each 0.5-mL dose of the DTaP-HepB-IPV vaccine (PEDIARIX) was supplied in a prefilled syringe from a single lot and was formulated to contain 25 Lf (limit of flocculation unit) diphtheria toxoid, 10 Lf tetanus toxoid, 25 µg pertussis toxoid, 25 µg filamentous hemagglutinin, 8 µg pertactin, 10 µg hepatitis B surface antigen (recombinant), 40 D-antigen units of type 1 (Mahoney), 8 D-antigen units of type 2 (MEF-1), and 32 D-antigen units of type 3 (Saukett) of polioviruses. The DTaP vaccine (Infanrix, GlaxoSmithKline Biologicals, Rixensart, Belgium) contained 25 Lf diphtheria toxoid, 10 Lf tetanus toxoid, 25 µg pertussis toxoid, 25 µg filamentous hemagglutinin, and 8 µg pertactin per 0.5-mL dose in a single-dose vial. The hepatitis B vaccine (Engerix-B, GlaxoSmithKline Biologicals, Rixensart, Belgium) contained 10 µg hepatitis B surface antigen (recombinant) per 0.5-mL dose in a prefilled syringe. Each 0.5-mL dose of the commercially available PCV-7 vaccine (Prevnar) contained 2 µg of each of the pneumococcal polysaccharide serotypes 4, 9V, 14, 18C, 19F, and 23F, and 4 µg of 6B coupled to the CRM197 carrier protein and was supplied in a single-dose vial. The Hib vaccine (HibTITER, Wyeth Pharmaceuticals, Inc, Philadelphia, Pa) contained 10 µg of Haemophilus b saccharide conjugated to CRM197 protein per 0.5-mL dose in a single-dose vial. The commercially available IPV vaccine (IPOL, Sanofi Pasteur SA, Lyon, France) contained 40 D-antigen units of type 1 (Mahoney), 8 D-antigen units of type 2 (MEF-1), and 32 D-antigen units of type 3 (Saukett) of poliovirus per 0.5-mL dose in a prefilled syringe.

Study Design

In this open-label study, infants eligible for inclusion were randomly assigned to one of three study groups, according to an Internet randomization system, with 10% randomness and the center as minimization factor. This is a classic randomization method of minimization in that it corrects the imbalance between treatments within a center in 90% of the assignments (ie, with 90% probability) and allocates randomly to one of the three groups in 10% of the assignments (ie, with 10% probability). The vaccination schedule for the study groups is presented in the Figure. Intramuscular injections of DTaP-HepB-IPV or DTaP vaccine were administered in the upper right anterolateral thigh; HepB vaccine was administered in the lower right anterolateral thigh; and PCV-7 and Hib vaccines were administered in the upper and lower left anterolateral thigh, respectively. When more than one vaccine was administered into the same leg, the injections were separated by at least 2 inches. IPV vaccine was administered by subcutaneous injection in the left deltoid.

Blood samples were collected from all subjects at 2 months of age (before the first vaccination) and 7 months of age (1 month after the third DTaP-HepB-IPV or DTaP vaccination).

Serologic Evaluations

Anti-S pneumoniae antibody testing only was performed in a blinded fashion at the GlaxoSmithKline Laboratory in
Rixensart, Belgium; all other assays were performed at the University of Rochester, Rochester, NY (MEP Laboratories). Serum samples were tested for the presence of antibodies to all vaccine antigens. Standardized enzyme-linked immunosorbent assays (ELISAs) were used to assess antibody concentrations to diphtheria and tetanus toxoids, pertussis toxoid (PT), filamentous hemagglutinin (FHA), pertactin (PRN), polyribosylribitol (PRP), and 7 S pneumoniae serotype-specific antibodies (to 4, 6B, 9V, 14, 18C, 19F, and 23F). The cut-off of the ELISA for diphtheria and tetanus toxoids was 0.1 IU/mL. A concentration of 0.1 IU/mL or above was considered seropositive and seroprotective. The cut-off of the ELISA for PT, FHA, and PRN were 5 ELISA units (EL.U)/mL. Vaccine response for PT, FHA, and PRN was defined as the appearance of antibodies in infants who were initially seronegative (ie, with concentrations less than the cut-off value) or at least maintenance of prevaccination antibody concentrations in subjects who were initially seropositive (ie, with concentrations greater than or equal to the cut-off value). Antibody concentrations for hepatitis B surface antigen (anti-HBsAg) were measured by ELISA, using a commercially available kit (AUSAB, Abbott Laboratories, Abbott Park, Ill), with the cut-off at 10 mIU/mL. Concentrations greater than or equal to this value were considered seropositive. For PRP, the cut-off of the ELISA test was 0.15 µg/mL. Concentrations of 0.15 µg/mL or above and 1.0 µg/mL or above were analyzed. Neutralizing antibodies against poliovirus types 1, 2, and 3 were determined by a microneutralization assay and were expressed as dilution titers. Results were expressed as the reciprocal of the highest dilution of serum showing 50% virus neutralization. Antibody titers of 1:8 or higher were considered seropositive and seroprotective. S pneumoniae serotype-specific antibody concentrations were determined by comparing the ELISA curves with a standard reference serum, with the cut-off at 0.05 µg/mL. Concentrations greater than or equal to this value were considered seropositive.

Safety Evaluations

All infants were observed for 30 minutes after each vaccination. Parents were asked to record temperatures, other general symptoms (irritability/irritability/fussiness, loss of appetite, and drowsiness), local symptoms (pain, redness, and swelling at the injection sites), and concomitant medications on the day of vaccination (day 0) and for the next 3 days (days 1 through 3). They were supplied with a diary to assist them in reporting symptoms, a gauge for measuring reactions (redness and swelling) at the injection sites, and a thermometer to record rectal temperatures. Unsolicited adverse events were recorded for the entire study period. For each symptom or event reported, parents were asked if they sought medical advice (defined as a visit with medical personnel). During the extended safety follow-up period (6 months after the last dose of study vaccine), serious adverse events, onset of chronic illness (eg, diabetes, autoimmune disease, asthma, and allergies), and emergency department visits and physician office visits not related to well-child care, vaccination, or common acute illnesses (eg, upper respiratory tract infections, otitis media, pharyngitis, and gastroenteritis) were reported.
Statistical Analysis

Primary immunogenicity analyses were based on the according-to-protocol cohort, which included all subjects who met the eligibility criteria, who complied with the procedures of the protocol, and for whom assay results were available for at least one study vaccine antigen 1 month after the three-dose primary vaccination series.

Geometric mean antibody concentrations (GMCs) or geometric mean antibody titers (GMTs) and seropositivity or seroprotection rates were calculated with their 95% confidence intervals (CIs) for each group at each blood-sampling time point. One month after the third dose, differences between the Combination Vaccine Group and each of the other two groups (Separate and Staggered Vaccine Groups) were computed with standardized asymptotic 95% CIs for pertussis vaccine response rates and seroprotection rates for diphtheria, tetanus, hepatitis B, poliovirus types 1, 2, and 3, and Hib. For each antigen, antibody GMC/GMT ratios (Separate Vaccine Group divided by Combination Vaccine Group, and Staggered Vaccine Group divided by Combination Vaccine Group) were computed with 90% and 95% CIs, using an analysis of covariance (ANCOVA) model on the logarithm10 transformation of the antibody concentrations/titers. The ANCOVA model included the vaccine group as fixed effect (all three groups) and the prevaccination concentration/titer as regressor.

The criteria for clinical noninferiority for the primary objective included 1) the upper limit of the 90% CI on the antibody GMC ratio (Separate Vaccine Group over Combination Vaccine Group) below 1.5 for each pertussis antigen; 2) the upper limit of the 95% CI on the absolute difference (Separate Vaccine Group minus Combination Vaccine Group) for seroprotection rate below 10% for diphtheria and tetanus antigens; and 3) the upper limit of the 95% CI on the absolute difference for seroprotection rate below 5% for the three poliovirus antigens. Noninferiority for hepatitis B was not assessed because HepB was not to be administered at 4 months of age to infants in the Separate Vaccine Group who received a dose of HepB before enrollment in the study. Additional exploratory analyses were performed to characterize group difference in immunogenicity between the combination vaccine group and each of the control groups: statistical difference was based on 0% being included in the 95% CI for group difference in seroprotection rate and on 1 being included in the 95% CI for GMC ratio.

All safety analyses were descriptive and based on the modified intent-to-treat cohort, which included all vaccinated infants for whom safety data were available. Safety data were categorized, and analyses were performed overall, per subject, for the three-dose vaccination series. The percentage of infants having specific symptoms within the 4 days after vaccination were computed with the exact 95% CI, according to the type of symptom, intensity, and relation to vaccination. Differences between groups in percentage of subjects reporting solicited symptom(s) were evaluated through two-sided Fisher’s exact tests. A P value below .05 was used to highlight possible differences. Serious adverse events reported during the entire study, including the extended safety follow-up period, were described.

RESULTS

Study Population

A total of 575 infants were enrolled and randomly assigned to the Separate Vaccine Group (n = 188), the Combination Vaccine Group (n = 199), and the Staggered Vaccine Group (n = 188). The modified intention-to-treat cohort for safety included 573 infants (188, 198, 187 in the Separate, Combination, and Staggered Vaccine Groups, respectively), and the according to protocol cohort for immunogenicity included 486 infants (156, 169, and 161 in the Separate, Combination, and Staggered Vaccine Groups, respectively). Among the 575 infants enrolled, the median age at first dose was 9 weeks, 51.5% were female, and 84% were Caucasian. The demographics were similar among the three groups.

During the course of the study, 43 infants were withdrawn (16 each from the Separate and Combination Vaccine Groups, and 11 from the Staggered Vaccine Group). The reasons for withdrawal were loss to follow-up (25 infants), withdrawal of consent (9 infants), moving from study area (7 infants), and protocol violation (2 infants). No subject was withdrawn because of an adverse event.

Immunogenicity

Criteria for noninferiority were met for all primary end points. The upper limits of the two-sided 95% CI on the absolute difference (Separate Vaccine Group minus Combination Vaccine Group) in the seroprotection rates for diphtheria (2.12%), tetanus (0.32%), and poliovirus types 1, 2, and 3 (2.24% for each) were below the predefined clinical limits of noninferiority (10% for diphtheria and tetanus, and 5% for poliovirus) (Table I; available at www.jpeds.com). Likewise, the upper limits of the 90% CI on the ratios of the adjusted antibody GMCs (Separate Vaccine Group/Combination Vaccine Group) for PT (0.70), FHA (1.00), and PRN (0.99) were below the predefined clinical limit for noninferiority of 1.5 (data not shown). Seroprotection rates for diphtheria, tetanus, and poliovirus were high in the Combination and Separate Vaccine Groups (Table I). Seroprotection rates for hepatitis B did not differ between the two groups, regardless of hepatitis B vaccination status before study entry. There were no statistically significant differences between the two groups in seroprotection rates for PRP. For anti–S pneumoniae antibodies, seropositivity was high in all groups. Although the comparison between the Combination and the Staggered Vaccine Groups was not a primary objective, the criteria for noninferiority as defined for the primary end points also were met for the comparisons between these two groups.

For all three groups after the third dose, seroprotection rates ranged from 98.7% to 100% for diphtheria and from 98.1% to 100% for tetanus (Table I). For antipertussis, postvaccination antibody GMCs were significantly higher in
Table II. Antibody GMCs or GMTs 1 month after third dose of DTaP or DTaP-HepB-IPV (according-to-protocol cohort for immunogenicity)

<table>
<thead>
<tr>
<th>Antibody</th>
<th>Combination Vaccine Group (n = 138-168)</th>
<th>Separate Vaccine Group (n = 126-156)</th>
<th>Staggered Vaccine Group (n = 135-158)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>GMC/GMT 95% CI</td>
<td>GMC/GMT 95% CI</td>
<td>GMC/GMT 95% CI</td>
</tr>
<tr>
<td>Anti-D (IU/mL)</td>
<td>1.987 1.761, 2.242</td>
<td>1.564† 1.354, 1.806</td>
<td>3.238‡ 2.889, 3.630</td>
</tr>
<tr>
<td>Anti-T (IU/mL)</td>
<td>2.428 2.180, 2.704</td>
<td>1.346† 1.166, 1.554</td>
<td>2.240 2.017, 2.487</td>
</tr>
<tr>
<td>Anti-PT (EL.U/mL)</td>
<td>48.7 42.8, 55.4</td>
<td>28.9† 25.2, 32.2</td>
<td>50.3 44.6, 56.7</td>
</tr>
<tr>
<td>Anti-FHA (EL.U/mL)</td>
<td>113.7 101.5, 127.3</td>
<td>96.4 85.5, 108.7</td>
<td>117.7 105.9, 130.9</td>
</tr>
<tr>
<td>Anti-PRN (EL.U/mL)</td>
<td>93.7 82.3, 106.7</td>
<td>79.1 66.8, 93.6</td>
<td>99.5 87.5, 113.2</td>
</tr>
<tr>
<td>Anti-HBsAg (mIU/mL)*</td>
<td>1123.6 912.0, 1384.2</td>
<td>667.5† 534.1, 834.3</td>
<td>1515.3 1250.6, 1835.9</td>
</tr>
<tr>
<td>Anti-PRP (mIU/mL)</td>
<td>678.0 568.1, 809.2</td>
<td>225.0‡ 191.3, 264.7</td>
<td>793.1 660.3, 952.7</td>
</tr>
<tr>
<td>Anti-poliovirus 1 titer</td>
<td>578.3 479.3, 697.8</td>
<td>228.1† 198.1, 262.7</td>
<td>608.8 500.8, 740.2</td>
</tr>
<tr>
<td>Anti-poliovirus 2 titer</td>
<td>1269.2 1061.3, 1517.8</td>
<td>454.2‡ 390.6, 528.2</td>
<td>1167.5 973.9, 1399.6</td>
</tr>
<tr>
<td>Anti–S pneumoniae Serotype (μg/mL)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>1.74 1.54, 1.98</td>
<td>2.07 1.81, 2.37</td>
<td>1.62 1.44, 1.83</td>
</tr>
<tr>
<td>6B</td>
<td>0.80 0.65, 0.99</td>
<td>0.67 0.52, 0.87</td>
<td>0.59 0.49, 0.72</td>
</tr>
<tr>
<td>9V</td>
<td>1.55 1.36, 1.77</td>
<td>1.60 1.39, 1.85</td>
<td>1.11† 0.97, 1.28</td>
</tr>
<tr>
<td>14</td>
<td>4.68 4.04, 5.43</td>
<td>6.32 5.39, 7.41</td>
<td>4.51 3.91, 5.19</td>
</tr>
<tr>
<td>18C</td>
<td>2.63 2.31, 3.00</td>
<td>2.96 2.53, 3.47</td>
<td>2.37 2.06, 2.72</td>
</tr>
<tr>
<td>19F</td>
<td>1.09 0.95, 1.25</td>
<td>1.05 0.91, 1.22</td>
<td>0.75† 0.66, 0.86</td>
</tr>
<tr>
<td>23F</td>
<td>1.48 1.23, 1.79</td>
<td>1.81 1.45, 2.25</td>
<td>1.29 1.09, 1.53</td>
</tr>
</tbody>
</table>

D indicates diphtheria; DTaP, diphtheria–tetanus–acellular pertussis vaccine; FHA, filamentous hemagglutinin; GMC, geometric mean concentration; GMT, geometric mean titer; HBsAg, hepatitis B surface antigen; HepB, hepatitis B vaccine; IPV, inactivated poliovirus vaccine; n, number of subjects; PRN, pertactin; PRP, polyribosylribitol; PT, pertussis toxin; T, tetanus.

*Total infants regardless of hepatitis B vaccination status before entry.
†Statistically significant difference between the Combination Vaccine Group and either the Separate or Staggered Vaccine Group.
‡Statistically significant difference between the Combination Vaccine Group and the Separate or Staggered Vaccine Groups.

the Staggered Vaccine Group compared with the Combination or Separate Vaccine Group (Table II). For antitetanus, postvaccination antibody GMCs were significantly lower in the Separate Vaccine Group compared with the Combination Vaccine Group (Table II). Postvaccination response rates among the three groups ranged from 91.7% to 98.7% for PT, FHA, and PRN (Table I). The postvaccination antibody GMC for PT was significantly lower in the Separate Vaccine Group compared with the Combination Vaccine Group (Table II). The antibody GMCs of serotypes 9V and 19F for PT were significantly lower in the Separate Vaccine Group compared with the Staggered Vaccine Group (Table II). The antibody GMCs of serotypes 9V and 19F were significantly lower in the Staggered Vaccine Group compared with the Combination Vaccine Group.

Safety

**General Symptoms.** Overall, irritability was the most frequently reported general symptom in all groups and was significantly higher in the Combination Vaccine Group compared with the Separate or Staggered Vaccine Groups (P = .002 and P = .005, respectively) (Table III; available at www.jpeds.com). Incidence rates of any drowsiness, irritability, and loss of appetite were significantly higher in the Combination Vaccine Group compared with the Staggered Vaccine Group (P < .05).

**Fever.** The overall incidence of fever of 100.4°F (38.0°C) or above in the Combination Vaccine Group was significantly higher than that reported in both the Separate and the Staggered Vaccine Groups (P < .05), and the incidence of fever
above 101.3°F (38.5°C) was significantly higher in the Combination Vaccine Group compared with the Staggered Vaccine Group (P < .05 [Table III]). Rates of fever higher than 103.1°F (39.5°C) were similarly low among all groups (12.5% to 25.0%). Among infants who had fever, the majority in all groups had it for only 1 day (50% to 76% of infants across the groups).

Overall, medical advice was sought for fever after 8 vaccine doses in 8 different infants. All had intermediate-grade fever (≤103.1°F or ≤39.5°C), and only 1 infant in the Combination Vaccine Group had fever above 103.1°F. Six of 8 infants with medically attended fevers were seen in outpatient settings (3 in the Separate, 1 in the Staggered, and 2 in the Combination Vaccine Groups), and the other 2 infants (1 each in the Combination and Staggered Vaccine Groups) were seen in the emergency department.

**Local Symptoms.** The rates of local reactions reported per subject were significantly higher at the DTaP-HepB-IPV vaccine injection site in the Combination Vaccine Group than at the DTaP injection site in the Separate Vaccine Group for grade 3 pain, swelling of any intensity, and swelling of more than 20 mm (Table III), in addition to grade 2 or 3 pain (31.8% vs 20.7%, P = .015), redness of more than 5 mm (26.8% vs 15.4%, P = .009), and swelling of more than 5 mm (18.7% vs 7.4%, P = .001) (data not shown). When all injection sites were considered, only the rate of swelling greater than 20 mm was higher in the Combination Vaccine Group than in the Separate Vaccine Group (Table III).

The rates of local reactions were significantly higher in the Combination Vaccine Group than in the Staggered Vaccine Group for grade 3 pain, swelling of any intensity, and swelling of more than 20 mm (Table III), in addition to grade 2 or 3 pain (31.8% vs 20.7%, P = .015), redness of more than 5 mm (26.8% vs 15.4%, P = .009), and swelling of more than 5 mm (18.7% vs 7.4%, P = .001) (data not shown). When all injection sites were considered, only the rate of swelling greater than 20 mm was higher in the Combination Vaccine Group than in the Separate Vaccine Group (Table III).

**Unsolicited Adverse Events.** Grade 3 unsolicited adverse events (ie, events that prevent normal, everyday activities) occurring within 31 days after vaccine administration were reported infrequently (≤4.3% in each group). Overall, the reported frequency of unsolicited adverse events of any intensity ranged from 81.5% to 87.7% among all three groups, with respiratory tract infections being reported most frequently (41.5% to 45.5%).

**Serious Adverse Events.** Among 22 infants, 28 serious adverse events were reported (12 events in the Combination Vaccine Group, 9 events in the Separate Vaccine Group, and 7 events in the Staggered Vaccine Group). The investigators did not consider these serious adverse events to be related to vaccination. There were no deaths in the study.

**Extended Safety Follow-up Period.** The frequency of adverse events reported during the extended follow-up period (5 months after the last study visit at 1 month after the last dose of study vaccine) in any one category (serious adverse events, chronic illness, physician office visits, and emergency department visits) was 15.4% in the Separate Vaccine Group, 12.4% in the Staggered Vaccine Group, and 9.2% in the Combination Vaccine Group. Respiratory illnesses were the most frequently reported adverse events resulting in a physician office visit. Injury and gastroenteritis were the most frequently reported adverse events resulting in an emergency department visit. Convulsions were reported only during the extended follow-up period: One child from the Combination Vaccine Group was hospitalized with onset of convulsions 66 days after the third dose of DTaP-HepB-IPV vaccine, and one child from the Staggered Vaccine Group was hospitalized with onset of a febrile convulsion 34 days after the last dose of PCV-7 vaccine.

**Discussion**

This study showed that the immunogenicity of a combined DTaP-HepB-IPV vaccine coadministered with Hib and PCV-7 vaccines as a three-dose primary series was at least as good as that of separately administered DTaP, HepB, IPV, Hib, and PCV-7 vaccines, with respect to diphtheria, tetanus, pertussis, and poliovirus antibody responses. Furthermore, seroprotection rates for hepatitis B were above 98% for all groups. Immunologic responses to Hib and PCV-7 vaccines showed no difference between the two groups.

The GMC ratios for antibodies against diphtheria were higher in the Combination Vaccine Group and lower than in the Staggered Vaccine Group. Infants in all groups received the same amount of diphtheria antigen from diphtheria toxoid and from PCV-7 vaccine, which contains *S. pneumoniae* saccharide conjugated to diphtheria CRM197 protein. However, infants in the Staggered Vaccine Group received diphtheria antigen at 6 time points compared with 3 time points in the Combination Vaccine Group. This may have resulted in higher antibody levels to diphtheria in the Staggered Vaccine Group.

Although there were no statistically significant differences among groups in vaccine response rates for the three pertussis antigens, the GMCs for antibodies against PT in the Separate Vaccine Group were lower than the corresponding antibody GMCs in the Combination Vaccine Group. However, GMCs for the pertussis antibodies were not different when PCV-7 was coadministered with DTaP-HepB-IPV vaccine or given 2 weeks later. Inconsistent differences in the response to pertussis have been observed in prescience clinical trials of PCV-7. For example, in one trial, infants who received PCV-7 coadministered with DTaP-IPV/Hib vaccine at 2, 3, and 4, and between 11 and 15 months of age had lower antibody GMCs for PRN than did children who received DTaP-IPV/Hib vaccine at 2, 3, and 4 months of age and PCV-7 at 6, 7, 8, and between 11 and 15 months of age. Likewise, in another study, when PCV-7 was coad-
administered with DTaP, lower antibody GMCs were noted for PT and FHA compared with the antibody response when PCV-7 was administered alone. Previous studies have demonstrated that coadministering PCV-7 with routine immunizations (DTaP, Hib, HepB, and IPV) results in higher rates of fever than when these vaccines are administered at different time points. In a study conducted by Schmitt et al, a significantly higher percentage of infants who received PCV-7 concomitantly with a DTaP-IPV/Hib combination vaccine had fever of 100.4°F (38.0°C) or higher after the first dose, compared with infants who received the DTaP-IPV/Hib vaccine alone (44.5% vs 29.9%; P = 0.035). In the present study, although there were significantly higher rates of fever in the Combination Vaccine Group compared with the Separate and Staggered Vaccine Groups, there were no significant differences in rates of fever at or above 102.2°F (39.0°C), and the fevers were short in duration and did not result in clinically important consequence.

When evaluating local symptoms in the Separate Vaccine Group, pain, redness, and swelling reported at the HepB and IPV injection sites (in addition to the DTaP site) should be considered. When any injection site was taken into account, no statistically significant differences in the incidence of pain or redness were observed between the Combination and Separate Vaccine Groups. Only the rate of swelling of more than 20 mm was significantly higher among infants in the Combination Vaccine Group compared with those in the Separate Vaccine Group (8.1% vs 2.7%, P = .024). During the 6 months after the third vaccine dose, the safety measures were similar among all three groups. The safety profile of DTaP-HepB-IPV vaccine also has been investigated in a study that compared DTaP-HepB-IPV vaccine coadministered with Hib and PCV-7 vaccines with administration of separate vaccines (DTaP, HepB, IPV, Hib, and PCV-7). The authors thank Len Friedland for his contribution to the preparation of this manuscript.

REFERENCES


APPENDIX

In addition to the authors, the other members of the 085 Study Group are J. Alvey, J. Casey, S. Christensen, S. A. Fagenholz, T. Klein, B. Nauert, B. Pistorius, R. Schaten, S. D. Senders, and M. Sperling.
Table I. Seroprotection, seropositivity, and vaccine response rates 1 month after third dose of DTaP or DTaP-HepB-IPV (according-to-protocol cohort for immunogenicity)

<table>
<thead>
<tr>
<th>End point</th>
<th>Combination Vaccine Group (n = 154-168)</th>
<th>Separate Vaccine Group (n = 141-156)</th>
<th>Difference (Separate minus Combination)</th>
<th>Staggered Vaccine Group (n = 149-158)</th>
<th>Difference (Staggered minus Combination)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anti-D ≥0.1 IU/mL</td>
<td>167 99.4</td>
<td>153 98.7</td>
<td>-0.70 -4.05 2.12‡ 157 100.0 0.60 -1.80 3.29</td>
<td>158 100.0</td>
<td>0.00 -2.37 2.24</td>
</tr>
<tr>
<td>Anti-T ≥0.1 IU/mL</td>
<td>168 100.0</td>
<td>152 98.1</td>
<td>-1.94 -5.54 0.32‡ 158 100.0 1.80 -0.60 5.15</td>
<td>154 100.0</td>
<td>-1.34 -5.44 2.26</td>
</tr>
<tr>
<td>Anti-HBsAg ≥10 mIU/mL‡</td>
<td>164 98.2</td>
<td>152 98.7</td>
<td>0.50 -3.01 4.01 158 100.0 1.80 -0.60 5.15</td>
<td>150 98.0</td>
<td>-0.66 -4.46 2.87</td>
</tr>
<tr>
<td>Vaccine response to PT</td>
<td>153 98.7</td>
<td>135 95.1</td>
<td>3.64 -8.69 0.31 148 97.4 -1.34 -5.44 2.26</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccine response to FHA</td>
<td>152 98.7</td>
<td>136 96.5</td>
<td>-2.25 -6.89 1.52 150 98.0 -0.66 -4.46 2.87</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vaccine response to PRN</td>
<td>143 91.7</td>
<td>135 95.1</td>
<td>3.40 -2.50 9.46 145 94.2 2.49 -3.44 8.62</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-poliovirus 1 ≥1:8</td>
<td>168 100.0</td>
<td>153 100.0</td>
<td>0.00 -2.45 2.24‡ 156 100.0 0.00 -2.40 2.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-poliovirus 2 ≥1:8</td>
<td>168 100.0</td>
<td>153 100.0</td>
<td>0.00 -2.45 2.24‡ 156 100.0 0.00 -2.40 2.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-poliovirus 3 ≥1:8</td>
<td>168 100.0</td>
<td>153 100.0</td>
<td>0.00 -2.45 2.24‡ 156 100.0 0.00 -2.40 2.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PRP ≥0.15 µg/mL</td>
<td>168 100.0</td>
<td>154 99.4</td>
<td>-0.65 -3.56 1.60 158 100.0 0.00 -2.37 2.24</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-PRP ≥1.0 µg/mL</td>
<td>161 95.8</td>
<td>141 91.0</td>
<td>-4.87 -10.91 0.55 150 94.9 -0.90 -6.02 3.97</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anti-S pneumoniae serotype 4</td>
<td>163 99.4</td>
<td>156 100.0</td>
<td>155 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6B</td>
<td>156 95.7</td>
<td>134 90.5</td>
<td>148 96.1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>9V</td>
<td>163 100.0</td>
<td>151 100.0</td>
<td>150 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>14</td>
<td>166 100.0</td>
<td>156 100.0</td>
<td>154 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>18C</td>
<td>166 99.4</td>
<td>153 100.0</td>
<td>156 100.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>19F</td>
<td>160 99.4</td>
<td>147 100.0</td>
<td>149 100.0</td>
<td></td>
<td></td>
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<tr>
<td>23F</td>
<td>158 97.5</td>
<td>141 96.6</td>
<td>150 98.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

D indicates diphtheria; DTaP, diphtheria–tetanus–acellular pertussis vaccine; FHA, filamentous hemagglutinin; HBsAg, hepatitis B surface antigen; HepB, hepatitis B vaccine; IPV, inactivated poliovirus vaccine; n, number of subjects with available results; PRN, pertactin; PRP, polyribosylribitol; PT, pertussis toxin; T, tetanus.

Vaccine response to PT, FHA, and PRN is defined as the appearance of antibodies in subjects who were initially seronegative (ie, with concentrations less than the cut-off value) or at least maintenance of prevaccination antibody concentrations in subjects who were initially seropositive (ie, with concentrations greater than or equal to the cut-off value).

*Regardless of hepatitis B vaccination status before study entry.

†No clinical limit defined for noninferiority; the 95% CIs of group differences in rates will not be used to draw conclusions on noninferiority of Combination Group to Separate Group but rather to provide information on the comparability of the two groups.

‡Noninferiority criterion met: Upper limit below the clinical limit for noninferiority.
### Table III. Overall incidence of solicited general and local symptoms reported within 4 days after vaccination in the per subject analysis

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Separate Vaccine Group n (%)</th>
<th>Combination Vaccine Group n (%)</th>
<th>P value</th>
<th>Staggered Vaccine Group n (%)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 188</td>
<td>n = 198</td>
<td></td>
<td>n = 187</td>
<td></td>
</tr>
<tr>
<td></td>
<td>n (%)</td>
<td>n (%)</td>
<td></td>
<td>n (%)</td>
<td></td>
</tr>
<tr>
<td>Rectal temperature</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥100.4°F</td>
<td>101 (53.7)</td>
<td>134 (68.0)</td>
<td>.005</td>
<td>87 (46.5)</td>
<td>.000</td>
</tr>
<tr>
<td>&gt;101.3°F</td>
<td>30 (16.0)</td>
<td>45 (22.8)</td>
<td>.095</td>
<td>21 (11.2)</td>
<td>.003</td>
</tr>
<tr>
<td>&gt;103.1°F</td>
<td>1 (0.5)</td>
<td>5 (2.5)</td>
<td>.216</td>
<td>1 (0.5)</td>
<td>.216</td>
</tr>
<tr>
<td>Irritability</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>157 (83.5)</td>
<td>185 (93.4)</td>
<td>.002</td>
<td>158 (84.5)</td>
<td>.005</td>
</tr>
<tr>
<td>Grade 3*</td>
<td>14 (7.4)</td>
<td>25 (12.6)</td>
<td>.128</td>
<td>14 (7.5)</td>
<td>.128</td>
</tr>
<tr>
<td>Drowsiness</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>145 (77.1)</td>
<td>159 (80.3)</td>
<td>.458</td>
<td>131 (70.1)</td>
<td>.024</td>
</tr>
<tr>
<td>Grade 3†</td>
<td>8 (4.3)</td>
<td>9 (4.5)</td>
<td>1.000</td>
<td>6 (3.2)</td>
<td>.602</td>
</tr>
<tr>
<td>Loss of appetite</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>101 (53.7)</td>
<td>116 (58.6)</td>
<td>.357</td>
<td>87 (46.5)</td>
<td>.019</td>
</tr>
<tr>
<td>Grade 3‡</td>
<td>2 (1.1)</td>
<td>3 (1.5)</td>
<td>1.000</td>
<td>2 (1.1)</td>
<td>1.000</td>
</tr>
<tr>
<td>Pain at any site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>118 (62.8)</td>
<td>126 (63.6)</td>
<td>.916</td>
<td>98 (52.4)</td>
<td>.030</td>
</tr>
<tr>
<td>Grade 3§</td>
<td>8 (4.3)</td>
<td>18 (9.1)</td>
<td>.068</td>
<td>10 (5.3)</td>
<td>.174</td>
</tr>
<tr>
<td>Pain at DTaP-HepB-IPV or DTaP site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>98 (52.1)</td>
<td>119 (60.1)</td>
<td>.124</td>
<td>95 (50.8)</td>
<td>.081</td>
</tr>
<tr>
<td>Grade 3§</td>
<td>3 (1.6)</td>
<td>15 (7.6)</td>
<td>.007</td>
<td>9 (4.8)</td>
<td>.297</td>
</tr>
<tr>
<td>Redness at any site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>125 (66.5)</td>
<td>135 (68.2)</td>
<td>.745</td>
<td>102 (54.5)</td>
<td>.007</td>
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<td>&gt;20 mm diameter</td>
<td>14 (7.4)</td>
<td>16 (8.1)</td>
<td>.851</td>
<td>20 (10.7)</td>
<td>.388</td>
</tr>
<tr>
<td>Redness at DTaP-HepB-IPV or DTaP site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>97 (51.6)</td>
<td>116 (58.6)</td>
<td>.184</td>
<td>96 (51.3)</td>
<td>.183</td>
</tr>
<tr>
<td>&gt;20 mm diameter</td>
<td>8 (4.3)</td>
<td>13 (6.6)</td>
<td>.373</td>
<td>18 (9.6)</td>
<td>.349</td>
</tr>
<tr>
<td>Swelling at any site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>84 (44.7)</td>
<td>102 (51.5)</td>
<td>.187</td>
<td>70 (37.4)</td>
<td>.006</td>
</tr>
<tr>
<td>&gt;20 mm diameter</td>
<td>5 (2.7)</td>
<td>16 (8.1)</td>
<td>.024</td>
<td>9 (4.8)</td>
<td>.219</td>
</tr>
<tr>
<td>Swelling at DTaP-HepB-IPV or DTaP site</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any</td>
<td>61 (32.4)</td>
<td>88 (44.4)</td>
<td>.016</td>
<td>64 (34.2)</td>
<td>.047</td>
</tr>
<tr>
<td>&gt;20 mm diameter</td>
<td>2 (1.1)</td>
<td>14 (7.1)</td>
<td>.004</td>
<td>9 (4.8)</td>
<td>.395</td>
</tr>
</tbody>
</table>

DTaP indicate diphtheria–tetanus–acellular pertussis vaccine; DTaP-HepB-IPV, diphtheria and tetanus toxoids and acellular pertussis adsorbed, hepatitis B (recombinant), and inactivated poliovirus vaccine.

*Grade 3 = crying that could not be comforted; prevented normal activity.
†Grade 3 = drowsiness that prevented normal activity.
‡Grade 3 = not eating at all.
§Grade 3 = cried when limb was moved/spontaneously painful.
Parents’ Perspectives Regarding a Physician-Parent Conference after Their Child’s Death in the Pediatric Intensive Care Unit

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Objective To investigate parents’ perspectives on the desirability, content, and conditions of a physician-parent conference after their child’s death in the pediatric intensive care unit (PICU).

Study design Audio-recorded telephone interviews were conducted with 56 parents of 48 children. All children died in the PICU of one of six children’s hospitals in the National Institute of Child Health and Human Development Collaborative Pediatric Critical Care Research Network (CPCCRN) 3 to 12 months before the study.

Results Only seven (13%) parents had a scheduled meeting with any physician to discuss their child’s death; 33 (59%) wanted to meet with their child’s intensive care physician. Of these, 27 (82%) were willing to return to the hospital to meet. Topics that parents wanted to discuss included the chronology of events leading to PICU admission and death, cause of death, treatment, autopsy, genetic risk, medical documents, withdrawal of life support, ways to help others, bereavement support, and what to tell family. Parents sought reassurance and the opportunity to voice complaints and express gratitude.

Conclusions Many bereaved parents want to meet with the intensive care physician after their child’s death. Parents seek to gain information and emotional support, and to give feedback about their PICU experience. (J Pediatr 2007;151:50-5)

In the United States, 53,000 children die annually. Most of these deaths occur in inpatient hospital settings, primarily intensive care units. Pediatric intensive care physicians are extensively involved in the care of dying children and their families. Such care includes communicating poor prognoses, treating pain and other symptoms, advising on decisions regarding life support, requesting permission for autopsy, and initiating organ donation. In managing the child’s death, intensive care physicians have a unique opportunity to help parents prepare for the death and begin a grief process that enables the family to remain functional and intact.

Previous studies have documented the need for greater parental support following the death of a child and better physician training to provide such support. Bereaved parents have expressed the need for comprehensive information regarding their child’s illness and death, emotional support, and consistent follow-up by their child’s physicians. Professional organizations such as the American Academy of Pediatrics, the Royal College of Paediatrics and Child Health, and the Society of Critical Care Medicine suggest that physician-parent meetings to discuss the death and review autopsy results may help meet families’ needs during bereavement. However, evidence regarding parents’ desire for such meetings, the most appropriate time and place, the topics to be discussed, and the participants to be involved is lacking. The paucity of evidence and inadequate training may contribute to physicians’ reluctance to meet with parents after a child’s death.
Family perspectives must be strongly considered when planning supportive interventions during the complex experience of bereavement. The objective of this study was to investigate parents’ perspectives regarding the desirability, content, and conditions of a physician-parent conference conducted after their child’s death in the pediatric intensive care unit (PICU).

METHODS

Setting

The Collaborative Pediatric Critical Care Research Network (CPCCRN) established by the National Institute of Child Health and Human Development consists of six clinical centers and a data coordinating center.17 Pediatric intensive care physicians have primary responsibility for the care of all medical patients and routinely provide consultation on surgical patients in the PICU at each center.

Participants

Parents or legal guardians were eligible to participate if their child died in the PICU at one of the CPCCRN sites between 3 and 12 months before the start of the study. The medical records of the deceased children were reviewed to obtain the parents’ contact information and primary language.18 Parents who did not speak English or Spanish were excluded. The study was approved by the Institutional Review Board at each site. Informed consent was obtained from all participants.

Recruitment

Parents were contacted consecutively beginning with those whose child died 12 months earlier. Initial contact occurred via a mailed letter that originated from the hospital where the child died. The letter asked parents to participate in a research interview. Parents were telephoned 2 weeks later to explain the details of the study and schedule interviews. If both parents of one child agreed to participate, separate interviews were scheduled.

Interviews

A committee of CPCCRN investigators developed an interview guide to elicit parents’ experiences with and perceptions about meeting with their child’s intensive care physician after their child’s death. The interview guide was based on the bereavement literature19-23 and the clinical experience of the investigators. Spanish versions of the interview guide were developed by forward and back translation. To standardize interview procedures, interviewers participated in training sessions that included didactics, modeling of interview techniques, role-playing, and feedback.

Interviews were conducted between January 19, 2006 and May 22, 2006 by research assistants from the clinical centers where the children died. Interviews were conducted in English or Spanish over the telephone and were digitally audio-recorded. Parents responded to questions about their contacts with hospital personnel since their child died; their desire to meet with their child’s intensive care physician; and the preferred timing, location, participants, and topics for such a meeting. Parents also ranked the importance of predefined topics and provided demographic information. Parents were asked to respond to all questions in the interview guide. If a parent indicated that he or she would not want to have a physician-parent conference, the parent was asked to explain the reason why not, and to respond hypothetically to further questions about the meeting. Parents selected their race and ethnicity from a predefined list to assess sample diversity. All interviews were monitored by one of two investigators (KM, SE) who provided feedback to the interviewer to maintain standardization and quality.

Medical Record Review

Medical records of the deceased children of participating parents were reviewed to obtain the child’s age, sex, trajectory of death, mode of death, and length of PICU and hospital stay. Mode of death was categorized as limitation of therapy, withdrawal of therapy, brain death, or death despite cardiopulmonary resuscitation.24

Data Analysis

Analysis was ongoing during data collection, and interviews were conducted until saturation was reached.25 Two investigators, a pediatric intensive care physician (KM) and a behavioral scientist with expertise in health communication (SE), analyzed the interviews. The behavioral scientist is bilingual; the physician analyzed the Spanish interviews with the assistance of a translator. The two investigators listened to each interview independent of each other and wrote detailed notes on parents’ responses to the questions.26 Responses to select questions were transcribed verbatim. Displays of emotion (e.g., crying) were noted. The two investigators compared their notes for accuracy and generated a combined data set. Discrepancies between investigators were resolved by listening to the audio-recording together and reaching consensus. A member of the data coordinating center reviewed 20% of the interviews with representation from each site to confirm the accuracy of the data set.

The data set was imported into a qualitative analysis software program (QSR N6, QSR International Pty Ltd., Doncaster, Australia) to facilitate data management. The two investigators used an iterative process to identify themes pertaining to the content and conditions of the physician-parent conference. This process included independent reading of the data set to identify themes, comparison of themes between investigators, and re-reading of the data set and discussion to refine themes and reach consensus on their meaning. Exemplars were taken from the transcribed sections of the interviews. To enhance the validity of the thematic analysis, two bereaved parents reviewed the manuscript to provide their opinions as to whether parents’ views were appropriately
represented. Categorical data were described as absolute counts and percentages, and continuous data were described as medians and ranges.

RESULTS

Parents of 161 deceased children were sent letters explaining the study; 56 parents of 48 children (30% of families) were interviewed, parents of 33 children (20%) refused, and parents of 79 children (49%) could not be contacted by telephone. One mother (1%) agreed to participate and was interviewed, but the recording device malfunctioned and the interview was lost (Tables I and II). Parents were interviewed a median of 8 months (range, 4-15 months) after their child’s death. Five interviews were conducted in Spanish.

Contacts with Hospital Personnel Since the Child’s Death

Thirty-five (63%) parents had spoken with one or more hospital workers since their child’s death. Sixteen (29%) parents had had contact with a physician; however, only seven (13%) had had a scheduled meeting with a physician to discuss their child’s death. Other physician contacts included expressions of condolence via telephone or at memorial services, and chance visits in corridors when parents returned to the hospital for other purposes.

Twenty-five (45%) parents had spoken with a nurse or ancillary health provider. Of these, 13 (52%) parents had had spontaneous social visits with staff, and 12 (48%) had planned professional contacts for psychosocial support. Eight (14%) parents had spoken with administrative personnel about hospital billing, charitable donations, or voluntary participation on hospital advisory boards.

Desirability of Meeting with an Intensive Care Physician

Thirty-three (59%) parents wanted to meet with their child’s intensive care physician, 19 (34%) did not want to meet, two (4%) were undecided, and two (4%) did not answer the question. Of those who did not want to meet with the intensive care physician, nine (47%) were satisfied with the information and support provided by the physician before the child’s death, seven (37%) were dissatisfied with the physician’s availability and communication skills, two (11%) gave no explanation, and one planned to meet with another physician to discuss the child’s death. For example, a satisfied parent explained, “They were very informative. When I left the hospital when my son died I knew of everything that I needed to know.” In contrast, a dissatisfied parent said, “They should have been there before she died. After the fact, it’s just a little late to discuss it and try to talk about it after she’s passed away.”

Place, Timing, and Meeting Participants

Of the 33 parents who wanted to meet with the intensive care physician, 27 (82%) were willing to return to the hospital to meet. One parent stated, “It would have been difficult but nevertheless I would have come.” Similarly, another parent responded, “Yes, although it’s not easy. But I would feel that it’s important enough because there are so many questions.” Parents’ preferred timing for meeting with the intensive care physician ranged from one day after the death to more than one year. Of those who wanted to meet, 15 (45%) wanted to
meet within the first 3 months, five (15%) between 3 and 6 months, four (12%) between 6 and 12 months, four (12%) after one year, one (3%) anytime, and four (12%) were undecided. One parent explained, “Early enough to have any benefit that you could have from it yet not just so close to the grieving time that you’re not hearing what anybody’s saying anyhow.” Twenty-six parents (79%) wanted their spouse/partner to attend the meeting, 12 (36%) wanted their own parents to attend, and 18 (55%) wanted a nurse who had cared for their child to attend. One parent responded, “Somebody you could trust. In my case, maybe my mother.” Another parent said, “I think it would be really helpful to have the primary care nurse there, too. They may ask questions in that meeting that you maybe didn’t think of from a medical standpoint.”

Among the 23 parents who did not want to meet with the intensive care physician, or who were undecided about meeting or did not answer that question, 16 (70%) felt that they would be willing to return to the hospital if they had decided to meet. Nine (39%) felt that the best time for such a meeting would be within the first 3 months, three (13%) between 3 and 6 months, five (22%) between 6 and 12 months, two (9%) after one year, one (4%) anytime, and three (13%) were undecided. Nineteen (83%) wanted to bring their spouse/partner, five (22%) wanted to bring their parents, and nine (39%) wanted to invite the child’s nurse, if they decided to meet.

Content of the Meeting

Parents most often mentioned their desire to gain information; next, their desire to provide feedback to the physician regarding their PICU experience; and to a lesser extent their need for emotional support. Informational topics spontaneously mentioned by parents included the chronology of events leading to PICU admission and death, cause of death, treatment, autopsy, genetic risk, medical documents, withdrawal of life support, ways to help others, bereavement support, and what to tell other family members (Table III; available at www.jpeds.com). Parents’ ranked responses to questions about the importance of predefined topics showed that information about treatment, autopsy, cause of death, medical records, and bereavement support was very important to most parents (Figure).

Parents wanted to provide feedback on several aspects of care including physician communication (Table IV; available at www.jpeds.com). Parents frequently perceived that information was withheld during their child’s PICU stay, especially regarding prognosis. Other communication issues included callous style, use of medical jargon, and conflicting information from different physicians. Additionally, many parents wanted to express gratitude for the care received and to provide feedback on other health providers, their degree of trust in physicians and the healthcare system, medical errors, and administrative issues.

Emotional support sought by parents included reassurance and the sense that the physician cared about them. One parent explained the need for reassurance, “And like I said, if there was anything else that we could have done. I don’t even know if knowing there was something else would be helpful but it’s always on your mind. Did we do everything we could have done? Were we good parents? It’s more about reassuring.” Another parent described a feeling of abandonment after the death and the need to know that the physician still cared, “It seems like they care so much while it’s going on and as soon as it’s done they forget about you. You build a pretty good trust with these people for a couple of months of your life and all of a sudden they aren’t there. I would have liked my doctor to have at least called me.”

DISCUSSION

Our findings indicate that many parents want to meet with their child’s intensive care physician to discuss the death of their child, and they are willing to return to the hospital to do so. However, our findings also indicate that such meetings rarely occur. Some parents wanted to meet with the physician early after the death, whereas others preferred to wait until the distress of acute grief had begun to subside. Parents envisioned the conference to be a small personal meeting with the intensive care physician and in some cases family members or hospital personnel who had close relationships with their child. Parents sought information about their child’s illness and death, the opportunity to provide feedback about their PICU experience, and emotional support. These findings support the published opinions of experienced clinicians and the scant research conducted on physician–family conferences during bereavement in other populations.19,21,22,27-29

The most important component of the physician-parent conference is the provision of information to parents. Parents reported that the emotional turmoil surrounding the child’s demise made it difficult for them to comprehend information provided at that time. Information most frequently sought by parents and ranked highest in importance was directly related to the child’s treatment and cause of death. Many parents felt that a review of the sequence of events leading to the child’s PICU admission and death would help them to make sense of what happened. Medical records
and autopsy reports were viewed by parents as additional sources of information that could increase their understanding of their child’s treatment and cause of death. Parents also wanted information about the risk of the illness in other children and steps that could be taken toward prevention. Our findings concur with those of Meyer et al that showed that complete and honest information is one of parents’ top priorities for quality care of dying children. Parents continue to seek information after their child’s death. Regarding the most appropriate timing for providing such information, social support theory suggests that soon after the death rather than later may be more beneficial. Information provided early on can help parents more accurately appraise the experience of their child’s death and their own adaptive capabilities, thereby promoting a healthier response to the loss.

Feedback that parents wanted to provide to the physician often concerned ineffective communication. Many parents reported that “bad news” was withheld or delivered poorly, leaving them with a sense of betrayal and loss of control. Although some parents believed that information was withheld to protect their hope, parents stated that they preferred to hear the truth to spare their child and themselves unnecessary suffering. Problems with communication at the end of a child’s life have been previously described. In a study by Contro et al, families reported the distress they experienced by the uncaring delivery of bad news, callous remarks made by staff, and the receipt of contradictory information about their child’s condition and prognosis. In this same study, physicians and staff reported feeling inexperienced in communicating with patients and families about end-of-life issues, and they described their own need for greater emotional, psychological, and social support when caring for dying patients. In our study, problems with communication during the child’s last hospitalization was a common reason given by parents for not wanting to meet with their child’s intensive care physician after the death.

Additional feedback that parents wanted to provide included complaints about people or events that they perceived as wrongful. Parents often explained that their negative feedback was intended to prevent other families from experiencing similar problems. Isolated incidents such as callous remarks and preventable oversights in care are long remembered by bereaved parents. Allowing families to speak and be heard at end-of-life conferences increases family satisfaction and reduces conflict with physicians. Careful listening may also help families during bereavement. Many parents wanted to provide feedback on positive aspects of care as well. For example, parents wanted to express gratitude to health professionals whom they perceived had gone beyond the call of duty in caring for their child.

The most frequent type of emotional support sought by parents was reassurance that the right decisions had been made and that no other plan of action would have altered the child’s outcome. Research conducted after neonatal death has shown that parents welcome reassurance from a source they perceive as authoritative. Parents also wanted to know that the physician cared about them after the child’s death. Although most parents did not rank physician inquiries about personal and family coping as very important, many parents explained that they would perceive such questions as a sign of caring. Parents did not expect the physician to provide grief counseling directly during the conference. Most parents ranked bereavement support as very important; however, several commented that referrals for such care could be made by social workers or chaplains.

The physician-parent conference is not the only forum by which emotional support can be offered to parents. Many of the parents in this study received emotional support through contacts with nurses, chaplains, social workers, and other hospital staff in the form of letters, telephone calls, and personal visits. MacDonald et al described the deep appreciation felt by parents toward staff who attended memorial services, sent sympathy cards, or performed other acts of kindness and commemoration after a child’s death. Parents’ perceptions of a caring emotional attitude from staff during the child’s illness and death have been associated with a decreased intensity of parental grief both immediately after the death and in the long term.

Limitations of this study include the large number of parents who could not be contacted and the predominance of mothers among participants. Differences in parents’ views based on demographics, the trajectory of death, or mode of death could not be evaluated because of the small sample size. Also, questions remain regarding whether parents would prefer to meet with a physician other than the one who cared for their child in the PICU. Strengths of this study include the geographic diversity of the participants and the collection of data directly from bereaved parents.

Parents should be invited to attend a physician-parent conference early after their child’s death, and this invitation should be kept open for those parents who want to meet with the physician at a later time. Physicians should be prepared to provide information, receive feedback from parents about their PICU experience, and offer emotional support. More research is needed to evaluate the therapeutic effects of a physician-parent conference on parental grief.

REFERENCES

Parents’ Perspectives Regarding a Physician-Parent Conference after Their Child’s Death in the Pediatric Intensive Care Unit


### Table III. Informational topics that parents want to discuss with the intensive care physician*

<table>
<thead>
<tr>
<th>Topic</th>
<th>Sample quotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chronology of events leading to PICU admission and death</td>
<td>“I would just like to clarify what happened. J was in a regular room and she kind of crashed. By the time I got back to the hospital, she went from being in a regular room to being in ICU and everything was just horrid. At that point, there really wasn’t a chance to go, “What happened!””</td>
</tr>
<tr>
<td>Cause of death</td>
<td>“Nobody ever really told me what was wrong with him. It was some different things that they had said could be but nothing was a fact. I just want to know why he died.”</td>
</tr>
<tr>
<td>Treatment</td>
<td>“I want to know about her medicines and the different beds they had her in and what role they played and what were they hoping to accomplish by putting her in those beds and with the machines that they used on her.”</td>
</tr>
<tr>
<td>Autopsy</td>
<td>“We had issues about the autopsy which I would have liked to have explained a little bit more.”</td>
</tr>
<tr>
<td>Genetic risk</td>
<td>“Is it something genetic? Is it something to look for in my other children?”</td>
</tr>
<tr>
<td>Medical documents</td>
<td>“The only question that we really had was on his death certificate. It was marked cerebral edema and we’re curious as to why that was, rather than marked as actually SIDS. Cause, they said that’s exactly what SIDS is, when they quit breathing.”</td>
</tr>
<tr>
<td>Limitation/withdrawal of life support</td>
<td>“What I’d like to ask is the whole difference between critical care and comfort care. You know we talked about it with the doctor in the conference room, when we made that decision, but that would probably be the topic that I’d want to talk about.”</td>
</tr>
<tr>
<td>Ways to help others</td>
<td>“My only thing now, is there anything I could do in terms of being there for other parents or helping them in that respect?”</td>
</tr>
<tr>
<td>Bereavement support</td>
<td>“Maybe talk to them about where you can get help. I think it would be important if they think about telling you what you could do and where you could go.”</td>
</tr>
<tr>
<td>What to tell other family members</td>
<td>“After the fact, we had a lot of questions asked to us, by our own family. Everybody. We tried answering the best we could but when everything is going on it’s really hard to communicate to the rest of the family all the details and everything.”</td>
</tr>
</tbody>
</table>

*Topics are listed in order of decreasing frequency of mention by parents.

### Table IV. Feedback that parents want to provide to the intensive care physician*

<table>
<thead>
<tr>
<th>Topic</th>
<th>Sample quotation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Communication</td>
<td></td>
</tr>
<tr>
<td>Withholding prognosis</td>
<td>“It was apparent they knew my baby was dying but none of them quite came out and said ‘your baby’s gonna die’ . . . So they knew and that irritated me that they didn’t come out and say it.”</td>
</tr>
<tr>
<td>Calloos delivery of “bad news”</td>
<td>“The way the news was delivered to me was horrible. It was very callous. I was not offered a chair. I was not offered a drink of water. I was alone.”</td>
</tr>
<tr>
<td>Use of medical jargon</td>
<td>“The head of PICU was very helpful in explaining everything in layman’s terms.”</td>
</tr>
<tr>
<td>Conflicting information</td>
<td>“I talked to one doctor and he told me not to have this procedure done this way. And I turned around and the intensive care doctor was doing the procedure that way . . . I think the doctors need to talk to one another.”</td>
</tr>
<tr>
<td>Gratitude</td>
<td>“I would really like to thank them and compliment them on how they handled the situation. They were very good about it and they tried to prepare us for everything.”</td>
</tr>
<tr>
<td>Other providers</td>
<td>“I realize that everywhere you go, there are different personalities, but some of these nurses there, were magnificent. But some of them were just doing it for a paycheck. They are not nurses.”</td>
</tr>
<tr>
<td>Degree of trust</td>
<td>“Why was they looking guilty when I came in the room, like they done did something to him . . . ? That made me think they killed my baby.”</td>
</tr>
<tr>
<td>Medical errors</td>
<td>“I would also like to talk about that in the future, when a child is so young and so delicate and also sick, that there should be much more care taken in following medical orders.”</td>
</tr>
<tr>
<td>Administrative issues</td>
<td>“The complaint process was very weak as well. I submitted 3 written complaints on the forms that are provided by the hospital and I never got any feedback.”</td>
</tr>
</tbody>
</table>

*Topics are listed in order of decreasing frequency of mention by parents.
APPENDIX

Acknowledgments: Children's Hospital of Michigan, Detroit, MI: Sabrina Heidemann, MD, Maureen Frey, PhD, RN; Karmanos Cancer Institute, Detroit, MI: Terrance L. Albrecht, PhD; Children's National Medical Center, Washington, DC: Michael Bell, MD, Jean Reardon, BSN, RN, Sandy Romero, CCLS; Arkansas Children's Hospital, Little Rock, AR: Parthak Prodhani, MD, Glenda Hefley, MSNc, RN; Seattle Children's Hospital, Seattle, WA: Thomas Brogan, MD, Ruth Barker, RRT; Children's Hospital of Pittsburgh, Pittsburgh, PA: Shekhar T. Venkataraman, MD, Alan Abraham, BA; Children's Hospital Los Angeles, Los Angeles, CA: Jeffrey I. Gold, PhD, Elizabeth Ferguson, RN, J. Francisco Fajardo, CLS (ASCP), RN, MD; Mattel Children's Hospital at University of California Los Angeles, Los Angeles, CA: Rick Harrison, MD; University of Utah (Data Coordinating Center), Salt Lake City, UT: Jeri Burr, BS, RNC, CCRC, Amy Donaldson, MS, Richard Holubkov, PhD, Devinder Singh, BS, Rene Enriquez, BS; National Institute of Child Health and Human Development, Bethesda, MD: Tammara Jenkins, MSN, RN.

The Following Individuals are Members of the Collaborative Pediatric Critical Care Research Network:

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Objective  To assess the impact of parenting style and disease severity on quality of life (QOL) in children with cerebral palsy (CP).

Study design  Thirty-nine children with CP, their siblings, and their parents participated in the study. Probands and siblings, ages 6 to 18 years, completed questionnaires on parenting style (accepting, rejecting, controlling, and autonomy allowing) using the Children’s Report of Parental Behavior Inventory. Parents completed generic (Child Health Questionnaire [CHQ]) and disease-specific (Pediatric Outcomes Data Collecting Instrument [PODCI]) QOL questionnaires for both children. A physician determined disease severity with the Gross Motor Function Classification System.

Results  In children with CP, parenting style positively correlated with the CHQ scores: physical summary and psychosocial summary ($r = 0.40$, $P < .01$) and family activities scale ($r = 0.34$, $P = .03$). Autonomy allowing parenting style impacted on psychosocial aspects of QOL, as reflected by CHQ scores, more than the degree of disability. In other domains of QOL, the effect of parenting style was greater than IQ, anxiety, and socioeconomic status.

Conclusions  Parenting style is a significant factor in QOL in CP and the only known factor to impact on the psychosocial domains of the CHQ, exceeding the effect of disease severity. Because QOL is an important treatment goal in children with CP, early family interventions, particularly those focusing on parenting style, should be considered. (J Pediatr 2007;151:56-60)

Traditionally, treatment of children with chronic diseases focused on physical aspects of the disease, and treatment efficacy was measured primarily by means of physical improvement. In the past decade, quality of life (QOL), defined as well-being across various broad domains, has become an important treatment goal in chronic diseases, including cerebral palsy (CP). CP is a non–progressive disorder of movement and posture caused by a defect or injury to the immature brain, and its impact is further exacerbated by disabilities other than the motor impairments, such as epilepsy, learning disabilities, and behavioral and emotional problems. Whereas treatment modalities for CP have been documented in a multitude of studies, there are few data on their subjective impact on children with CP and their families.

Earlier studies revealed that children with only mild CP have lower QOL scores than the healthy population. Although QOL scores in the physical domains correlated well with the level of motor disability, this was not the case for scores in the psychosocial scales. This finding points to the importance of factors affecting QOL other than level of motor disability.

Parenting style is one of the most important family variables in the child's psychosocial development. Parenting behaviors are complex, but can be represented in a 2-dimensional model for clinical and research work. One dimension is parental control and restriction versus autonomy allowance: parental control is extensive regulation of the child's behaviors and actions, autocratic parental decision-making, overprotection, and instructions to the child about how he or she should think or feel. Parental autonomy allowance is characterized by ways parents enable their child to act freely and be independent. The second dimension is parental rejection and hostility versus acceptance and support: parental criticism is characterized by coldness, indifference, and lack of pleasure from being with the child, whereas parental acceptance is a warm and welcoming attitude, active listening, and emotional involvement in the child's life.

The goal of this study was to assess the impact of parenting style on the QOL of children with CP and their siblings.
METHODS

Participants
Families of children with CP (n = 47) who fulfilled these criteria were identified: 1) an unequivocal diagnosis of CP made by a pediatric neurologist; 2) IQ score ≥75; 3) age 6 to 18 years; 4) a school-age sibling with normal intelligence; 5) parents with a working knowledge of Hebrew. The 8 families who declined participation had similar demographic features to the 39 who participated. The mean age of probands was 12.0 ± 3.1 years (58% boys), and the mean age of siblings was 13.2 ± 2.9 (46% boys). Eighteen siblings (42%) were older than the probands, 15 (34%) were younger, and 7 (24%) were the same age (twins). Full scale IQ of probands was 94 ± 17, and it was 109 ± 12 for siblings. Socioeconomic status was high for 31% of the families, middle for 33% of the families, and low for 36% of the families.

Each proband was examined at home by a pediatric neurologist (A.A.) who reviewed the medical chart and assessed motor disability. The parents and the children completed the tools outlined below independently. In accordance with the instructions of each tool, clarifications to queries were, or were not, provided by the psychologist or the pediatric neurologist. For those children who were not competent readers, the questions were read aloud, and a brief explanation or example was provided, when necessary.

Measures
The Gross Motor Function Classification System (GMFCS) measures severity of CP and rates outcome of motor function in a scale ranging from I to V and was completed by the physician (A.A.). CP was graded as mild when the child could walk independently (I/II), moderate when the child was ambulant with assistive devices (III), and severe when the child was wheelchair dependent (IV/V).15,24 Table I provides information on CP subtype, severity of motor deficit and comorbid conditions.

Parental Questionnaires
The Child Health Questionnaire parent form-50 (CHQ PF-50; validated Hebrew version) is a generic questionnaire that evaluates aspects of QOL, with 50 questions. A score of 0 represents the worst health state, and a score of 100 represents the best health state. The CHQ has 2 summary scores: physical and psychosocial.25,26

The Pediatric Outcomes Data Collection Instrument (PODCI) is a 55-question disease-specific questionnaire that assesses parent-perceived functional health status of physical function, pain/comfort, expectations from treatment, and happiness with physical condition (100 reflects best health status).27,28 The version used was translated into Hebrew specifically for this study.

Demographic Questionnaire
Relevant medical information, comorbid conditions, and socioeconomic status were provided by the parents.29

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mild (n = 12)</th>
<th>Moderate (n = 13)</th>
<th>Severe (n = 14)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Type of CP (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hemiplegia (n = 8)</td>
<td>6 (15.5)</td>
<td>2 (5.0)</td>
<td>0</td>
</tr>
<tr>
<td>Diplegia (n = 12)</td>
<td>6 (15.5)</td>
<td>5 (13.0)</td>
<td>1 (2.5)</td>
</tr>
<tr>
<td>Quadriplegia (n = 19)</td>
<td>0</td>
<td>6 (15.5)</td>
<td>13 (33.0)</td>
</tr>
<tr>
<td>Comorbid conditions (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>ADHD and/or LD (n = 20)</td>
<td>8 (20.5)</td>
<td>4 (10.0)</td>
<td>8 (20.5)</td>
</tr>
<tr>
<td>Epilepsy (n = 7)</td>
<td>1 (2.5)</td>
<td>2 (5.0)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Speech and language disorders (n = 8)</td>
<td>3 (7.5)</td>
<td>1 (2.5)</td>
<td>4 (10.0)</td>
</tr>
<tr>
<td>Etiology of CP (%)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prematurity (n = 27)</td>
<td>7 (18.0)</td>
<td>11 (28.0)</td>
<td>9 (23.0)</td>
</tr>
<tr>
<td>Complicated labor at term</td>
<td>2 (5.0)</td>
<td>3 (7.5)</td>
<td></td>
</tr>
<tr>
<td>Moderate (n = 5)</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Mild, GMFCS levels 1-2; Moderate, level 3; Severe, levels 4-5; ADHD, attention deficit hyperactivity disorder; LD, learning disabilities.

Questionnaires Completed by the Children
The Children’s Report of Parental Behavior Inventory (CRPBI), validated Hebrew version, is a 40-item questionnaire evaluating 4 domains of the mother’s parenting style as experienced by the child: accepting, rejecting, controlling, and autonomy allowing.20,30

The Revised Children’s Manifest Anxiety Scale (RCMAS), validated Hebrew version,31,32 is a 37-item questionnaire that evaluates the child’s level of anxiety according to self-report.

The Wechsler Intelligence Scale for Children-Revised (WISC-R), Hebrew version was also used.33

Statistical Analysis
Demographic, descriptive, and comparative statistics were computed with the SPSS statistical package software (version 11). Internal consistency was estimated by computing Cronbach’s α coefficient for each scale. Because we used multidimensional instruments, Cronbach’s α was computed for each subscale. The concurrent validity of the different instruments was assessed by examining the association between different scales that measure the same health domain. The t test for dependent samples was used to compare the CHQ scores between the child with CP and the sibling.

Multivariate regression analysis was used to compare the CHQ and PODCI scores of the children with CP according to the severity of the disability.

RESULTS
QOL in Children with CP and Their Siblings
As expected, the parental report on health of children with CP was poorer for every subscale of the CHQ (Table II). The physical summary score and the psychosocial summary score were more than 2 SD lower than that of their healthy siblings.
Table II. Child Health Questionnaire scores of probands with cerebral palsy, siblings, and normative sample

<table>
<thead>
<tr>
<th>Scale</th>
<th>CP probands (n = 39)</th>
<th>Siblings (n = 39)</th>
<th>t value*</th>
<th>P value</th>
<th>Normative sample (n = 64)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Functioning</td>
<td>41.3 ± 32.1</td>
<td>94.8 ± 16.6</td>
<td>7.77</td>
<td>.000</td>
<td>97.2 ± 12.2</td>
</tr>
<tr>
<td>Role/Social Limitations-Physical</td>
<td>56.4 ± 39.7</td>
<td>96.7 ± 11.3</td>
<td>6.23</td>
<td>.000</td>
<td>98.7 ± 7.5</td>
</tr>
<tr>
<td>Role/Social Limitations-Emotional Behavioral</td>
<td>71.8 ± 31.6</td>
<td>95.9 ± 12.9</td>
<td>4.66</td>
<td>.000</td>
<td>99.3 ± 3.4</td>
</tr>
<tr>
<td>Bodily Pain/Discomfort</td>
<td>74.1 ± 24.4</td>
<td>88.3 ± 13.8</td>
<td>3.69</td>
<td>.001</td>
<td>92.7 ± 14.3</td>
</tr>
<tr>
<td>Behavior</td>
<td>71.4 ± 16.3</td>
<td>81.8 ± 10.1</td>
<td>3.16</td>
<td>.003</td>
<td>83.8 ± 14.6</td>
</tr>
<tr>
<td>Mental Health</td>
<td>64.6 ± 14.3</td>
<td>77.0 ± 10.6</td>
<td>4.39</td>
<td>.000</td>
<td>80.1 ± 9.2</td>
</tr>
<tr>
<td>Self-Esteem</td>
<td>68.5 ± 17.2</td>
<td>87.5 ± 11.5</td>
<td>5.94</td>
<td>.000</td>
<td>90.9 ± 11.2</td>
</tr>
<tr>
<td>General Health</td>
<td>59.4 ± 16.9</td>
<td>73.0 ± 14.8</td>
<td>3.92</td>
<td>.000</td>
<td>77.8 ± 13.3</td>
</tr>
<tr>
<td>Parent Impact-Emotional</td>
<td>58.1 ± 27.3</td>
<td>82.4 ± 20.0</td>
<td>4.47</td>
<td>.000</td>
<td>87.2 ± 14.9</td>
</tr>
<tr>
<td>Parent Impact-Time</td>
<td>76.1 ± 27.0</td>
<td>93.7 ± 16.9</td>
<td>3.34</td>
<td>.002</td>
<td>95.3 ± 10.2</td>
</tr>
<tr>
<td>Family Activities</td>
<td>72.8 ± 22.2</td>
<td>89.2 ± 15.8</td>
<td>3.78</td>
<td>.001</td>
<td>91.9 ± 12.4</td>
</tr>
<tr>
<td>Family Cohesion</td>
<td>64.1 ± 26.8</td>
<td>65.7 ± 29.1</td>
<td>0.00</td>
<td>1.00</td>
<td>84.2 ± 17.3</td>
</tr>
<tr>
<td>Physical Summary Score</td>
<td>35.7 ± 17.6</td>
<td>50.9 ± 10.9</td>
<td>9.15</td>
<td>.000</td>
<td>54.6 ± 3.3</td>
</tr>
<tr>
<td>Psychosocial Summary Score</td>
<td>43.9 ± 14.5</td>
<td>51.2 ± 7.9</td>
<td>6.26</td>
<td>.000</td>
<td>54.6 ± 6.2</td>
</tr>
</tbody>
</table>

* t test was used to compare probands to siblings.

Table III. Child Health Questionnaire scores as a function of the motor severity of cerebral palsy according to Gross Motor Function Classification System

<table>
<thead>
<tr>
<th>Scale scores ± SD/severity of CP</th>
<th>Mild (n = 13)</th>
<th>Moderate (n = 12)</th>
<th>Severe (n = 14)</th>
<th>F</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Physical Functioning</td>
<td>73.1 ± 22.3</td>
<td>41.2 ± 22.9</td>
<td>11.9 ± 12.7</td>
<td>32.8</td>
<td>.000</td>
</tr>
<tr>
<td>Role/Social Limitations-Physical</td>
<td>74.4 ± 35.8</td>
<td>61.1 ± 37.2</td>
<td>35.7 ± 38.0</td>
<td>3.8</td>
<td>.031</td>
</tr>
<tr>
<td>Role/Social Limitations-Emotional Behavioral</td>
<td>73.5 ± 31.3</td>
<td>80.6 ± 28.9</td>
<td>62.7 ± 33.9</td>
<td>1.1</td>
<td>.356</td>
</tr>
<tr>
<td>Bodily Pain/Discomfort</td>
<td>74.1 ± 24.4</td>
<td>78.3 ± 19.9</td>
<td>68.6 ± 33.2</td>
<td>0.6</td>
<td>.568</td>
</tr>
<tr>
<td>Behavior</td>
<td>76.2 ± 16.1</td>
<td>81.3 ± 10.1</td>
<td>69.0 ± 17.4</td>
<td>3.9</td>
<td>.028</td>
</tr>
<tr>
<td>Mental Health</td>
<td>64.6 ± 13.9</td>
<td>69.6 ± 13.4</td>
<td>60.4 ± 14.9</td>
<td>1.4</td>
<td>.264</td>
</tr>
<tr>
<td>Self-Esteem</td>
<td>73.4 ± 20.5</td>
<td>72.6 ± 13.5</td>
<td>60.4 ± 14.6</td>
<td>2.6</td>
<td>.086</td>
</tr>
<tr>
<td>General Health</td>
<td>61.5 ± 17.4</td>
<td>60.1 ± 19.7</td>
<td>56.8 ± 14.5</td>
<td>0.3</td>
<td>.770</td>
</tr>
<tr>
<td>Parent Impact-Emotional</td>
<td>63.5 ± 20.0</td>
<td>71.5 ± 29.8</td>
<td>41.7 ± 23.8</td>
<td>5.1</td>
<td>.011</td>
</tr>
<tr>
<td>Parent Impact-Time</td>
<td>76.9 ± 27.8</td>
<td>92.6 ± 11.9</td>
<td>92.6 ± 11.9</td>
<td>5.4</td>
<td>.009</td>
</tr>
<tr>
<td>Family Activities</td>
<td>79.2 ± 19.6</td>
<td>84.7 ± 14.3</td>
<td>56.5 ± 21.3</td>
<td>8.4</td>
<td>.001</td>
</tr>
<tr>
<td>Family Cohesion</td>
<td>67.3 ± 25.8</td>
<td>66.7 ± 26.8</td>
<td>58.9 ± 28.8</td>
<td>0.4</td>
<td>.676</td>
</tr>
<tr>
<td>Physical Summary Score</td>
<td>42.5 ± 15.3</td>
<td>36.9 ± 10.0</td>
<td>28.6 ± 13.7</td>
<td>15.2</td>
<td>.000</td>
</tr>
<tr>
<td>Psychosocial Summary Score</td>
<td>43.9 ± 16.4</td>
<td>47.4 ± 12.1</td>
<td>40.9 ± 12.7</td>
<td>2.8</td>
<td>.076</td>
</tr>
</tbody>
</table>

and a historical Israeli control group.26 Scores in all the physical scales of the CHQ were markedly lower for children with severe CP compared with those for children with moderate and mild CP (Table III). Responses to the PODCI questionnaire, which taps the ability to function in activities of daily life, were similar (Table IV; available at www.jpeds.com). Parents gave higher scores on the psychosocial and parent impact-time scales of the CHQ for their children who had moderate CP than for their children with mild CP (psychosocial summary score 47.4 ± 12.1 and 43.9 ± 16.4, respectively; parent impact-time score 92.6 ± 11.9 and 76.9 ± 27.8, respectively). For siblings, there was no correlation between parents’ CHQ scores and the level of disability of their sibling with CP.

Effect of Parenting Style on Health Status

The parenting style questionnaire has 4 variables: parental acceptance, parental rejection, parental control, and parental autonomy allowance. We were able to collapse the 4 groups into 2 categories, “acceptance/rejection” and “autonomy/control,” because these pairs were negatively correlated in our study (r = −0.69 for acceptance and rejection, and r = −0.29 for parent control and autonomy allowance).21

We found a positive correlation between the autonomy allowing parenting style and the CHQ physical summary (r = 0.400, P = .012), psychosocial summary (r = 0.403, P = .012), and family activities scale scores (r = 0.341, P = .030). The accepting parenting style also positively correlated with the psychosocial summary score (r = 0.363, P = .022; Table V). We also documented a positive correlation between the autonomy allowing parenting style and the PODCI “Happiness with Physical Condition” domain (r = 0.550, P = .003).

For the siblings, there was no correlation between the autonomy allowing parenting style for any of the CHQ scale scores. The accepting parenting style correlated positively only with parent impact-emotional scale (r = 0.433, P =
Parenting style influenced QOL as reflected by CHQ scores in children with CP after controlling for the level of disability and its influence was greater than these factors: age, IQ, anxiety level, and socioeconomic status (Table V). The parenting style did not correlate with the socioeconomic status or education of the parents.

**DISCUSSION**

We found that parenting style was a most important factor affecting the psychosocial aspects of QOL of children with CP. The impact of parenting style on psychosocial aspects of QOL was far greater than other factors assessed in this study, including severity of illness, IQ, anxiety level, and socioeconomic status. Parenting style influenced QOL as reflected by CHQ scores in children with CP after controlling for the level of disability and its influence was greater than these factors: age, IQ, anxiety level, and socioeconomic status (Table V). The parenting style did not correlate with the socioeconomic status or education of the parents.

All siblings had normal QOL regardless of the presence of a sibling with CP or disease severity. The effect of parenting style was specific to the children with CP. Perhaps healthy children can cope with various parenting styles, whereas a handicapped child, who is at risk for lower QOL, is particularly likely to benefit from an autonomy allowing and accepting parenting style.

The accepting parenting style influenced only the psychosocial domains of the CHQ, while the autonomy allowing parenting style also affected physical and family activities scale scores. The results point to the importance of autonomy allowance in these children despite their physical disability. The effect of parenting style during childhood on QOL of children with CP might be even more significant later in their lives. Recent studies suggest that both the physical and psychosocial well-being of children with CP can deteriorate with the transition from youth to adulthood. The autonomy allowing parenting style during childhood may prepare children with CP for more independent lives as adults.

Level of disability is a proven determinant of QOL. We found that children with CP, regardless of their level of disability, had lower QOL scores than their siblings and healthy control subjects. Furthermore, the physical scale scores were significantly lower in children with severe disability than children with mild or moderate disability. However, the degree of disability did not affect the psychosocial scores in a similar manner. The scores on the psychosocial scales were lower for children with mild CP than for children with moderate CP. A possible explanation is that children with mild CP measure themselves and are compared by their parents to the healthy population, although children with moderate CP exclude themselves from the “healthy group.”

Studies on QOL are highly subjective in nature and as such have inherent limitations. Although we used common and conventional tools, we were fully aware of their limitations being parent-based rather than child-based. We therefore used multiple questionnaires, generic and disease-specific, and found that they exhibit high internal consistency and correlate well with each other.

The consensus that improving QOL is an important treatment goal in children with CP mandates measures and treatment that enhance this goal. This study demonstrates that parenting style is not only a significant factor in QOL in children with CP, but is the only known factor to impact on psychosocial aspects of QOL, emphasizing the overwhelming importance of parenting style on the well being of these children. This offers a therapeutic option for enhancing QOL for those with this debilitating, life-long disease. Parent-child interaction approaches such as the “filial therapy training” or the “stepping stones triple P program” are examples of effective interventions for enhancing parenting style while impacting children’s behavior and emotional stability. We suggest, therefore, that family-directed interventions be instituted early in the course of the treatment program of the child with CP, particularly those focusing on parenting style.

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**Table V. Correlations between the Child Health Questionnaire scores and parenting style, anxiety level, age, IQ, and socioeconomic status in children with cerebral palsy**

<table>
<thead>
<tr>
<th>Parenting style—Acceptance/Rejection</th>
<th>Physical summary score</th>
<th>Psychosocial summary score</th>
<th>Family cohesion</th>
<th>Family activities</th>
<th>Parent impact—emotional</th>
<th>Parent impact—time</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.18 (.167)</td>
<td>0.36 (.022)†</td>
<td>0.04 (.414)</td>
<td>0.06 (.382)</td>
<td>0.13 (.235)</td>
<td>0.28 (.067)</td>
</tr>
<tr>
<td>Parenting style—Autonomy/Control</td>
<td>0.40 (.012)†</td>
<td>0.40 (.012)†</td>
<td>0.28 (.061)‡</td>
<td>0.34 (.030)‡</td>
<td>0.20 (.142)</td>
<td>0.04 (.418)</td>
</tr>
<tr>
<td>Anxiety level (RCMAS)</td>
<td>0.14 (.215)</td>
<td>0.24 (.091)</td>
<td>0.14 (.228)</td>
<td>0.32 (.038)‡</td>
<td>0.20 (.140)</td>
<td>0.47 (.003)†</td>
</tr>
<tr>
<td>Age</td>
<td>0.30 (.070)</td>
<td>0.09 (.340)</td>
<td>0.28 (.094)</td>
<td>0.10 (.316)</td>
<td>0.10 (.321)</td>
<td>0.46 (.012)†</td>
</tr>
<tr>
<td>IQ</td>
<td>0.18 (.194)</td>
<td>0.05 (.399)</td>
<td>0.06 (.395)</td>
<td>0.07 (.372)</td>
<td>0.12 (.290)</td>
<td>0.12 (.291)</td>
</tr>
<tr>
<td>Socioeconomic status</td>
<td>0.13 (.268)</td>
<td>0.19 (.185)</td>
<td>0.29 (.087)</td>
<td>0.25 (.118)</td>
<td>0.10 (.318)</td>
<td>0.09 (.341)</td>
</tr>
</tbody>
</table>

*RCMAS, Revised Children’s Manifest Anxiety Scale.

*After controlling for severity of the motor disability, 1-tailed significance: r (P).

†P < .05.
REFERENCES

Table IV. Pediatric Outcomes Data Collecting Instrument scores as a function of the motor severity according to the Gross Motor Function Classification System

<table>
<thead>
<tr>
<th>PODCI subscales</th>
<th>Mild (n = 13)</th>
<th>Moderate (n = 12)</th>
<th>Severe (n = 14)</th>
<th>F*</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Upper Extremity and Physical Function</td>
<td>77.6 ± 20.2</td>
<td>67.7 ± 15.6</td>
<td>32.1 ± 23.9</td>
<td>18.00</td>
<td>.000</td>
</tr>
<tr>
<td>Transfers and Basic Mobility</td>
<td>92.5 ± 7.3</td>
<td>60.3 ± 12.9</td>
<td>27.9 ± 10.0</td>
<td>33.02</td>
<td>.000</td>
</tr>
<tr>
<td>Sports and Physical Functioning</td>
<td>70.9 ± 17.9</td>
<td>30.4 ± 12.5</td>
<td>20.2 ± 24.6</td>
<td>26.38</td>
<td>.000</td>
</tr>
<tr>
<td>Pain/Comfort</td>
<td>88.6 ± 13.5</td>
<td>86.9 ± 15.7</td>
<td>75.0 ± 16.2</td>
<td>1.04</td>
<td>.366</td>
</tr>
<tr>
<td>Expectations from Treatment</td>
<td>75.2 ± 17.0</td>
<td>91.2 ± 18.9</td>
<td>79.9 ± 19.3</td>
<td>2.67</td>
<td>.087</td>
</tr>
<tr>
<td>Happiness with Physical Condition</td>
<td>72.5 ± 23.4</td>
<td>66.7 ± 11.3</td>
<td>52.6 ± 14.9</td>
<td>2.66</td>
<td>.088</td>
</tr>
<tr>
<td>General Function and Symptoms†</td>
<td>82.4 ± 10.9</td>
<td>61.3 ± 0.7</td>
<td>38.8 ± 1.0</td>
<td>24.34</td>
<td>.000</td>
</tr>
</tbody>
</table>

*F, P: The comparison, using the general linear model, was between probands with mild and moderate disability and those with severe disability.
†This summary score is computed as a composite of the first 4 domains (the 3 physical function domains and the pain and comfort domains).
Walking Age Does Not Explain Term Versus Preterm Difference In Bone Geometry

HAIFA ABOU SAMRA, RN, MSIM, AND BONNY SPECKER, PhD

Objective  To elucidate the relationship between bone geometry and onset of walking in former term and preterm children.

Study design  We conducted a cross-sectional study of 128 preschool children aged 3 to 5 years who underwent peripheral quantitative computerized tomography measures of bone size at the distal tibia. Linear models were developed, stratifying by sex, to determine whether bone differences between children born term and preterm were caused by differences in walking age.

Results  Children with a history of preterm birth walked later than children born at term (12.4 ± 0.5 versus 10.9 ± 0.2 months; \textit{P} = .004); however, gestation-corrected walking age (11.4 ± 0.5 for children born preterm) did not differ. In multiple regression analysis, boys born preterm had larger periosteal and endosteal circumferences and smaller cortical thickness and area than boys born term (least square means, 49.7 ± 1.3 mm, 43.0 ± 1.8 mm, 1.1 ± 0.11 mm, and 49.3 ± 3.2 mm² versus 47.0 ± 0.5 mm, 38.5 ± 0.7 mm, 1.4 ± 0.04 mm, and 56.9 ± 1.2 mm², respectively; all \textit{P} < .05). Preterm birth remained statistically significant after adding the age of walking to the models, but no longer significant when current activity levels were included.

Conclusion  Greater periosteal and endosteal circumferences, with smaller cortical bone thickness and area, were found in former preterm boys, but not girls, and were explained by differences in current activity levels, not age of walking. (\textit{J Pediatr} 2007;151:61-6)

Although genetics is the major determinant of bone mass, modifiable environmental influences, such as physical activity and diet, have been shown to optimize an individual’s genetic potential.\textsuperscript{1} In addition, infant birth weight and growth are thought to influence adult bone geometry.\textsuperscript{2} An association between adult and childhood bone has been suggested, and peak bone mass attained in early life is considered to be a major factor in predicting future osteoporosis risk.\textsuperscript{1,3} Beneficial bone effects of early childhood activity may persist beyond the period of the increased activity,\textsuperscript{4} and several longitudinal studies have shown high childhood activity to be associated with high adult bone density.\textsuperscript{5,6} The Department of Health and Human Services has recommended that physical activity levels increase in early childhood to optimize bone health.\textsuperscript{7}

Walking is thought to place significant strain on bone, and adult studies have shown that walking enhances bone density.\textsuperscript{8} Increased bone loading also is associated with increased bone size.\textsuperscript{9} Theoretically, walking during infancy should exert the same effect by placing strains on the skeleton leading to a beneficial bone response, and walking at an earlier age will lead to greater cumulative loading than walking at a later age. In a longitudinal study of 20 children, Ruff investigated the relationship between body size, muscle size, and bone structural development and strength. Data were obtained from serial radiographs that were taken at approximately 6-month intervals from near birth to late adolescence.\textsuperscript{10} He found that femoral bone strength velocity increased earlier during the second year with the beginning of walking, and humeral strength velocity declined as crawling stopped and assistance in standing and walking began. Because the peaks in bone strength velocities were not accompanied by changes in body size, Ruff suggested that bone strength is strongly dependent on mechanical loading.\textsuperscript{10} This study is the only one that we are aware of that has examined the association between bone strength and the beginning of walking.

The aim of this study was to investigate the relationship between bone size and the onset of independent walking in preschool children, and to determine whether walking age explains the bone differences between preschool children born preterm compared with children born at term. We previously reported similar periosteal circumferences but larger...
endosteal circumference and smaller cortical area of the tibia in preschool children who were reported to be born preterm than in preschool children born at term. In this study, we hypothesized that: 1) children who walk earlier will have larger periosteal circumference and cortical area and thickness and smaller endosteal circumference than children who walk later; and 2) children with history of preterm birth begin to walk at an older age compared with children born at term and this difference in walking age will explain the larger endosteal circumference and smaller cortical bone area that is observed in children with history of preterm birth.

METHODS
Baseline data from 239 children aged 3 to 5 years who participated in a 1-year, randomized, placebo-controlled physical activity and calcium supplementation trial were used for this analysis. Of the 239 children, 110 had tibia bone measurements with movement and were excluded from the study. A comparison between children who were included and those who were not showed that the children who were excluded were younger, lighter, and shorter (all \( P < .001 \)). There were no differences in sex, history of preterm birth, physical activity levels, and walking age between children who were excluded and children who were not excluded.

Parents completed questionnaires that provided information on sex, race, feeding history during the first month of life, and parents’ education. Parents were asked to recall the age the child first walked. Mothers also recalled whether the child was born early and, if so, how many weeks on the basis of their due date as determined by their obstetrician. Children were considered preterm when they were born \( \pm 37 \) weeks gestation. The average length of prematurity was 4.1 \( \pm 2 \) weeks. Fifty-six of the study participants were classified as Caucasian, and 8 were classified as non-Caucasian. Information on ethnicity and birth weight was not obtained. All children were evaluated for completeness by study personnel, and a nutrient intake analysis was performed with the Nutritionist V database (First Data Bank, San Bruno, CA). Quartiles of calcium intake (mg/day) were determined from lowest to highest as \(<709 \) mg/day, 709 to 875 mg/day, 876 to 1036 mg/day, and \( >1036 \) mg/d. The study was approved by the South Dakota University Human Subjects Committee, and parental written informed consent was obtained.

Data were entered onto an Access database, and analyses were performed with JMP statistical software (SAS Institute, Cary, NC). Data were tested for normality and relationships between bone measures and anthropometric measurements (height, weight, \%BF), demographic characteristics, and potential cofounders were examined. Data were stratified by sex caused by differences in bone measures, percent body fat, and activity levels. Significant predictors \((P \leq .05)\) of periosteal and endosteal circumferences, cortBA, and cortical thickness were determined with stepwise regression analysis. Once multiple regression models for each of the outcome variables were obtained, walking age and gestation-corrected walking age were individually added to the model to determine whether either factor explained a significant amount of the remaining error. The significance of all 2-way interactions was tested for significance. Walking age was adjusted by subtracting the months born early (number of weeks/4.3) from walking age. Data given are mean \( \pm \) SEM unless otherwise stated.

RESULTS
There were 129 children with pQCT scans at baseline who had no movement. However, 1 of these children had a reported walking age of 7 months and an adjusted walking age of 5.6 months \((<3 \) SD from the mean). This was considered a recall error, and this child was excluded from the analysis. There were 64 girls and 14 children with a history of preterm birth. Overall, children with a history of preterm birth walked later than children with term birth (12.4 \( \pm 0.5 \) versus 10.9 \( \pm 0.2 \) months; \( P = .004 \)), but this difference was not significant for gestation-corrected age at walking (11.4 \( \pm 0.5 \) versus 10.9 \( \pm 0.2 \) months; \( P = .34 \); Table I). There were no sex differences in any of the bone measures, although boys had lower \%BF and greater activity levels than girls (Table I). In both boys and girls, bone measures were not associated with race, type of feeding during the first month, calcium intake, or season of enrollment.

Boys
Preterm boys were older and had larger periosteal and endosteal circumferences than term boys (Table I). None of the bone measurements were associated univariately with walking age (Table II; available at www.jpeds.com). Periosteal circumference was univariately associated with weight \((r = \)
respectively). Thickness, or cortBA (respectively), was univariately associated with age (r = .46, P = .001), height (r = .64, P < .001), and weight (r = .52, P < .001). Age and height were not significant when weight was included in the analysis. Endosteal circumference was univariately associated with weight (r = .39, P = .06), and weight (r = .52, P < .001), and height (r = .05). Cortical thickness was univariately associated with age (r = .38, P = .002), height (r = .54, P < .001), and weight (r = .48, P < .001). In multiple regression analysis, height was the only variable associated with cortical thickness (Table IV; available at www.jpeds.com) and least square means for bone outcomes did not differ (Figure 2; available at www.jpeds.com). CortBA was univariately associated with age (r = .48, P < .001), height (r = .66, P < .001), and weight (r = .65, P < .001). In multiple regression analysis, cortBA was associated with both height and weight (Table IV).

Walking age was not associated with any of the bone measures (Table II) and similar findings were observed when walking age corrected for gestational age was added to the final regression models. Inclusion of percent time in moderate plus vigorous activity did not alter these findings.

### Discussion

Because of previous reports of persistent effects of mechanical loading on bone geometry, we examined the effect of age of walking on tibial bone size. We hypothesized that children who walked at a younger age would have differences in bone geometry compared with children who walked at an older age, and because age of walking may differ between term and preterm children, we proposed that differences in age at walking would explain bone differences between term and preterm girls. There were no significant differences in any of the bone or anthropometric measures between term and preterm girls. Term and preterm girls had similar height, weight, %BF, and physical activity levels. Periosteal circumference was univariately associated with age (r = .33, P = .007), height (r = .43, P < .001), and weight (r = .53, P < .001). Age and height were not significant when weight was included in the analysis. Endosteal circumference was univariately associated with weight (r = .24, P = .05). Cortical thickness was univariately associated with age (r = .38, P = .002), height (r = .54, P < .001), and weight (r = .48, P < .001). In multiple regression analysis, height was the only variable associated with cortical thickness (Table IV; available at www.jpeds.com) and least square means for bone outcomes did not differ (Figure 2; available at www.jpeds.com). CortBA was univariately associated with age (r = .48, P < .001), height (r = .66, P < .001), and weight (r = .65, P < .001). Multiple regression analysis, cortBA was associated with both height and weight (Table IV).

When walking age was added to the regression models, significant differences between term and preterm birth persisted (Table III). Cortical thickness was univariately associated with age (r = .55, P < .001), height (r = .54, P = .001), and weight (r = .52, P < .001). In multiple regression analysis, cortBA was associated with age, weight, and height of preterm birth (Table III). Least square means from the multiple regression models are shown in Table I.

When walking age was added to the regression models, significant differences between term and preterm birth persisted (Table III). Similar findings were observed when walking age corrected for gestational age was added. However, when percent time in moderate plus vigorous activity was added to each of the multiple regression models shown in Table III, history of preterm birth no longer was a significant predictor of periosteal and endosteal circumferences, cortical thickness, or cortBA (P = .46, P = .26, P = .06, and P = .11, respectively).

**Girls**

There were no significant differences in any of the bone or anthropometric measures between term and preterm girls. Term and preterm girls had similar height, weight, %BF, and physical activity levels. Periosteal circumference was univariately associated with age (r = .33, P = .007), height (r = .43, P < .001), and weight (r = .53, P < .001). Age and height were not significant when weight was included in the analysis. Endosteal circumference was univariately associated with weight (r = .24, P = .05). Cortical thickness was univariately associated with age (r = .38, P = .002), height (r = .54, P < .001), and weight (r = .48, P < .001) in multiple regression analysis, height was the only variable associated with cortical thickness (Table IV; available at www.jpeds.com) and least square means for bone outcomes did not differ (Figure 2; available at www.jpeds.com). CortBA was univariately associated with age (r = .48, P < .001), height (r = .66, P < .001), and weight (r = .65, P < .001) in multiple regression analysis, cortBA was associated with both height and weight (Table IV).

Walking age was not associated with any of the bone measures (Table II) and similar findings were observed when walking age corrected for gestational age was added to the final regression models. Inclusion of percent time in moderate plus vigorous activity did not alter these findings.

### Discussion

Because of previous reports of persistent effects of mechanical loading on bone geometry, we examined the effect of age of walking on tibial bone size. We hypothesized that children who walked at a younger age would have differences in bone geometry compared with children who walked at an older age, and because age of walking may differ between term and preterm children, we proposed that differences in age at walking would explain bone differences between term and preterm girls. There were no significant differences in any of the bone or anthropometric measures between term and preterm girls. Term and preterm girls had similar height, weight, %BF, and physical activity levels. Periosteal circumference was univariately associated with age (r = .33, P = .007), height (r = .43, P < .001), and weight (r = .53, P < .001). Age and height were not significant when weight was included in the analysis. Endosteal circumference was univariately associated with weight (r = .24, P = .05). Cortical thickness was univariately associated with age (r = .38, P = .002), height (r = .54, P < .001), and weight (r = .48, P < .001) in multiple regression analysis, height was the only variable associated with cortical thickness (Table IV; available at www.jpeds.com) and least square means for bone outcomes did not differ (Figure 2; available at www.jpeds.com). CortBA was univariately associated with age (r = .48, P < .001), height (r = .66, P < .001), and weight (r = .65, P < .001) in multiple regression analysis, cortBA was associated with both height and weight (Table IV).

Walking age was not associated with any of the bone measures (Table II) and similar findings were observed when walking age corrected for gestational age was added to the final regression models. Inclusion of percent time in moderate plus vigorous activity did not alter these findings.

### Table I. Population characteristics by history of preterm birth and sex

<table>
<thead>
<tr>
<th>Variables</th>
<th>Term* girls (n = 58)</th>
<th>Preterm girls (n = 6)</th>
<th>Term boys (n = 56)</th>
<th>Preterm boys (n = 8)</th>
<th>Girls vs boys†</th>
<th>Term vs preterm†</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcome variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Periosteal circumference (mm)</td>
<td>46.5 ± 0.4</td>
<td>46.1 ± 1.3</td>
<td>46.9 ± 0.5</td>
<td>50.4 ± 1.4</td>
<td>.18</td>
<td>.08</td>
</tr>
<tr>
<td>Endosteal circumference (mm)</td>
<td>38.5 ± 0.5</td>
<td>39.0 ± 1.5</td>
<td>38.5 ± 0.7</td>
<td>42.9 ± 1.9</td>
<td>.49</td>
<td>.04</td>
</tr>
<tr>
<td>Cortical thickness (mm)</td>
<td>1.3 ± 0.03</td>
<td>1.1 ± 0.1</td>
<td>1.3 ± 0.04</td>
<td>1.1 ± 0.1</td>
<td>.35</td>
<td>.09</td>
</tr>
<tr>
<td>Cortical bone area (mm²)</td>
<td>54.1 ± 1.4</td>
<td>47.9 ± 4.4</td>
<td>56.2 ± 1.5</td>
<td>54.2 ± 4.0</td>
<td>.23</td>
<td>.24</td>
</tr>
<tr>
<td>Predictive variables</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Walking age (months)</td>
<td>10.9 ± 0.2</td>
<td>11.8 ± 0.7</td>
<td>11.0 ± 0.2</td>
<td>12.8 ± 0.6</td>
<td>.50</td>
<td>.004</td>
</tr>
<tr>
<td>Gestation-corrected walking age (months)</td>
<td>10.9 ± 0.2</td>
<td>11.1 ± 0.7</td>
<td>11.0 ± 0.2</td>
<td>12.6 ± 0.6</td>
<td>.34</td>
<td>.64</td>
</tr>
<tr>
<td>Age (years)</td>
<td>4.1 ± 0.1</td>
<td>3.9 ± 0.2</td>
<td>4.1 ± 0.1</td>
<td>4.6 ± 0.2</td>
<td>.39</td>
<td>.31</td>
</tr>
<tr>
<td>Height (cm)</td>
<td>103.8 ± 0.8</td>
<td>100.1 ± 2.4</td>
<td>103.7 ± 0.7</td>
<td>107.3 ± 1.8</td>
<td>.46</td>
<td>.76</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>17.2 ± 0.3</td>
<td>15.9 ± 1.1</td>
<td>17.3 ± 0.3</td>
<td>18.6 ± 0.9</td>
<td>.47</td>
<td>.83</td>
</tr>
<tr>
<td>Body fat (%)</td>
<td>28 ± 0.6</td>
<td>29 ± 1.8</td>
<td>24 ± 0.6</td>
<td>22 ± 1.6</td>
<td>&lt;.001</td>
<td>.61</td>
</tr>
<tr>
<td>Calcium intake (mg/d)</td>
<td>875 ± 38</td>
<td>781 ± 122</td>
<td>908 ± 40</td>
<td>876 ± 103</td>
<td>.48</td>
<td>.51</td>
</tr>
<tr>
<td>% time in moderate + vigorous activity</td>
<td>11.7 ± 0.5</td>
<td>8.9 ± 1.6</td>
<td>13.8 ± 0.4</td>
<td>11.9 ± 1.3</td>
<td>.003</td>
<td>.05</td>
</tr>
</tbody>
</table>

Data are mean ± SEM.

*Term is defined as >37 weeks gestation.

†Significance value based on 2-way analysis of variance.

‡Significantly different from term infants within the same sex, P < .05.
and preterm children. We did not, however, find any association between measures of bone size.

We examined the relationship between bone size and age at walking in preschool children. Onset of walking is influenced by several developmental factors, which are of environmental, neural, and biomechanical origins, and the interactions among these factors. Biomechanical changes, such as redistribution of leg fat and muscle mass, strengthening of back and abdominal muscles, and lowering of the infant’s center of mass, are necessary for walking to occur. Mechanical loading to bones of the lower extremities that occurs at the initiation of walking is associated with increased femoral bone strength and decreased humeral bone strength velocities. Whether age at attainment of independent walking has a persistent effect on the size of weight-bearing bones later in life remains unclear. We did not, however, observe such a relationship in this study.

Earlier studies that have investigated age of walking attainment in infants with history of preterm birth find that, even after adjusting for gestational age differences, preterm infants have later onset of walking attainment and different patterns of locomotion compared with term infants. Investigators speculated that children with history of preterm birth, in the absence of cerebral palsy and pathological conditions, might have subtle problems with muscle power coordination and timing. Muscle deficiencies were believed to be caused by delayed motor function and inability of preterm infants to coordinate flexion-extension activities. When such limitations in muscle function exist in preterm infants the activity levels may be affected, and, consequently, mechanical loading of bone may be diminished. Although we found significant differences between term and preterm infants in the age of independent walking attainment, this difference was no longer significant when corrected for gestational age differences.

In our earlier analysis of these data, we reported a 2.1% lower total body bone mineral content in children born preterm than in children born at term. We also found a larger endosteal circumference, with a similar periosteal circumference, and a lower cortical bone area in children born preterm compared with children born at term. Former preterm children had significantly lower activity levels than former

### Table III. Multiple regression models predicting bone measures in boys

<table>
<thead>
<tr>
<th></th>
<th>Estimate</th>
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<tr>
<td><strong>Periosteal circumference</strong> (mm)—full model</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>Intercept</td>
<td>48.98</td>
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<td></td>
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<td>Weight (kg)</td>
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<td>1.06</td>
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</tr>
<tr>
<td>%BF</td>
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<td>.003</td>
<td></td>
<td>-36.09</td>
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<td>.006</td>
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<tr>
<td>Age (years)</td>
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<td>.01</td>
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<td>.06</td>
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<td>Walking age (months)</td>
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<td>−</td>
<td></td>
<td>-0.21</td>
<td>0.31</td>
<td>.51</td>
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<tr>
<td>R²</td>
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<td></td>
<td></td>
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<td>0.31</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

| **Endosteal circumference** (mm)—full model |          |     |         |                   |          |     |         |
| Intercept            | 49.87    | 6.09| <.001   |                   | 57.66    | 9.27| <.001   |
| Weight (kg)          | 1.02     | 0.36| .007    |                   | 0.9      | 0.39| .03     |
| % BF                 | -40.04   | 17.73|.03     |                   | -37.5    | 18.74|.05    |
| Age (years)          | -4.21    | 1.36| .003    |                   | -4.46    | 1.42| .003    |
| History of Preterm (no) | -2.23  | 0.98| .03     |                   | -2.83    | 1.33| .02     |
| Walking age (months) | −        | −   | −       |                   | -0.44    | 0.43| .31     |
| R²                   | 0.22     |     |         |                   | 0.24     |     |         |

| **Cortical thickness** (mm)—full model |          |     |         |                   |          |     |         |
| Intercept            | 0.016    | 0.264|.95     |                   | -0.514   | 0.453|.26    |
| Age (years)          | 0.288    | 0.060| <.001   |                   | 0.332    | 0.065| <.001   |
| History of preterm (no) | 0.141  | 0.057|.02     |                   | 0.184    | 0.063| .005    |
| Walking age (months) | −        | −   | −       |                   | 0.029    | 0.023| .22     |
| R²                   | 0.29     |     |         |                   | 0.33     |     |         |

| **Cortical area (cm²)—full model** |          |     |         |                   |          |     |         |
| Intercept            | -4.58    | 9.2 | .62     |                   | -25.68   | 15.29|.10    |
| Weight (kg)          | 1.47     | 0.49 | .004    |                   | 1.76     | 0.52 | .001    |
| Age (y)              | 7.72     | 2.04 | <.001   |                   | 8.74     | 2.1  | <.001   |
| History of Preterm (no) | 3.77  | 1.71| .03     |                   | 5.29     | 1.88 | .007    |
| Walking age (months) | −        | −   | −       |                   | 0.99     | 0.71 | .17     |
| R²                   | 0.42     |     |         |                   | 0.47     |     |         |
Walking Age Does Not Explain Term Versus Preterm Differences in Bone Geometry

Boys born preterm, even by only 3 to 4 weeks, were shown to have lower survival rates than female infants and show more signs of neurological dysfunction than female infants. Our study is the first to find differences in bone measures between groups. However, in our earlier analysis we did not stratify by sex. After stratifying by sex for this analysis, history of preterm birth was no longer a significant predictor of bone measures in girls, and there were no significant differences in any of the outcome and predictive variables between term and preterm girls. Preterm boys did, however, have larger periosteal and endosteal circumferences, with smaller cortical thicknesses and cortical area than former term boys, consistent with our earlier analysis of boys and girls combined. These findings are consistent with those of Backstrom et al, who found that prematurity-associated bone effects were more pronounced in male subjects than female subjects. Lower bone stress index in the weight bearing tibia, caused by a smaller cortical bone area and a smaller cross-section at the mid-shaft, was apparent in male subjects aged 18 to 27 years who had history of preterm birth, but not in female subjects. Backstrom et al speculated that weaker muscle performance in younger men born preterm could have contributed to their findings.

Earlier studies showed that male infants born preterm have lower survival rates than female infants and show more signs of neurological dysfunction than female infants. Boys born preterm, even by only 3 to 4 weeks, were shown to have lower motor and social developmental scores than their female counterparts. Our study is the first to find differential sex effects of gestational age on bone measures in preschool children. These differences between term and preterm boys remained significant when walking age was included in the analysis. When physical activity measures were included in the statistical analysis, however, history of preterm birth was no longer a significant predictor of bone size. Differences in physical activity between term and preterm children need be investigated further.

One of the limitations of this study is that our measure of activity levels was only available for 94% of the girls and 83% of the boys. It is possible that the small number of boys with activity measures makes it more difficult to detect term-preterm differences in a multiple regression analysis that includes activity levels. However, if these findings were confirmed in larger populations, it would have significant implications for developmental assessment and interventions in boys with history of preterm birth. This study has other limitations. First, parents’ recall of their child’s age of independent walking and gestational age may be inaccurate because they were asked about events that occurred as long as 4 years before data were collected. In addition, parents may have been biased toward reporting an earlier walking age than a later one, especially in children with history of preterm birth, because delayed onset of walking might imply that their child had developmental limitations. In addition, defining walking age might vary from one parent to another, depending on the number of steps a child would take before the parents consider the child to be walking independently.

The average weeks of lost gestation in our study participants with history of preterm birth was approximately 4 weeks. Therefore, these infants were not likely to be very low birth weight or to have had complicated postnatal medical history. Detecting a difference in outcome variables between groups of term children and preterm children is more difficult than detecting a difference between term children and very preterm children and would require a larger sample size. If delayed onset of independent walking is associated with preterm birth and also a predictor of bone size, a larger sample size may be needed to detect such a relationship. Our post hoc power for detecting a difference in bone measures between children in the lowest and highest walking quartiles was 48% for boys and 21% for girls, with a sample size of 64. Our results also could have been confounded by other important factors, such as birth weight, appropriateness of weight for gestational age, presence of complications during delivery, intensive care nursery admission, length of stay, medication use, and maternal drug use and smoking. Unfortunately, such information was not available to us and could not be included in the analysis.

In summary, bone size in preschool children was not associated with age of walking in either boys or girls. However, bone size was associated with history of preterm birth, but only among boys. Greater periosteal and endosteal circumferences, but thinner bones with smaller cortical bone area were found in former preterm boys. Our findings need to be confirmed in larger populations, and factors contributing to these differences need to be further explored.

REFERENCES


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<tr>
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<th>Girls</th>
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<tr>
<td>Gestation-corrected walking age</td>
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<td>Cortical thickness (mm)</td>
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<td>% time in moderate + vigorous activity</td>
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<tr>
<td>Cortical bone area (mm²)</td>
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<td>−0.03</td>
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<tr>
<td>Table IV. Multiple regression models predicting bone measures in girls</td>
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</tr>
<tr>
<td>R² = 0.28</td>
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</table>

Periosteal circumference (mm)—full model

| Intercept | 35.22 | 2.31 | <.001 |
| Weight (kg) | 0.66 | 0.13 | <.001 |
| Walking age (months) | - | - | - |

R² = 0.06

Endosteal circumference (mm)—full model

| Intercept | 32.55 | 3.07 | <.001 |
| Weight (kg) | 0.35 | 0.18 | .05 |
| Walking age (months) | - | - | - |

R² = 0.28

Cortical thickness (mm)—full model

| Intercept | -1.16 | 0.5 | .02 |
| Weight (kg) | 0.02 | 0.00 | <.001 |
| Walking age (months) | - | - | - |

R² = 0.46

Cortical area (cm²)—full model

| Intercept | -38.92 | 24.37 | .11 |
| Weight (kg) | 1.36 | 0.78 | .086 |
| Height (cm) | 0.67 | 0.34 | .05 |
| Walking age (months) | - | - | - |

R² = 0.46

Figure 2. Mean (±SEM) periosteal (Peri-C) and endosteal circumferences (Endo-C), cortical thickness (CortThk), and cortical area (Cort A) in term (black bars) and preterm females (grey striped bars).

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Headache in Children with Sickle Cell Disease: Prevalence and Associated Factors

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Objective To compare the prevalence of frequent headache in children with sickle cell disease (SCD) to that of black control subjects and to assess factors associated with headache in SCD.

Study design In this cross-sectional study, a headache questionnaire was administered to subjects with SCD and black control subjects. Subjects answered supplementary questions about SCD complications. Clinical and radiographic information were abstracted from medical charts for subjects with SCD.

Results Children (n = 241) with SCD and 141 control subjects were studied; 32.4% (95% CI 26.5%-38.7%) of subjects with SCD reported having headaches at least weekly, similar to control subjects at 27% (95% CI 19.8%-35.1%, P = NS); however, in children <13 years, headache was more common in subjects with SCD than in control subjects (24% vs 9.7%, P = .013). The prevalence of headache was similar among the different SCD genotypes. Factors associated with frequent headaches in subjects with SCD included older age, frequent vaso-occlusive pain episodes, symptoms of obstructive sleep apnea, and cerebral vessel stenosis detected by magnetic resonance angiography.

Conclusion The prevalence of headaches in children with SCD is similar to the general population; however, younger children with SCD report headaches more frequently than control subjects. The cause of headache is likely multifactorial, and SCD-specific factors may contribute. (J Pediatr 2007;151:67-72)

Children with sickle cell disease (SCD) frequently complain of headaches, but there are limited data concerning the prevalence and causes of headache in these children. The prevalence of headache in healthy children has been reported to range from 8% to 60%, depending on the population studied and the definition used. The most common causes of headache in childhood are tension and migraine headache. Children with SCD may have headaches related to these common causes, but headaches also may be related to their underlying SCD such as bony infarction, severe anemia, or frequent opioid medication use. One particular concern is that headache may be a manifestation of cerebrovascular disease. A child with SCD who presents with frequent headache often undergoes an extensive evaluation, which may include brain computed tomography, magnetic resonance imaging (MRI) and angiography (MRA), and transcranial Doppler (TCD) ultrasonography, to determine the cause and to rule out cerebral vasculopathy and stroke. It is unclear, however, how often headache is the sole symptom of central nervous system disease, or whether frequent headache signifies the presence of cerebrovascular disease.

This cross-sectional study was performed to determine the prevalence of frequent headache in children with SCD compared with healthy black control subjects. Our secondary aim was to investigate whether specific SCD-related factors such as vaso-occlusive pain episodes (VOE) and cerebrovascular disease are associated with frequent headache. These data could facilitate the evaluation of headache in the pediatric population with SCD and suggest areas for future study.
METHODS

Patients

This study was approved by the Institutional Review Boards at The Children’s Hospital of Philadelphia (CHOP) and Duke University Medical Center. Subjects with SCD were consecutively recruited from the Sickle Cell Center at CHOP, and black control subjects were recruited from the General Pediatrics and Adolescent Medicine clinics at CHOP between February and October 2004. Inclusion criteria for subjects with SCD were ages 6 to 21 years and any SCD genotype (SS, SC, Sβ+thalassemia, and Sβ−thalassemia). Eligibility criteria for control subjects included black ethnicity, age 6 to 21 years, and without known sickle cell trait or a diagnosis of significant chronic medical illness that required hospitalization at least once a year. Informed consent was obtained from guardians, and assent was obtained from children 7 years and older.

Data Collection

During routine clinic visits, subjects with SCD and control subjects, with the help of their caretakers, completed an identical headache questionnaire. The study questionnaire was developed on the basis of the International Headache Society (IHS) classification system for headache in children and asked historical questions about headache, including frequency, characteristics, and associated symptoms, as well as questions about medication use and activity limitations related to headache (sample questions appear in the Appendix; available at www.jpeds.com).

Subjects with SCD. Subjects with SCD also answered additional questions pertaining to SCD, including current medication use, history of transfusion therapy, number of VOE in the preceding 12 months, history of acute chest syndrome, symptoms of obstructive sleep apnea (OSA), and history of silent or overt infarction. Clinical information was abstracted from the medical charts of subjects with SCD and included SCD genotype, history of overt stroke, history of dactylitis before age 1 year (a marker of severe disease), and most recent pulse oximetry value. Complete blood count results were recorded, and the average hemoglobin level, white blood cell count, platelet count, and reticulocyte count from the last 3 well clinic visits were calculated. In addition, the fetal hemoglobin level within 6 months of the study date was recorded. Records were reviewed for all acute visits to the emergency department or other hospital-based sites and for all inpatient hospitalizations for the year before the study visit, and the final discharge diagnosis(es) were recorded.

Magnetic Resonance Imaging and Angiography

All brain magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) study results obtained for clinical reasons within 6 months of the study visit date were analyzed. In addition, for MRI analyses, study results obtained more than 6 months before the study visit date were included if the earlier study showed infarction because infarctions are unlikely to regress. Earlier abnormal MRA results were not included because vascular stenosis may change over time. Indications for the MRI/MRA were abstracted from the radiology report and chart review. Our institutional screening protocol to assess for central nervous system abnormalities includes screening brain MRI/MRA for children with SCD-SS and SCD-Sβ-thalassemia, as well as follow-up studies for those with elevated TCD velocities, prior silent infarcts, or other risk factors for stroke. All MRI/MRA scanning was performed on a 1.5 T Siemens Magnetom Vision (Siemens Medical Systems, Iselin, NJ) with our standard SCD imaging protocol.

At least 2 neuroradiologists reviewed each study. All images were read, and a clinical report was dictated by one of several neuroradiologists. All images were reviewed by the study neuroradiologist (A.P.), who was blinded to the clinical report, the subject’s clinical history, and questionnaire responses. The study neuroradiologist’s findings were then compared with the clinical report to assess for agreement. A second neuroradiologist (R.Z.) reviewed all studies where there was a discrepancy between the initial clinical report and the study neuroradiologist’s (A.P.) review, and consensus was reached.

Cerebral infarction was defined as an area of abnormally increased signal on T2-weighted and fluid-attenuated inversion recovery imaging. The number, size, and location of lesions were recorded. Lesions were classified as punctate (<5 mm), medium (5-15 mm), or large (>15 mm). Limited abnormality was defined as a single, punctate lesion, whereas more extensive abnormality was defined as multiple or moderate-to-large lesions. Silent infarction was defined as the presence of lesions without a clinical history of stroke. Stenosis was defined as an area of narrowing or focal signal dropout in an artery of the circle of Willis and graded as mild, moderate, severe, or occlusion. Significant stenosis was defined as at least 1 vessel with a stenosis grade of moderate or higher. The presence or absence of moyamoya disease, a significant bone marrow hyperplasia (graded moderate or severe), and sinusitis also were recorded. TCD results also were reviewed, and the highest time-averaged mean velocities on both the most recent study and on any study to date were recorded.

Data Analysis

The study was powered to detect a 15% difference in the prevalence of frequent headache between subjects and control subjects with β = 0.8 and α = 0.05. Frequent headache was defined as headache reported to occur at least once a week. The prevalence of frequent headache with 95% confidence interval (CI) was calculated for subjects and control subjects and compared by use of χ² analysis. Headaches
that met the following characteristics were classified as migraine headache on the basis of IHS criteria: duration of longer than 2 hours; at least 2 of the following: unilaterality, pulsating quality, moderate to severe intensity (applied in this study as resulting in missed school), or aggravation by routine physical activity; and at least 1 of the following: nausea and vomiting or photophobia and phonophobia.

Clinical factors associated with headache were evaluated only for subjects with SCD. Frequent VOE was defined as 3 or more pain episodes per year for which medical attention was sought. Evidence of OSA was recorded as positive if the subject reported at least 1 of the following on questionnaire: snoring “most of the time” or witnessed pauses in breathing. Only subjects with SCD-SS or SCD-SCβ0-thalassemia were included in the analysis of cerebrovascular disease and headache association, because stroke is most common in SCD-SS,11 SCD-SCβ0-thalassemia has clinical severity similar to that of SCD-SS, and current recommendations for screening for stroke risk include only these 2 genotypes.12 Laboratory results were analyzed separately for those with SCD-SS or SCD-SCβ0-thalassemia and for those with SCD-SC or SCD-SCβ+ thalassemia. Subjects receiving chronic transfusions were excluded from laboratory analyses.

Data analysis was performed with STATA 9 software (StataCorp, College Station, Texas). Statistical assessment was carried out with Student’s t test for continuous variables and χ² tests and Fisher’s exact tests for binomial variables, with P < .05 considered to be statistically significant. Logistic regression was performed using variables with P < .1 in the univariate analysis. Spearman correlation coefficients were used to assess correlations between patient report and chart review results for clinical events.

RESULTS

Characteristics of Subjects with SCD and Control Subjects

A total of 241 children with SCD and 141 black control subjects were enrolled. Clinical characteristics of subjects and controls are shown in Table I.

Prevalence of Frequent Headache

The prevalence of frequent headache in children with SCD and control subjects is shown in Table I. In both groups, headache was more common with increasing age (P < .001). There were no significant differences in the prevalence of headache between SCD genotypes (SCD-SS: 30.5%; SCD-SC: 32.0%; SCD-SCβ+ thalassemia: 46.7%; SCD-SCβ0-thalassemia: 41.7%). In those with frequent headache, 22.1% of subjects with SCD and 21.1% of control subjects reported headache characteristics that met IHS criteria for migraines (P = NS).

Analysis of Factors Associated with Headaches in Subjects with SCD

CLINICAL AND LABORATORY FEATURES. Factors associated with frequent headache in subjects with SCD are shown in Table II. These associations remained when the group with SCD-SS or SCD-SCβ0-thalassemia and the group with SCD-SC or SCD-SCβ+ thalassemia were analyzed sepa-

<table>
<thead>
<tr>
<th>Table I. Characteristics of subjects with SCD and control subjects</th>
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</tr>
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<td>Receiving hydroxyurea†</td>
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<td>Prevalence of frequent headache‡ (%), 95% CI</td>
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<td>Age of those with frequent headache‡, years (mean ± SD)</td>
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<td>Prevalence of frequent headache‡, children &lt;13 years old (%), 95% CI</td>
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</table>

*Indications for chronic transfusion were abnormal TCD (n = 19), history of stroke (n = 13), recurrent acute chest syndrome (n = 9); other (n = 3).
†Indications for hydroxyurea therapy were recurrent pain and/or acute chest syndrome (n = 29) and abnormal TCD with refusal of transfusion (n = 1).
‡Frequent headache defined as headache occurring at least once a week; Includes all SCD genotypes.
BRAIN IMAGING STUDIES. Brain MRI studies were available for 114 (64.8%) and MRA for 97 (55.1%) of 176 children with SCD-SS or SCD-Sβ-thalassemia. Thirty-two of 55 (58.2%) children with frequent headache had MRI studies compared with 82 of 121 (67.8%) without frequent headache (P = NS). The indications for brain MRI were elevated TCD velocities (29.8%), routine screening (14%), headaches (12.3%), hypoxia or nocturnal hypoxemia (11.4%), history of stroke (9.6%), history of silent infarct (7%), worsening academic performance (7.9%), acute neurologic symptoms (6.1%), and other (1.8%).

The proportion of subjects with TCD and brain MRI/ MRA abnormalities is shown in Table I. Most silent infarcts were located in the frontal or parietal deep white matter or periventricular regions. In those with silent infarcts, 58% had only punctate-sized lesions, 78% had more than 1 lesion, and 50% had bilateral infarcts. Cerebral vessel stenosis was graded as mild (33.3%), moderate (28.6%), severe (28.6%), and occluded (9.5%). The internal carotid artery was involved in all but 1 case. Stenosis of the anterior cerebral artery also was seen in 4 (19%) and of the middle cerebral artery in 6 children (28.6%).

Results of the univariate analysis of the association of neuroimaging abnormalities with frequent headache are shown in Table IV. Stenosis detected by brain MRA was significantly associated with both elevated TCD velocity (P = .005) and history of overt stroke (P < .001). All but 4 children with vessel stenosis (3 mild, 1 moderate) had either abnormal TCD or a history of overt stroke. Only 4 children with available brain MRA had moyamoya disease; 3 reported frequent headache (P = .066). There was no association of frequent headache with MRI evidence of sinus disease (data not shown).
DISCUSSION

We report the prevalence of frequent headache in children with SCD compared with a control group and we investigate the association of headache with SCD-related factors. Interestingly, the overall prevalence of frequent headache of 32.4% in subjects with SCD was not significantly different than that of black control subjects. Our study group was representative of our clinic population, except there was a slightly higher proportion of children receiving long-term transfusions (25% compared with 20% in our population with SCD-SS and SCD-Sβ-thalassemia), probably because these children are seen in clinic more frequently and subjects were recruited consecutively. Our control subjects were drawn from primary health care clinics at an urban tertiary health care center, and it is possible that this represented a sicker population than that of a community physician’s office. Nonetheless, the prevalence of frequent headache in our study is comparable to the prevalence reported in healthy Canadian children of 26.3% in 12- to 13-year-olds and 31.2% in 14- to 15-year-olds. The prevalence is also strikingly similar to the 32.1% prevalence of frequent headache in a prior report of 42 children with SCD. The frequency of migraine headache also was comparable between subjects with SCD (22.1%) and control subjects (21.1%) and is similar to rates quoted in the literature of 10.6% for children ages 5 to 15 and 28% for adolescents ages 15 to 19. Thus frequent headache appears to be a common complaint in black children with and without SCD. Causes of headache in the general population, such as migraine and tension headache, should be considered in the evaluation of headache in children with SCD.

In younger children, frequent headache was significantly more common in children with SCD compared with control subjects. In addition, the average age of children with SCD who reported frequent headache was younger than that of control subjects. Clinical factors associated with headache in the young children were similar to those found in the entire cohort, although not all associations reached statistical significance. This suggests that SCD may contribute to the cause of headache.

The overall prevalence of headache was similar among the different SCD genotypes, although smaller numbers of patients with SCD-Sβ-thalassemia genotypes limited these analyses. Clinical factors associated with headache (excluding neuroimaging findings) also were comparable across genotypes. Thus clinicians should address symptoms of headache in patients with all SCD genotypes. The laboratory findings of higher hemoglobin levels and higher platelet counts in those with SCD-SC or SCD-Sβ-thalassemia and frequent headache are intriguing and may reflect increased viscosity and inflammation, respectively.

In our study, children with frequent headache were more likely both to report frequent VOE and to experience headache and VOE concurrently. Vaso-occlusive pain and headache may have a common underlying pathophysiology related to vascular damage and increased blood viscosity. Vaso-occlusion with infarction of facial bones or of the skull also may directly cause headache. In addition, headache is a known side effect of opioid pain medications frequently used for treatment of VOE. Interestingly, bone marrow hyperplasia, perhaps because of pain from bony expansion or reflecting a marker of more severe disease, also was associated with frequent headache.

Overall, children with SCD and frequent headache also were more likely to have symptoms suggestive of OSA, specifically snoring and witnessed periods of apnea, but this finding was not significant in multivariate analysis in the younger children. OSA may cause headache in healthy children and may also cause headache in children with SCD. Given that most children in our study did not have formal polysomnography, which is the gold standard for diagnosis of

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Table IV. Association of neuroimaging abnormalities with frequent headache in subjects with SCD-SS or SCD-Sβ-thalassemia

<table>
<thead>
<tr>
<th></th>
<th>SCD-SS/Sβ-thalassemia, all ages (+) Headache</th>
<th>SCD-SS or Sβ-thalassemia &lt;13 years* (-) Headache</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Highest TCD velocity† (cm/s)</td>
<td>152 ± 38</td>
<td>157 ± 36</td>
<td>.39</td>
</tr>
<tr>
<td>History of overt stroke</td>
<td>9.1%</td>
<td>6.6%</td>
<td>.56</td>
</tr>
<tr>
<td>Silent infarct</td>
<td>25.9%</td>
<td>39.2%</td>
<td>.22</td>
</tr>
<tr>
<td>Extensive silent infarct</td>
<td>14.8%</td>
<td>23.0%</td>
<td>.42</td>
</tr>
<tr>
<td>Any cerebral infarction (overt or silent)</td>
<td>37.5%</td>
<td>45.1%</td>
<td>.46</td>
</tr>
<tr>
<td>Cerebral vessel stenosis</td>
<td>44.4%</td>
<td>12.9%</td>
<td>.001</td>
</tr>
<tr>
<td>Moderate to severe cerebral vessel stenosis</td>
<td>33.3%</td>
<td>7.1%</td>
<td>.001</td>
</tr>
<tr>
<td>Confirmed abnormal TCD</td>
<td>17.0%</td>
<td>14.0%</td>
<td>.63</td>
</tr>
<tr>
<td>Abnormal TCD with cerebral vessel stenosis by MRA</td>
<td>26.1%</td>
<td>4.6%</td>
<td>.003</td>
</tr>
<tr>
<td>Bone marrow hyperplasia</td>
<td>31.3%</td>
<td>14.8%</td>
<td>.047</td>
</tr>
</tbody>
</table>

*Includes only children younger than 13 years old; number with brain MRI = 65 and number with brain MRA = 57.
†Highest TCD velocity to date; subjects with history of stroke excluded.
OSA, further prospective study with more specific testing is warranted.

Neither silent cerebral infarction nor history of overt stroke was associated with headache in our study, which suggests that chronic brain parenchymal disease is not a significant cause of frequent headache in SCD. However, headache may be a symptom of cerebral vasculopathy in SCD given the observed association of both cerebral vessel stenosis and moyamoya disease with frequent headache. Furthermore, in the general population headache is a known symptom of moyamoya disease, a severe vasculopathy. In our study, although higher TCD velocities, suggestive of large-vessel stenosis, were not associated with headache, the combination of abnormal TCD and abnormal MRA was associated with frequent headache. This combination appears to signify a more specific vasculopathy since in the Stroke Prevention Trial in Sickle Cell Anemia, among children with abnormal TCD, stroke risk was higher in those with abnormal MRA than in those with normal MRA. Given that almost all children with abnormal MRA in our study had either a history of stroke or an abnormal TCD result, screening with TCD may be beneficial in the evaluation of frequent headache. In those with frequent headache and abnormal TCD or known stroke, MRA may aid in assessing for the presence or progression of vessel stenosis. Our study is limited because not all of our subjects with SCD-SS and SCD-β-thalassemia had brain MRI/MRA, and these studies were obtained for a variety of clinical reasons. Thus the relationship between cerebrovascular abnormalities and headache in SCD deserves further investigation.

The symptom of headache in SCD is probably multifactorial and may be related to migraine, VOE, bone marrow hyperplasia, OSA, or cerebral vessel stenosis. These factors should be considered in the evaluation of headache in SCD; most can be assessed with a thorough history, and the addition of TCD and other ancillary studies, if indicated. Future prospective, multicenter studies are warranted to study these associations further and to evaluate therapeutic interventions.

We thank Dr. T. Haacker and Dr. J. Pletcher for allowing us to study patients under their care in the General Pediatrics and Adolescent Medicine Clinics at CHOP.
Appendix. Selected items from questionnaire

Does your child ever experience headaches or migraines (yes, no)?
How often does your child have headaches or migraines (almost every day, 2-3 times a week, once a week, 2 times a month, once a month)?
How long does each headache typically last (less than 2 hours, more than 2 hours)?
Does the headache get worse with bright lights (yes, no, don’t know)?
Does the headache get worse with loud noises (yes, no, don’t know)?
Does your child snore most of the time (yes, no, don’t know)?*
How many pain episodes has your child had in the past 12 months that you saw a doctor for (for example: Hematology Acute Care Unit visit, emergency department visit, overnight stay in the hospital)?*

*SCD subject questionnaire only.
Neuropsychological Performance in School-Aged Children with Surgically Corrected Congenital Heart Disease

MARIJKE MIATTON, DIPSYCH, DANIEL DE WOLF, MD, PHD, KATRIEN FRANÇOIS, MD, EVERT THIERY, MD, AND GUY VINGERHOETS, PHD

Objective  As surgical management of children with congenital heart disease (CHD) advanced, developmental outcome became the main focus of contemporary research. In this study, we specify the cognitive profile of children with CHD, 6 to 12 years postoperatively.

Study design  Patients with CHD (n = 43, mean age 8 years, 8 months) and healthy controls (n = 43, mean age 8 years, 11 months), were examined with an abbreviated intelligence scale (Wechsler Intelligence Scale for Children-3rd edition, Dutch version) and a developmental neuropsychological assessment battery (NEPSY [a developmental NEuroPSYchological assessment]).

Results  We identified significantly lower scores for the CHD group on Estimated Full Scale IQ ($P < .01$). Neuropsychological assessment revealed lower scores for the CHD group on the cognitive domains of Sensorimotor Functioning ($P < .001$), Language ($P < .001$), Attention and Executive Functioning ($P < .05$), and Memory ($P < .05$). Children with CHD displayed more impulsive test behavior than healthy peers. No differences on IQ or cognitive domains were found between the cyanotic and the acyanotic CHD group.

Conclusions  Six to 12 years postoperatively, children with CHD display a neuropsychological profile with mainly mild motor deficits and subtle difficulties with language tasks. Attention/executive functioning and memory also appear involved but to a lesser degree. Long-term follow-up of children with surgically corrected CHD, even when hemodynamically successful, is warranted, as they are at risk for neurodevelopmental delay at school age. (J Pediatr 2007;151:73-8)

The incidence of congenital heart disease (CHD) in the Western industrialized world is estimated at 12 per 1000 live births.1 Significant advances in surgical management and decreases in mortality and morbidity have made functional outcome the primary focus of contemporary research. Follow-up studies have identified developmental and neurological abnormalities in as many as 25% of survivors. Neurological evaluation mostly revealed deficits in neurocognitive (language, attention) or motor functions (balance, hopping).2 On developmental testing, children with CHD showed poorer hand-eye coordination and lower scores on locomotor functioning, personal/social, and speech and hearing scales than healthy control subjects.3 Assessment of IQ in school-aged children with CHD showed scores within the expected range,4,5 although lower than in the general population.6 Neuropsychological assessment was mostly performed on isolated diagnostic groups and revealed problems on several cognitive functions.7-16

The purpose of the present study is to characterize the neuropsychological outcome of school-aged children with a surgically corrected cyanotic or acyanotic CHD and compare their performances with those of age- and sex-matched healthy controls. In contrast to most studies, enrollment of children was not limited to those with a specific diagnosis but covered the whole spectrum of CHD, to draw conclusions on the neuropsychological functioning of children with CHD in general and not to link specific neurocognitive profiles to specific diagnoses. We evaluated differences in neuropsychological outcome between cyanotic and acyanotic CHD.

METHODS

Patient Characteristics and Medical Data

Patients with various CHDs, operated in the Ghent University Hospital between 1995 and 1999, with a birth weight >2000 g, without perinatal problems, and without...
noncardiac malformations or genetic abnormalities (Down syndrome, Velocardiofacial syndrome, and DiGeorge syndrome) were contacted and invited to participate in the study (n = 163). Several candidates had moved and could not be located (n = 17). Various reasons were given by nonparticipants: presence of a developmental disorder (n = 2); one boy with autism, one boy with a severe learning disorder), testing being too time consuming (n = 27), awaiting new cardiac surgery for the child (n = 2), not wanting to confront the child with something that happened a long time ago (n = 19), and stating that the child has no cognitive problems and participating would imply that he or she does (n = 8). Reasons for not participating remained unclear in 31 case patients who did not respond in any way to the invitation. All children with tetralogy of Fallot and all other children showing characteristic features of genetic abnormalities at birth had a genetic screening (fluorescence in situ hybridization, FISH) to exclude for DiGeorge/velocardiofacial syndrome. In total, 57 patients were included in the project, of which we selected only those patients who had undergone an open-heart procedure (n = 43; 21 girls, 22 boys). We compared the total CHD group with the healthy controls and the cyanotic CHD group (n = 26) with the acyanotic CHD group (n = 17). For each child in the patient group, a healthy sex-, age-, and educational-level matched control was included. The healthy children were contacted through their school boards. All parents gave written informed consent. At the time of testing, all children (i.e., the total CHD group and healthy controls) attended school full-time, and according to their parents, they participated actively in sports and school activities. We calculated the Hollingshead Four Factor Index of Social Status to quantify the socioeconomic status of each family. This index uses education, occupation, sex, and marital status to determine a composite social status. The score was computed by multiplying the Occupation scale value by a weight of 5 and the Education scale by 3, and then summing these products. The raw scores range from 8 to 66, with higher scores reflecting higher socioeconomic status.17 The local Ethical Committee approved the study. Procedures were in accordance with the recommendations found in the Helsinki Declaration of 1975.18

Medical data were collected from the patients’ files. We included birth weight and length, and Apgar scores immediately after birth and after 10 minutes. All children had undergone an open-heart operation with full-flow cardiopulmonary bypass under moderate hypothermia (25°C-33°C).

Neuropsychological Assessment

After agreeing to participate, children were invited for a neuropsychological assessment of 2 to 3 hours duration. The child was tested with a short form of the Wechsler Intelligence Scale for Children-3rd edition, Dutch version (WISC-III NL). The short form included two verbal subtests (Information and Vocabulary) and two performance subtests (Picture Completion and Block Design).19 A deviation IQ was calculated using the procedure suggested by Sattler.20 On subtest level, a mean performance of 10 (SD = 3) is expected. Mean Estimated Full Scale IQ is 100 (SD = 15).

The neuropsychological battery consisted of all core subtests of the NEPSY (a developmental NEuroPSYchological assessment). The NEPSY tests the child’s neuropsychological development in five functional domains to detect subtle deficiencies, within and across these functional domains, which can interfere with learning in preschool and school-aged children.21 In the domain Attention and Executive Functioning, children had to perform three tasks. The subtest Tower measures nonverbal planning and problem solving. The subtest Auditory Attention and Response Set tests vigilance, sustained auditory attention, and the ability to shift and maintain new and complex sets. The subtest Visual Attention reports on speed and accuracy of selectively focusing and maintaining attention on a visual target. The domain Memory includes both verbal (Narrative Memory, Memory for Names) and visual memory tasks (Memory for Faces). The subtests Phonological Processing (phonemic awareness), Speeded Naming (access to and production of names of recurring colors, sizes, and shapes), and Comprehension of Instructions (ability to process and respond quickly to verbal instructions of increasing complexity) form the domain Language. Visual-spatial Skills were assessed by two subtests: Arrows (line orientation and directionality) and Design Copy (ability to copy two-dimensional geometric figures). Fingertip Tapping (finger dexterity and motor speed), Imitation of Hand/Finger Positions (ability to imitate hand/finger positions), and Visuomotor Precision (fine motor skills, hand-eye coordination) were the tasks to perform in the domain Sensorimotor Functioning. A mean performance on the domains is 100 (SD 15); on subtest level a mean performance of 10 (SD 3) is expected.

Statistical Analysis

Statistical analyses were performed using the Statistical Package for the Social Sciences for Windows (version 12.0) (SPSS, Chicago, Ill). Demographics (age at the moment of testing, sex, and educational level of both parents, Hollingshead Index), medical characteristics (birth weight, Apgar scores), and outcome measures (Estimated IQ, NEPSY) were compared between the total CHD group and the healthy control group. Nominal data were analyzed with χ² statistics. Normality was checked by Kolmogorov-Smirnov tests. If the data were not normally distributed, the nonparametric Wilcoxon’s signed rank test was used. For normally distributed data, multivariate analyses of variance were used with group (total CHD group vs healthy control group, cyanotic CHD group vs acyanotic CHD group) as a between-subject factor; the WISC-III subtests, Estimated Full Scale IQ, NEPSY domains and subtests as dependent variables; and educational level of the father as covariate.

RESULTS

Patient Characteristics and Medical Data

The mean age at testing of the total CHD group was 8 years, 8 months ± 1 year, 6 months. As a result of matching,
no significant group differences on sex and age at testing were found between the total CHD group and the healthy control group. There was a significantly lower length at birth ($P < .05$) in the total CHD group compared with the healthy control group. We did not find significant differences in Apgar scores between these groups. No group differences were found on maternal level of education or on socioeconomic status, as rated by the Hollingshead Four Factor Index. The majority of children came from middle-class families (skilled craftsmen, clerical and sales workers). We did find a lower paternal educational level in the CHD group, so this variable was entered as a covariate in all statistical analyses.

We divided the CHD group in a cyanotic ($n = 26$) and an acyanotic CHD group ($n = 17$). No differences on demographic, medical, or socioeconomic variables could be identified between these patient groups. Medical data are presented in Table I. Demographic and socioeconomic results are summarized in Table II.

### Neuropsychological Assessment

On the short-form intelligence scale, we found a significantly lower Estimated Full Scale IQ for the total CHD group ($P < .01$). Two of the subtests included were significantly lower in the patient group: Picture Completion ($P < .01$) and Vocabulary ($P < .05$). Children with CHD performed significantly lower on the domains of Sensorimotor Functioning ($P < .001$) and Language than healthy controls. Each subtest included in both domains yielded significantly lower results. Attention and Executive Functioning ($P < .05$) as well as Memory ($P < .05$) also were significantly lower in the CHD group. Visual-spatial skills did not elicit group differences. Results on all included subtests are summarized in Table II. Boxplots of the group differences on estimated IQ, Sensorimotor Functioning, and Language can be found in Figure 1. In Figures 2 and 3 (available at www.jpeds.com), boxplots on the subtests of the domain Sensorimotor Functioning and Language can be found. No significant differences between the cyanotic and the acyanotic CHD group emerged on the intellectual and neuropsychological evaluation.

### DISCUSSION

The current study results indicate a significant relation between surgically corrected CHD and specific neurocognitive difficulties during school age. In accordance with previously published studies,4-6 we found the estimated intellectual capacities of children with surgically corrected CHD to fall within the expected range, although significantly lower than in our healthy group. Because it was not our intention to replicate IQ findings, we used only a short-form intelligence battery. Nevertheless, one can appreciate the differences we found on subtest level. Children with CHD showed significantly worse performances on the verbal subtest Vocabulary, in accordance with studies that included Full Scale IQ testing.7 This lower score on the verbal subtest reflects the deficits in expressive and receptive language that children with CHD display.10,11 Picture Completion also yielded significant differences. This subtest demands visual recognition and asks the child to compare the given picture with the image in their visual long-term memory.

On the neuropsychological evaluation, the most striking deficits are on sensorimotor functioning and language. We found that up to 25% of the children with CHD performed worse than expected on motor tasks. Children with CHD had significantly reduced skills for imitating hand and finger positions, were slower on motor tasks, showed worse hand-eye coordination, showed less accuracy in fine visuomotor skills, and they used an impulsive strategy compared with the healthy control group. Inefficient processing of tactile and kinesthetic information seems the most plausible explanation for these poor sensorimotor performances in the CHD group.21 The slowing down in more complex motor tasks and worse hand-eye coordination have been repeatedly reported.7,11,12 In an attempt to identify the cause of these sensorimotor deficits, recent research revealed children with preoperative hypoxemia in infancy to be at higher risk for...
motor dysfunction than children with cardiac insufficiency. In the brain, the basal ganglia are most sensitive to hypoxia and ischemia. Previous studies even demonstrated decreases in the basal ganglia N-acetylaspartate levels to be correlates for neuropsychological sequelae, as for instance motor speed. Both findings lead to the suspicion of the involvement of the basal ganglia in the motor deficits found in our study. In addition, hypoxia-ischemia has also been considered as a major cause for the occurrence of an important white matter disease called periventricular leukomalacia that results in neurodevelopmental delay, and that occurs in more than 50% of neonates who have undergone cardiac surgery. Children with obvious neurological dysfunction were excluded in this study; however, the presence of periventricular leukomalacia or other brain damage in our study group is unknown. Future functional magnetic resonance imaging studies can be useful in clarifying the relationship between motor deficits and brain (dys)function of the child with CHD.

A second neuropsychological function in which children with CHD performed significantly lower is language. At 2 years, 5 months of age, delays of 2 to 4 months in language were demonstrated for children with CHD. These results are in accordance with other previous studies. No differences were found in the other neuropsychological functions that were studied.

Table II. Demographics, mean performances, and standard deviations on IQ and NEPSY, F, and P value

<table>
<thead>
<tr>
<th>Variable</th>
<th>Patient group n = 43</th>
<th>Control group n = 43</th>
<th>F§</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>8 y, 8 mo ± 1 y, 6 mo</td>
<td>8 y, 11 m ± 1 y, 7 mo</td>
<td>.644</td>
<td>.829</td>
</tr>
<tr>
<td>Sex</td>
<td>22 6 21 9</td>
<td>22 6 21 9</td>
<td>.047†</td>
<td>.571</td>
</tr>
<tr>
<td>Education father (y)</td>
<td>12.9 6 2.0</td>
<td>14.3 6 3.4</td>
<td>4.86</td>
<td>.031*</td>
</tr>
<tr>
<td>Education mother (y)</td>
<td>13.0 6 1.9</td>
<td>13.7 6 2.9</td>
<td>1.70</td>
<td>.197</td>
</tr>
<tr>
<td>Mean Hollingshead SES</td>
<td>32.4 6 8.4</td>
<td>35.6 6 11.2</td>
<td>2.80</td>
<td>.135</td>
</tr>
<tr>
<td>INTELLIGENCE</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Estimated Full Scale IQ:</td>
<td>95.6 6 15.4</td>
<td>107.0 6 15.1</td>
<td>.833</td>
<td>.005*</td>
</tr>
<tr>
<td>Picture Completion</td>
<td>8.4 6 3.7</td>
<td>10.6 6 3.5</td>
<td>8.26</td>
<td>.005*</td>
</tr>
<tr>
<td>Block Design</td>
<td>9.2 6 3.0</td>
<td>10.1 6 2.9</td>
<td>1.67</td>
<td>.200</td>
</tr>
<tr>
<td>Information</td>
<td>10.3 6 2.8</td>
<td>11.7 6 2.6</td>
<td>3.57</td>
<td>.062</td>
</tr>
<tr>
<td>Vocabulary</td>
<td>9.6 6 2.3</td>
<td>11.5 6 2.7</td>
<td>6.93</td>
<td>.010*</td>
</tr>
<tr>
<td>NEPSY</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Attention and Executive Function</td>
<td>112.5 6 10.1</td>
<td>117.8 6 9.2</td>
<td>5.80</td>
<td>.018*</td>
</tr>
<tr>
<td>Tower</td>
<td>12.8 6 2.2</td>
<td>14.0 6 2.1</td>
<td>5.31</td>
<td>.024*</td>
</tr>
<tr>
<td>Auditory Attention and Response set</td>
<td>12.3 6 1.2</td>
<td>12.1 6 1.3</td>
<td>–.850‡</td>
<td>.395</td>
</tr>
<tr>
<td>Visual Attention</td>
<td>9.8 6 2.8</td>
<td>11.0 6 2.3</td>
<td>5.30</td>
<td>.024*</td>
</tr>
<tr>
<td>Memory</td>
<td>98.4 6 14.0</td>
<td>105.7 6 12.3</td>
<td>6.56</td>
<td>.012*</td>
</tr>
<tr>
<td>Memory for Faces</td>
<td>103.0 6 3.1</td>
<td>106.6 6 3.1</td>
<td>.443</td>
<td>.508</td>
</tr>
<tr>
<td>Memory for Names</td>
<td>87.3 6 3.4</td>
<td>111.1 6 2.4</td>
<td>12.72</td>
<td>.001***</td>
</tr>
<tr>
<td>Total over trials (max 24)</td>
<td>12.9 6 5.1</td>
<td>16.5 6 4.1</td>
<td>–3.12‡</td>
<td>.002*</td>
</tr>
<tr>
<td>recall (max 8)</td>
<td>4.6 6 2.3</td>
<td>5.9 6 1.8</td>
<td>6.51</td>
<td>.013*</td>
</tr>
<tr>
<td>Narrative Memory</td>
<td>105.5 6 3.0</td>
<td>108.4 6 1.9</td>
<td>.301</td>
<td>.585</td>
</tr>
<tr>
<td>Language</td>
<td>102.0 6 11.8</td>
<td>115.8 6 14.0</td>
<td>20.0</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>phonological processing</td>
<td>12.0 6 3.0</td>
<td>13.9 6 2.7</td>
<td>7.06</td>
<td>.010*</td>
</tr>
<tr>
<td>Speeded Naming</td>
<td>8.9 6 2.2</td>
<td>10.8 6 2.3</td>
<td>11.89</td>
<td>.001*</td>
</tr>
<tr>
<td>time (sec)</td>
<td>135.5 6 5.4</td>
<td>145.5 6 5.7</td>
<td>5.60</td>
<td>.020*</td>
</tr>
<tr>
<td>errors</td>
<td>0.9 6 1.3</td>
<td>0.3 6 0.5</td>
<td>–2.76‡</td>
<td>.006*</td>
</tr>
<tr>
<td>Comprehension of Instructions</td>
<td>105.5 6 1.9</td>
<td>125.5 6 2.2</td>
<td>15.65</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>Visual-spatial Skills:</td>
<td>115.0 6 15.7</td>
<td>120.2 6 12.7</td>
<td>1.27</td>
<td>.263</td>
</tr>
<tr>
<td>Design Copy</td>
<td>13.9 6 3.0</td>
<td>14.7 6 2.5</td>
<td>.593</td>
<td>.444</td>
</tr>
<tr>
<td>Arrows</td>
<td>10.9 6 2.8</td>
<td>11.7 6 2.5</td>
<td>.797</td>
<td>.375</td>
</tr>
<tr>
<td>Sensorimotor Functioning</td>
<td>88.8 6 13.4</td>
<td>101.1 6 9.6</td>
<td>18.59</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>Imitating Hand Positions</td>
<td>8.5 6 2.8</td>
<td>11.4 6 2.3</td>
<td>18.61</td>
<td>&lt;.001***</td>
</tr>
<tr>
<td>Finger-tap Tapping</td>
<td>9.5 6 1.5</td>
<td>10.5 6 1.8</td>
<td>–2.48‡</td>
<td>.013*</td>
</tr>
<tr>
<td>Visuomotor Precision</td>
<td>7.3 6 3.2</td>
<td>9.1 6 2.2</td>
<td>7.17</td>
<td>.009*</td>
</tr>
<tr>
<td>time (sec)</td>
<td>135.5 6 5.4</td>
<td>145.5 6 4.8</td>
<td>.455</td>
<td>.302</td>
</tr>
<tr>
<td>errors</td>
<td>19.8 6 20.9</td>
<td>4.9 6 7.9</td>
<td>–3.90‡</td>
<td>&lt;.001**</td>
</tr>
</tbody>
</table>

Ses Socioeconomic status.

*P < .05 (level of significance).

**P < .001 (level of significance).

†χ² value.

‡Kruskall-Wallis test.

§Degrees of freedom [1, 81].
In our study, we focused on the specific area of concern in older children with surgically corrected CHD: language. The CHD group displayed lower phonological awareness, difficulty with naming color, size, and shape, and lacked the ability to process and respond quickly to verbal instructions of increasing difficulty. During the subtest Speeded Naming, children with CHD again displayed an impulsive strategy, as revealed by a shorter time to complete the task compared with healthy controls, yet accompanied by more errors. These results corroborate with studies in children with transposition of the great arteries, in which dysfunction of speech was found in 40%. In infants with transposition of the great arteries or with ventricular septal defect, expressive language was found to be reduced in 34.4%; receptive language was defective in 28.1%. Poorer performances in reading and spelling are recognized as well. This deficient access to names of color, size, and shape suggests a deficient retrieval of sound-symbol associations and spoken-written word connections involved in reading acquisition.

In contrast to previous studies, the children with CHD showed significantly lower results on Memory. The subtest Memory for Names as well as the short-form IQ subtest Picture Completion specifically demand a cross-modal association between visual and semantic information that is particularly difficult for children with CHD. They display difficulties with both learning and recalling the names associated with the faces presented.

Further, our results indicate lower performances for the CHD group on the subtests Tower and Visual Attention. Focused auditory attention revealed no problems, but behavioral observation during the testing again revealed poor impulse control on the subtest Tower. Impulsivity in behavior has been previously reported in children with acyanotic CHD. Another explanation to bear in mind is a deficiency in frontal lobe functioning, which has been postulated before in adult patients with CHD. Concerning Visual Attention, several factors such as poor hand-eye coordination or impulsive strategy might have caused the lower performances in the CHD group.

In contrast to previous studies, visual-spatial skills did not elicit differences between the CHD group and the healthy controls. The children had no problems copying designs and estimating line orientation and directionality.

Although it is expected that because of the difference in severity of the symptoms cyanotic forms of CHD result in lower functional outcome than acyanotic forms of CHD, in this study, we could not identify any differences on the intellectual and neuropsychological evaluation between the cyanotic and the acyanotic CHD-group, which is supported by other clinical studies.

The current study should be interpreted in light of certain limitations. First, enrollment was voluntary, which creates a selection bias. Possibly the small sample size prevented us from finding significant differences between the cyanotic and acyanotic CHD group. Second, we did not include a “sick” control group, and thus we are unable to report on the impact of factors such as hospitalization, school absence, restrictions on sports/social activities, and overprotection by parents on neuropsychological functioning. Future studies should enhance this factor to clarify the respective contribution of postsurgery restrictions or of surgery itself to adverse neuropsychological outcome. Impulsive test behavior was observed and objectified in the CHD group. These differences were observed throughout the group and were not merely caused by a few individual cases.

Previously studied broad cognitive functions can now be narrowed to specific deficits, and we can now rate the degree of these relative deficits. This specification in the neuropsychological profile might lead to interventional programs tailored to the child’s needs, significantly improving functional cognitive outcome. In future, functional magnetic resonance imaging can elucidate the involvement of specific brain areas in the neuropsychological deficits we observed.

REFERENCES
17. Hollingshead AB. Four Factor Index of Social Status. New Haven, CT: Yale University, Department of Sociology; 1975.
Figure 2. Boxplots on group differences of all subtests of the domain Sensorimotor Functioning.

Figure 3. Boxplots on group differences of all subtests of the domain Language.
Epidemiology and Outcome of Necrotizing Fasciitis in Children: An Active Surveillance Study of the Canadian Paediatric Surveillance Program

IHUOMA ENELI, MD, MS, AND H. DELE DAVIES, MD, MS

Objective To describe the epidemiology, management, and outcome of pediatric necrotizing fasciitis (NF) in Canada before full implementation of varicella immunization programs.

Study design This was a prospective cohort study of all children under age 16 years identified by the Canadian Paediatric Surveillance Program (CPSP).

Results Between November 1, 2001 and October 31, 2003, 36 NF cases were identified (mean age, 5.9 ± 5 years). Group A streptococcus (GAS)-related and non–GAS-related NF accounted for 2.12 and 0.81 cases per million children, respectively. The annual incidence was substantially higher in children under age 5 years (5.9 vs 1.8 per million; P = .0002). Males over age 1 year had the highest disease burden, with 12 cases per million, versus 3.2 cases per million for females under age 1 year (P < .0001). Most (15/26; 58%) GAS-related cases were associated with varicella. Complications occurred in 29 children (78%), and 2 children (5.4%) died.

Conclusion In the prevaccine era, NF occurred most commonly in Canadian children under age 5 years, with a peak incidence in males under age 1 year. There is substantial associated morbidity and about 5% mortality. The data provide baseline incidence of disease and a surveillance mechanism for NF after the implementation of publicly funded varicella immunization programs in Canada. (J Pediatr 2007;151:79-84)

Necrotizing fasciitis (NF) is a serious infection involving the subcutaneous tissue, fascia, and fat, with substantial morbidity and mortality.1 NF may be classified into 2 types based on the causative organism. The first type includes those cases due to mixed infection from anerobes, most commonly Bacteroides and Peptostreptococcus species, facultative anaerobes such as non-group A [beta]-hemolytic streptococci, and Escherichia coli, Enterobacter species, Klebsiella, and Proteus species.2 The other type of NF is caused by invasive group A streptococcus (GAS).3 NF occurs at a higher frequency in patients with chronic disease, after varicella infection, possibly in association with use of nonsteroidal anti-inflammatory drugs (NSAIDs), and in patients with a history of recent surgery or trauma.1,3-9

The last 2 decades have brought an increase in the number of invasive GAS infections, including NF.5,10,11 Some studies have focused on an altered virulence of the bacteria to explain the resurgence, identifying an association between M1 and M3 strains and invasive disease.7-10

Although there are many case reports and case series of NF,2,4,12-17 there are no population-based studies of incidence involving primarily children. Population-based epidemiologic data for NF in children are needed to guide and monitor prevention programs, clinical recognition, and management. Using a national population-based, active surveillance system, we describe the epidemiology, management, and outcome of NF in Canada and compare epidemiologic features of GAS-related and non–GAS–related NF.

METHODS

Between November 1, 2001 and October 31, 2003, we identified all reported cases of NF in children age 0 to 16 years from the Canadian Paediatric Surveillance Program (CPSP), an active surveillance collaborative program between the Canadian Paediatric Society and Public Health Agency of Canada. The CPSP system has been used to...
successfully monitor and ascertain rare conditions (see http://www.phac-aspc.gc.ca/publicat/ccdr-mntc/04pdf/30s2_e.pdf). Approximately 2300 of the estimated 2500 pediatricians and pediatric subspecialists in Canada participate in this monthly surveillance system on a voluntary basis. Because of the severity of NF in Canada, management always occurs at a tertiary health care center, with care coordinated by a pediatrician. On identification of a case, an initial “check-off” form is used, followed by a detailed reporting form. The detailed reporting form serves to confirm the accuracy of diagnosis of NF before it is considered a case. The information on the form includes demographic data, presenting signs and symptoms, risk factors, primary site of infection, management, outcome, and microbiological test results. The average monthly response rate is 82%, with a >95% completion rate for detailed case reporting.

The present study was approved by the University of Calgary’s Conjoint Medical Ethics Committee.

Case Definition

NF was defined as cases with positive culture from either blood or connective or fascial tissue plus histopathology results demonstrating necrosis of superficial fascia, polymorphonuclear infiltrate, and edema of the reticular dermis, subcutaneous fat, and superficial fascia, or in the absence of histology, gross fascial edema and necrosis detected at surgery or frank cutaneous necrosis seen on physical examination.\(^1\)

Microbiology

A case was defined as GAS-related when group A streptococcus was isolated or as non–GAS-related when the isolates included other organisms, such as anaerobes, one or more facultative anaerobes such as streptococci (non–GAS), and/or Enterobacteriaceae.

Other Definitions

A risk factor was considered present if the case had any of the following conditions: contact with persons with confirmed GAS pharyngitis or invasive GAS disease, streptococcal pharyngitis, recent surgery, history of trauma, chronic illness, varicella within the past month, in out-of-home childcare attendance, hospitalization before onset of illness, or use of a NSAID within a week of hospitalization.\(^5\) When onset of NF occurred more than 48 hours after a hospital admission, the case was classified as nosocomial. A history of underlying chronic illness was ascertained. A case was classified as having a complication if any of the following developed: adult respiratory distress syndrome, amputation, need for multiple surgical procedures, need for skin graft, or death.\(^5\) Coagulopathy was defined as a platelet level at or below normal for age or the presence of disseminated intravascular coagulation. The criteria for hepatic and renal impairment were a 2-fold or greater elevation in serum alanine aminotransferase, aspartate aminotransferase, or total bilirubin and creatinine level.

RESULTS

Between November 1, 2001, and October 31, 2003, 36 cases of NF were identified in children under age 16 years; 10 of these cases met the case definition for non–GAS-related NF, and 26 cases did so for GAS-related NF. Cases were reported from 7 of the 13 Canadian provinces and territories (British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Quebec, and the Northwest Territories), which account for 92% of the population of Canada. The remaining provinces did not report any cases. The mean age of cases was 5.9 ± 5 years, with males constituting 49% of cases. The total annual incidence was 2.93 cases per million population under age 16 years (0.81 per million for non–GAS-related vs 2.12 per million for GAS-related; \(P < .01\)). The incidence was greatest during the first 5 years of life, with an overall annual incidence of 5.9 per million in this age group versus 1.8 per million in children age 5 years and older (\(P = .0002\)) (Figure 1). Males under age 1 year had the highest overall rates of disease (12 cases per million, vs 3.2 per million for females under age 1 year; \(P < .0001\)) (Figure II; available at www.jpeds.com).

Risk Factors

Twenty-nine children (81%) had at least 1 identified risk factor for NF. Seventeen cases (46%) cases occurred...
within 1 month of a varicella infection. Seven cases (19%) had at least 1 underlying chronic condition, including Down syndrome; congenital neutropenia; Klippel-Trenaunay-Weber syndrome, with lower leg hemangioma, bronchopulmonary dysplasia, and recurrent bronchitis; cerebral palsy, with hydrocephalus and ventriculoperitoneal shunting; and schizophrenia, with mild developmental delay. Compared with GAS-related cases, there was a trend of non–GAS-related NF cases being nosocomial (2/10 [20%] vs 0/24 [0%]; *P* = .08), being associated with recent surgery (3/10 [30%] vs 1/26 [4.0%]; *P* = .05); and having a recent history of trauma (3/10 [30%] vs 2/23 [8.0%]; *P* = .12). A documented history of varicella in the preceding month was more common in GAS-related cases (15/25 [60%] vs 2/10 [20%; *P* = .05]), as was a trend for contact with a person with pharyngitis (9/25 [36%] vs 0/10 [0%]; *P* = .07). GAS-related cases were more likely to report the use of a NSAID within a week before the onset of disease (10/17 [58.8%] vs 0/6 [0%; *P* = .01).

Among the 5 patients under 1 year old, only 1 had a history of a chronic condition, although 4 (80%) had a predisposing risk factor (Table I). The only infant death occurred in a 3-week-old male who had an abdominal infection originating the umbilical stump.

**Clinical Presentation**

The median time from onset of illness to coming to medical attention (excluding the 2 nosocomial cases) was 1 day. The most common presenting symptoms were localized pain (34/35; 97.1%), chills (12/34; 35.3%), and vomiting (9/33; 27.2%). Sore throat and respiratory symptoms (eg, cough, shortness of breath, sore throat) were more common in GAS-related NF (Table II).

Rash was the predominant finding on physical examination. Of the 26 cases that presented with a rash, generalized rash was seen only in GAS-related cases (*P* < .05). Desquamation during the hospital stay occurred in only 1 case (2.8%), a GAS-related NF case. Toxin-mediated physical examination findings (eg, generalized rash, conjunctivitis, strawberry tongue) occurred more frequently in GAS-related NF (14/26 vs 0/10; *P* = .006).

The median temperature at presentation was 38.6°C (range, 36.1 to 40.3°C). Seven of the 36 patients (19%) were hypotensive, requiring treatment with pressor agents. The majority (5/7; 71.4%) of the hypotensive patients were over 5 years old. Hypotension was more frequent in non–GAS-related cases (4/10; 40%) than in GAS-related cases (3/26; 11.5%), but the difference was not statistically significant (*P* = .07). Sixteen cases (44%) involved the lower extremities or groin area; 7 (19%) involved the upper extremities; 8 (22%) involved the head, neck, and chest; and 3 (8%) involved the abdomen. One infant had lesions in multiple sites. Non–GAS-related NF occurred more commonly on the lower extremities, groin, and abdomen (Figure 3); only 1 child had a lesion on the head and neck area.

### Table I. Risk factors, presentation, and outcome of necrotizing NF in patients under 1 year old

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>NF type</th>
<th>Chronic condition</th>
<th>Risk factor</th>
<th>Site</th>
<th>Source of organism</th>
<th>Complication</th>
<th>Length of stay (days)</th>
<th>Outcome</th>
<th>Source of organism</th>
<th>Complication</th>
<th>Length of stay (days)</th>
<th>Outcome</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3 weeks</td>
<td>M</td>
<td>Non–GAS*</td>
<td>No</td>
<td>Trauma</td>
<td>Abdomen (umbilical stump)</td>
<td>Tissue Aspirate</td>
<td>Coagulopathy</td>
<td>Rash</td>
<td>Renal impairment</td>
<td>3</td>
<td>Death</td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>8 months</td>
<td>M</td>
<td>GAS</td>
<td>No</td>
<td>Surgery NSAID</td>
<td>Abdomen Groin</td>
<td>Blood Tissue Aspirate</td>
<td>Rash</td>
<td>10</td>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>45 months</td>
<td>F</td>
<td>Non–GAS†</td>
<td>Yes‡</td>
<td>Prematurity Surgery</td>
<td>Cheek and neck§</td>
<td>Blood Aspirate</td>
<td>Rash</td>
<td>53</td>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>5 weeks</td>
<td>M</td>
<td>GAS</td>
<td>No</td>
<td>None</td>
<td>Abdomen</td>
<td>Tissue</td>
<td>Rash</td>
<td>36</td>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>10.5 months</td>
<td>M</td>
<td>GAS</td>
<td>No</td>
<td>Varicella</td>
<td>Abdomen</td>
<td>Tissue Aspirate</td>
<td>Rash</td>
<td>10</td>
<td>Survival</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Staphylococcus aureus.†Group B streptococcus.‡Down syndrome.§Suspected spider bite.¶Amputation of part of the left ear.

**BPD,** Bronchopulmonary dysplasia; **IVH,** intraventricular hemorrhage.
All of the patients had a positive blood, tissue, or aspirate culture. The highest yields were from tissue cultures—50% for non–GAS-related cases and 85% for GAS-related cases (Table III; available at www.jpeds.com). However, there was a significantly greater likelihood of isolation of the organism from skin or tissue culture in GAS-related cases (Table III). Isolated organisms in non–GAS-related cases included group B streptococcus, Staphylococcus epidermis, S. aureus, E. coli, Bacteriodes fragilis, K. pneumoniae, and Clostridium septicum.

Management and Outcome

Penicillin and clindamycin were the most commonly administered antibiotics. All 10 non–GAS-related patients received penicillin or another beta-lactam antibiotic (with 2 patients receiving a first-generation cephalosporin and 4 receiving a third-generation cephalosporin); 8 also received clindamycin. Other antibiotics used for non–GAS-related cases included vancomycin, aminoglycosides, and metronidazole. All GAS-related patients received clindamycin in combination with either penicillin (22/25 cases; 1 case undocumented) or another beta-lactam antibiotic (3/26 cases). Nineteen patients (14/26 [54%] GAS-related and 5/10 [50%] non–GAS-related) received unspecified blood products.

NF-related complications were observed in 28 cases (78.0%). The frequency of complications was similar in both types of NF (Table II) except for fatal outcome, which occurred only in non–GAS-related cases. All of the patients underwent a surgical procedure; 78% (28/36) had a second surgery, and 50% (18/36) had a third surgery. Skin graft rates were similar in GAS-related and non–GAS-related cases.

The median length of hospital stay was 12 days (range, 5 to 81 days). The length of stay was longer for non–GAS-related cases (median, 33 days; range, 12 to 81 days) compared with GAS-related cases (median, 12 days; range, 5 to 36 days; P = .004). The proportion of cases admitted to the intensive care unit was similar in both types of NF (60% vs 62%; P = 1.0). Non–GAS-related cases had a greater tendency to receive pressor agents (4/10 vs 3/26; P = .17) and to require mechanical ventilation (5/10 vs 7/26; P = .24). Two deaths (both non–GAS cases) occurred during the surveillance period, for an overall case fatality rate of 5.4%. One death was the youngest patient, and the other was a 5-year-old boy with congenital neutropenia who presented with a rapidly spreading violaceous lesion on the right buttock. K. pneumoniae and C. septicum were isolated from a biopsy specimen of the lesion.

DISCUSSION

In this population-based active surveillance study, we found an annual incidence of NF in children of 2.93 cases per million population per year, with 0.81 per million for non–GAS-related cases and 2.12 per million for GAS-related cases. Although there are no comparable baseline rates for non–GAS-related NF, our GAS-related NF rate is somewhat higher than the annual total population incidence rate of 1.3 cases per million in the population of Ontario between 1992

### Table II. Clinical presentation of non–GAS-related and GAS-related NF

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Non–GAS-related NF</th>
<th>GAS-related NF</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>3/10 (30%)</td>
<td>2/25 (8.0%)</td>
</tr>
<tr>
<td>Chills</td>
<td>4/9 (44%)</td>
<td>8/25 (32%)</td>
</tr>
<tr>
<td>Confusion</td>
<td>1/10 (10%)</td>
<td>1/25 (4.0%)</td>
</tr>
<tr>
<td>Cough</td>
<td>0/9 (0%)</td>
<td>4/25 (16%)</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>0/10 (0%)</td>
<td>3/25 (12%)</td>
</tr>
<tr>
<td>Diffuse myalgia</td>
<td>0/10 (0%)</td>
<td>2/25 (8.0%)</td>
</tr>
<tr>
<td>Headache</td>
<td>1/10 (10%)</td>
<td>2/25 (8.0%)</td>
</tr>
<tr>
<td>Local pain</td>
<td>10/10 (100%)</td>
<td>24/25 (96%)</td>
</tr>
<tr>
<td>Shortness of breath</td>
<td>1/9 (11%)</td>
<td>0/24 (0%)</td>
</tr>
<tr>
<td>Sore throat</td>
<td>0/9 (0%)</td>
<td>6/25 (24%)</td>
</tr>
<tr>
<td>Syncope</td>
<td>1/10 (10%)</td>
<td>1/24 (4.2%)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>1/9 (11%)</td>
<td>8/25 (33%)</td>
</tr>
<tr>
<td><strong>Physical examination and laboratory findings</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rash</td>
<td>6/10 (60%)</td>
<td>21/26 (73.0%)</td>
</tr>
<tr>
<td>Generalized*</td>
<td>0/6 (0%)</td>
<td>13/20 (65.0%)</td>
</tr>
<tr>
<td>Local*</td>
<td>6/6 (100%)</td>
<td>7/20 (35)</td>
</tr>
<tr>
<td>Desquamation</td>
<td>0/10 (0%)</td>
<td>1/25 (4.0%)</td>
</tr>
<tr>
<td>Mucous membrane hyperemia</td>
<td>1/10 (10%)</td>
<td>6/24 (25)</td>
</tr>
<tr>
<td>Conjunct</td>
<td>0/4 (0%)</td>
<td>4/12 (33)</td>
</tr>
<tr>
<td>Strawberry tongue</td>
<td>0/4 (0%)</td>
<td>2/13 (15)</td>
</tr>
<tr>
<td>Pharyngeal exudates</td>
<td>0/4 (0%)</td>
<td>4/14 (28)</td>
</tr>
<tr>
<td>Elevated liver enzymes</td>
<td>1/10 (10%)</td>
<td>2/26 (7.7)</td>
</tr>
<tr>
<td>Renal impairment</td>
<td>2/10 (20)</td>
<td>2/26 (7.7)</td>
</tr>
<tr>
<td>Coagulopathy</td>
<td>5/10 (50)</td>
<td>5/26 (19)</td>
</tr>
<tr>
<td><strong>Complications</strong></td>
<td>7/10 (70)</td>
<td>21/26 (81)</td>
</tr>
<tr>
<td>Adult respiratory distress syndrome</td>
<td>1/10 (10)</td>
<td>2/26 (7.7)</td>
</tr>
<tr>
<td>Amputation†</td>
<td>1/10 (10)</td>
<td>0/26 (0)</td>
</tr>
<tr>
<td>Fatal outcome</td>
<td>2/10 (20)</td>
<td>0/26 (0)</td>
</tr>
<tr>
<td>Skin graft</td>
<td>4/10 (30)</td>
<td>6/26 (23.1)</td>
</tr>
<tr>
<td>Multiple surgical procedures</td>
<td>7/10 (70)</td>
<td>21/26 (81)</td>
</tr>
</tbody>
</table>

*P < .05.
†Amputation of left ear in a 4.5-month-old male.
and 1996, but similar to the 2.5 cases per million reported in a study of invasive GAS in Alberta between 2000 and 2002. For both of these studies, the focus was not on NF (each had fewer than 10 pediatric NF cases), but on invasive GAS infections. As reported previously for other invasive GAS diseases, males and younger children had the highest incidence. Although morbidity, defined as the presence of complications, was similar for both non–GAS-related and GAS-related cases, GAS-related NF was associated with a lower case fatality rate (0% vs 20%; \(P = .07\)).

NF typically occurs in the presence of disruptions to the skin, respiratory tract, or perineal region or genital tract. Our study confirmed that in children, more than 50% of non–GAS-related NF occurs in individuals with an underlying medical condition, such as diabetes mellitus, trauma, or recent surgery. In contrast, few children with GAS-related NF (11%) have an underlying medical condition (except for recent varicella). The prevalence of an underlying condition in GAS-related NF is similar to the estimate of 16% previously noted for pediatric invasive GAS infections in general.

Non–GAS-related NF occurs most frequently in infants under age 1 year. All of the infants in our study had either an existing risk factor (eg, prematurity) or an underlying chronic condition that likely increased their susceptibility to the infection. In the under-1-year group, non–GAS-related NF involving the abdomen occurs most commonly in association with omphalitis. The majority of GAS–related cases (~60%) had a preceding varicella infection, a finding consistent with other studies showing an association with invasive GAS disease.

This information will be useful for monitoring the impact of Canadian varicella vaccine programs on NF. Patel et al found that the incidence of varicella-associated invasive GAS hospitalizations in Chicago decreased from 27% in the prevaccination era (1993 to 1995) to as low as 2% during a period of widespread vaccine use between 1999 and 2001. Varicella infection is postulated to lead to a breakdown in the protective barrier in the skin, oral mucosa, or respiratory tract, thereby increasing the susceptibility to infection from GAS. Furthermore, a predominance of TNF-\(\alpha\)-type response in association with a varicella infection may lead to a relative decrease in humoral immune response and predispose to bacterial infections.

Although varicella vaccine was licensed in Canada in 1998, at the onset of our study in January 2003, only 3 of 10 provinces (Alberta, Nova Scotia, and Prince Edward Island,) and 2 of the 3 territories (Northwest Territories and Nunavut) had fully implemented a publicly funded immunization program. Since 2004, most Canadian provinces and territories have initiated programs for catch-up immunization of high-risk patients and universal varicella vaccination at age 12 months. Our study will enable further evaluation of the impact of these new vaccination programs.

In this study, at least 50% the patients with GAS-associated NF had taken a NSAID within 1 week before presentation. NSAIDs impair granulocyte functions, including chemotaxis, phagocytosis, and bactericidal activity. Some investigators have hypothesized that NSAI increase the risk of GAS–related NF, especially in children with varicella. Although 1 case-control study found an association between ibuprofen use and GAS–related NF, 5 subsequent cohort studies have not confirmed such an association. However, only 1 of these studies was specifically designed to test the hypothesis that NSAIDs increase the risk of severe invasive GAS disease and NF; the association with NSAIDs in that study was thought to be due to confounding by indication. It is unclear whether NSAIDs contribute to GAS–related NF or lead to a spurious association due to their use for pain and fever control in the early phases of the illness. Nonetheless, clinicians managing patients at risk for or with suspected NF or invasive GAS disease should prescribe NSAIDs cautiously, especially in children with varicella.

In the present study, all patients were treated with penicillin or a beta-lactam antibiotic, and 83% were treated with clindamycin. GAS remains universally sensitive to penicillin. However, concerns about the clinical failure rates with penicillin despite microbiologic sensitivity in other GAS infections, findings of improved outcomes in animal models, and clinical studies have led to the frequent addition of clindamycin to antibiotic regimens. The only multicenter controlled trial of IGIV as an adjunctive therapy for streptococcal toxic shock syndrome was terminated after about 20% of the planned enrollment had occurred, due to slow enrollment. Yet in another study, 7 adult patients with NF in Ontario were treated with high-dose IGIV in conjunction with antibiotics and needed only minimally invasive procedures or no surgery. In 1 patient who underwent serial biopsies, investigators detected a quantitative decline in GAS, superantigen, and cytokine levels 66 hours after a high dose of IGIV. A potential limitation of our study is the reliance on reporting from physicians in the CPSP, which may lead to an underestimation of the real incidence. However, the provision of active monthly surveillance reminders to all participating physicians reduces the likelihood of missed cases and helps tracks participation rates. The participation rate in this CPSP study was >80%, and the 7 provinces from which cases were reported represent 92% of the total population of Canada. Using a 2-tiered data collection system improved the completeness of data collection. In addition, the incidence rates that we found were similar to rates reported in studies using other methodologies.

Previous reports on NF in the pediatric population have been case reports or series or have focused on NF as a component of invasive GAS disease, with only limited characterization of the risk factors, clinical presentation, and outcomes of NF. One strength of surveillance tools such
as the CPSP is that they promote better understanding of the epidemiology of rare diseases such as NF. Our findings serve as baseline population data for rates, clinical presentation, and outcomes as varicella immunization uptake expands in Canada.

We thank all of the pediatricians in Canada who diligently report cases to the Canadian pediatric surveillance network.

REFERENCES


Figure 2. NF incidence rate per million population by sex. ■ Male, □ Female.

Table III. Source of pathogens in NF classified by type

<table>
<thead>
<tr>
<th>Site</th>
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<tr>
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<td>%</td>
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*P < .05.
Diagnostic Sweat Testing: The Cystic Fibrosis Foundation Guidelines

VICKY A. LEGRYS, DRA, JAMES R. YANKASKAS, MD, LYNN M. QUITTELL, MD, BRUCE C. MARSHALL, MD, AND PETER J. MOGAYZEL, JR, MD, PHD

The Cystic Fibrosis Foundation (CFF) accredits cystic fibrosis (CF) centers, located in teaching and community hospitals nationwide, which provide comprehensive diagnosis and treatment for people with CF. The CF centers are evaluated by the CFF Center Committee according to specific criteria covering the areas of clinical care, teaching, and research. There are specific requirements for sweat testing, and adherence to them is required for accreditation. In 2006, the CFF Center Committee distributed a sweat testing guidelines memorandum to the CF center directors. Although the guidelines are based on the Clinical Laboratory Standards Institute (CLSI), formerly National Committee for Clinical Laboratory Standards, sweat testing document C34-A2 and the College of American Pathologists (CAP) Laboratory Accreditation Program Inspection Checklist items for sweat testing, they are more prescriptive for uniformity and are focused on diagnostic rather than screening sweat tests. The guidelines are applicable to patients of all ages undergoing sweat chloride testing.

Adherence to the guidelines is mandatory for CFF centers; however, the requirements are appropriate and adaptable for any facility performing diagnostic testing for CF. Although it may be ideal for sweat testing to be centralized at CF centers, in practice this does not occur. According to enrollment in a national proficiency testing program for sweat analysis, more than 600 laboratories performed sweat testing in 2006. With widespread implementation of newborn screening programs for CF, the reliance on a well-performed and well-interpreted sweat test is critical to the success of accurately diagnosing CF. Sweat chloride testing should be performed on all infants with a positive newborn screen even in cases in which two CF-causing mutations have been identified.

The following represent the 2006 CFF sweat testing guidelines, along with commentary discussing the specific guidelines.

GUIDELINES AND COMMENTARY

Guideline 1

The laboratory must perform quantitative pilocarpine iontophoresis sweat chloride testing according to the procedures outlined in CLSI document C34-A2 without modification.

**Commentary.** A quantitative sweat test for diagnosis includes four steps described in detail in the CLSI C34-A2 document:

- Stimulation of sweat using pilocarpine iontophoresis
- Collection of sweat into gauze, filter paper, or Macroduct coils (Wescor, Logan, UT)
- Evaluation of the amount collected either in weight (milligrams) or volume (microliters)
- Measurement of the sweat chloride concentration. This process is described in Guideline 12

Measurement of sweat conductivity, for example, Sweat Chek or Nanoduct (Wescor, Logan, UT) is not acceptable for diagnosis.

Guideline 2

The laboratory must have access to a copy of the above-referenced CLSI Guidelines document C34-A2, either paper copy or through electronic file (www.clsi.org).

**Commentary.** Personnel performing the sweat collection and analysis should be knowledgeable about the contents of the CLSI document.
Guideline 3
The iontophoresis equipment must be battery powered and regularly inspected.

COMMENTARY. For safety reasons, the iontophoretic current source needs to be battery powered. Inspection for current control and leakage must be periodically performed by biomedical engineering according to the manufacturer’s recommendations.

Guideline 4
The minimum age for testing is 48 hours.

COMMENTARY. Sweat electrolytes may be transiently elevated during the first 24 hours of age. If after 48 hours of age, an adequate sweat sample can be obtained, sweat testing is appropriate.

Guideline 5
Only the arms or legs are to be used as collection sites. The iontophoresis current should not cross the heart.

COMMENTARY. Sweat is stimulated and collected from the patient’s lower arm or upper leg, from a site that is free from inflammation, rash, or cuts to avoid contamination of the sample with serous fluid or blood.

Guideline 6
Sweat must be collected on gauze or filter paper or in a Macroduct coil (Wescor, Logan, UT) after iontophoresis.

a. If gauze or filter paper collection is used, the stimulated area must be $2 \times 2$ inches (total area, 4 square inches). A slightly smaller electrode (eg, $1\frac{1}{2} \times 1\frac{1}{2}$ inches) is used for iontophoresis. Other electrode sizes are permissible if they cover greater than 50% of the $2 \times 2$ inch area (ie, an area of greater than 2 square inches). The iontophoresis should be carried out using USP grade pilocarpine for 5 minutes. After stimulation, the sample must be collected from a single site, using $2 \times 2$ inch gauze or filter paper. The minimum sample weight using this method is 75 mg in 30 minutes.

b. If a Macroduct coil is used for collection, then sweat must be stimulated with a disposable Pilogel electrode using the Webster Sweat Inducer (Wescor, Logan, UT) for 5 minutes. After a 30-minute collection, the minimum acceptable sample is 15 μL.

COMMENTARY. Adherence to a minimum sweat weight or volume from a single site is critical to obtain valid sweat testing results. The requirement for a minimum amount is to ensure an appropriate sweat rate and sweat electrolyte concentration. Sweat electrolyte concentration is related to sweat rate. At low sweat rates, sweat-electrolyte concentration decreases, and the opportunity for sample evaporation increases.

Guideline 7
Sweat must be collected for no more than 30 minutes.

COMMENTARY. If the collection time exceeds 30 minutes, the requirement for the amount of sweat needed to ensure adequate stimulation must increase. Extending the collection time can allow additional opportunity for sweat evaporation and practically does not increase the sweat yield significantly.

Guideline 8
The incidence of insufficient samples (ie, quantity not sufficient, or QNS samples) must be investigated and resolved if it exceeds 5% for patients older than 3 months of age.

COMMENTARY. Achieving a QNS rate below 5% for patients older than 3 months of age should not be a problem if the procedure in the CLSI document and the manufacturer’s recommendations are followed. Factors influencing sweat collection include age, weight, race, skin condition, and collection system. For example, infants weighing less than 2000 grams, younger than 38 weeks age at testing, or of African-American race have an increased likelihood of producing an insufficient sample. Higher failure rates with the Macroduct coil compared with gauze collection have been reported. The calculation of a QNS rate is based on the percentage of tests where an adequate sweat sample is not obtained. If a bilateral (duplicate) sweat collection is performed, then the test is considered QNS only if an adequate sweat sample is not obtained from either site. For example, in an institution performing bilateral testing, a patient initially yields inadequate sweat samples on both sites (100% QNS). The same patient returns 1 week later and yields an adequate sample on one site and an inadequate sample on the other site (0% QNS). For this example, the overall QNS rate would be 50%.

Guideline 9
It is recommended that the collection and analysis be performed in duplicate.

COMMENTARY. Duplicate testing is recommended but not required as one mechanism for quality assurance. It should be noted that for diagnosis, duplicate testing done on the same day does not represent independent repeat testing.

Guideline 10
Insufficient samples should not be analyzed and must not be pooled for analysis.
**Guideline 11**

Collection and analytical procedures must be designed to minimize evaporation and/or contamination. For specific techniques, refer to CLSI document C34-A2, Sections 8.1.3.1 and 8.1.4.

**COMMENTARY.** Sweat collected in gauze, once reweighed, can be stored with or without diluent in a tightly sealed container for up to 3 days at refrigerator temperature. Studies concerning the stability of sweat stored in Macroduct coils have not been published; therefore, laboratories should validate storage conditions.

**Guideline 12**

Sweat must be quantitatively analyzed for chloride by one of the following methods:

a. Chloride by coulometric titration, using a chloridometer
b. Chloride by a manual titration, using the Schales and Schales mercuric nitrate procedure
c. Chloride by automated analyzers, using ion-selective electrodes that have been systematically validated against the methods described in a or b, above.

Analytical methods requiring the addition of extraneous chloride standard to patient samples to increase the analytical sensitivity should not be used.

**COMMENTARY.** Automated analyzers designed for quantifying serum chloride may lack the sensitivity needed for sweat analysis; therefore, validation studies using specimens with low chloride concentration (eg, 10 mmol/L) must be performed before use. It should be noted that automated analyzers using ion-selective electrodes for sweat chloride are different from the in situ or direct reading chloride electrode applied to the patient's skin.

**Guideline 13**

Perform and evaluate quality control with every sweat analysis run, using two levels of controls per the Clinical Laboratory Improvement Act of 1988 (CLIA, 1988).10,11

**COMMENTARY.** A positive and negative control should be assayed with each patient run. If sweat is collected on gauze or filter paper, the control material should be applied directly to the collection surface and eluted for analysis.

**Guideline 14**

It is recommended that the sweat test be included in the laboratory's overall evaluation of CQI (continuous quality improvement).

**COMMENTARY.** The CQI evaluation should include the annual percentage of QNS samples, borderline and positive results, and adverse skin reactions. These variables should be reviewed by the CF Center Director and the laboratory director.

**Guideline 15**

Sweat samples must be appropriately labeled for patient identification throughout sweat collection and analysis. Reagents must be appropriately labeled.

**Guideline 16**

Appropriate reference values for sweat chloride must be used: <40 mmol/L = negative; 40 to 60 mmol/L = borderline/indeterminate; >60 mmol/L = consistent with the diagnosis of CF.

Note: Sweat chloride values <40 mmol/L have been documented in genetically proven CF patients. Clinical correlation is necessary.12

**COMMENTARY.** Results from sweat testing performed in infants suggest that sweat chloride values greater than 30 mmol/L should be considered abnormal, requiring further patient evaluation.5,13-17

**Guideline 17**

The lower limit of detection should be determined by the laboratory and should be ≤10 mmol/L. The upper end of reportable results should be no more than 160 mmol/L.

**COMMENTARY.** The analytical method should be able to accurately measure sweat chloride at the mean normal concentration (around 10 mmol/L).18 Sweat concentrations below the lower limit of detection should be reported as "less than," for example, "<10 mmol/L." Although analytical instruments may have an upper measurement range of >160 mmol/L, concentrations above this are not physiologically possible and should not be reported.19 A patient with a sweat chloride of >160 mmol/L should be retested, as contamination or technical error is likely.

**Guideline 18**

All laboratories must document successful performance in the CAP proficiency testing survey for sweat test analysis.

**COMMENTARY.** In the sweat testing proficiency testing program, the CAP prepares three specimens that are mailed to laboratories twice a year. Laboratories are provided with feed-
Patients with borderline sweat tests should be monitored for respiratory problems and nutritional status. If the repeated tests remain borderline, ancillary testing such as genotyping, assessment of pancreatic function, respiratory tract microbiology, and urogenital evaluation may be used for CF screening, repeat sweat chloride testing is often required for diagnostic confirmation. Sweat chloride tests should also be repeated in patients with confirmed CF who do not follow the expected clinical course. Sweat chloride tests must be confirmed by repeating it at a different time or another diagnostic test for CF.

All positive tests must be confirmed with a repeat sweat chloride test at a different time or another diagnostic test for CF.

For diagnosis, a positive sweat chloride test must be confirmed by repeating it at a different time or confirmed by identification of two cystic fibrosis transmembrane conductance regulator gene (CFTR) mutations known to cause CF, or abnormal electrophysiological studies of nasal epithelium. All borderline sweat test results should be repeated. It is suggested that borderline sweat tests in patients identified by newborn screening be repeated within 1 to 2 months. If the repeated tests remain borderline, ancillary tests such as genotyping, assessment of pancreatic function, respiratory tract microbiology, and urogenital evaluation may be helpful. Patients with borderline sweat tests should be monitored for respiratory problems and nutritional status. Sweat chloride tests should also be repeated in patients with confirmed CF who do not follow the expected clinical course.

CFTR mutation analysis can be performed as a confirmation of an abnormal sweat test result. However, because many mutations are not detected by typical mutation panels used for CF screening, repeat sweat chloride testing is often required for diagnostic confirmation.

Sweat testing must be available at least 2 days per week. Wait time for scheduling routine tests should be less than 2 weeks.

Sweat testing must be performed on a sufficient number of patients by a limited number of experienced, well-trained personnel who pass periodic documented competency testing. CLIA 1988 requires that new employees demonstrate competency every 6 months for the first year and annually thereafter.

Misdiagnosis of patients has been attributed to laboratories performing too few tests to maintain proficiency. However, the determination of what constitutes a "sufficient number" of sweat tests is subjective and not easily quantified. In not specifying the minimum number of sweat tests to be performed, the CFF has allowed each laboratory to determine the number of tests required for proficiency. The requirement that QNS rates be monitored and that the center director be involved in the review of sweat test results should ensure that laboratories are proficient at performing these tests.

It is not appropriate to perform the sweat test using:

- Direct application of a chloride electrode to the patient’s skin.
- Chloride precipitation reaction by placing a patch directly on the patient’s skin.
- Measuring only potassium or sodium.
- Osmolality.
- Conductivity including Sweat Chek or Nanoduct (Wescor, Logan, UT).
- Any other screening (nonquantitative) tests.

The above methods are not appropriate for diagnosis at CF centers; however, the CFF has approved the Wescor Macroduct Sweat-Chek conductivity analyzer for screening at clinical sites, such as community hospitals, using the criteria that an individual having a sweat conductivity ≥50 mmol/L should be referred to an accredited CF care center for a quantitative sweat chloride test.

Despite the availability of genetic testing, a quantitative pilocarpine iontophoresis sweat chloride test remains the gold standard for the diagnosis of CF. Therefore, appropriate performance of sweat tests is vital to the function of the CF center. This is especially true as newborn screening for CF becomes more widespread.

**REFERENCES**

Transmission of Panton-Valentine Leukocidin-Positive *Staphylococcus Aureus* between Patients with Cystic Fibrosis

**ARNON ELIZUR, MD, RACHEL C. ORSCHELN, MD, THOMAS W. FERKOL, MD, W. MICHAEL DUNNE, JR, PHD, GREGORY A. STORCH, MD, AND CAROLYN L. CANNON, MD**

Panton-Valentine leukocidin-producing *Staphylococcus aureus* is an emerging pathogen worldwide, causing necrotizing lung infections in otherwise healthy individuals. We describe 2 episodes of patient-to-patient transmission of Panton-Valentine leukocidin-producing *S aureus*, resulting in acute, life-threatening pulmonary complications in patients with cystic fibrosis. Appropriate infection control measures may be warranted to prevent similar episodes. (*J Pediatr* 2007;151:90-2)

Staphylococcus aureus can chronically infect the lungs of patients with cystic fibrosis (CF), beginning early in life.\(^1\) Strains of *S aureus*, expressing the virulence factor Panton-Valentine leukocidin (PVL), are emerging worldwide.\(^2\) Composed of 2 distinct proteins that are secreted separately but act together, namely LukS-PV and LukF-PV, PVL is a pore-forming toxin that targets and lyses mononuclear and polymorphonuclear cells.\(^3\) PVL\(^+\) strains of both methicillin-sensitive *S aureus* (MSSA) and methicillin-resistant *S aureus* (MRSA) can cause rapidly progressive necrotizing pneumonias in otherwise healthy individuals.\(^4\) In this report, we describe 2 separate instances of PVL\(^+\) MSSA transmission between patients with CF leading to severe lung infections and acute respiratory failure in younger siblings.

**EPISODE 1**

In September, 2005, a 3-year-old girl with CF was admitted to the St. Louis Children’s Hospital (SLCH) Pediatric Intensive Care Unit with acute respiratory failure. The patient was generally well with only 1 admission for pulmonary exacerbation until 7 months before the current illness. She had chronic airway colonization with MSSA (PVL not tested), sensitive to penicillin and erythromycin, on the basis of the Kirby-Bauer disk diffusion method. During the 2 months preceding her admission, the patient was admitted twice, and an MSSA isolate, resistant to penicillin and erythromycin, was recovered (PVL not tested). The patient’s 11-year-old brother with CF had been admitted 9 months earlier with acute left lower lobe pneumonia. PVL\(^+\) MSSA resistant to penicillin and erythromycin was first isolated from the brother during that admission.

On presentation, the patient had tachypnea and hypoxemia. Her lungs had bibasilar crackles on auscultation. Chest imaging demonstrated a multilobar pneumonia (Figure, A). Initial laboratory findings included peripheral leukocytosis; blood culture results were negative. Antibiotic therapy, consisting of ticarcillin-clavulanate, tobramycin, and oxacillin, was initiated. The patient subsequently underwent bronchoscopy, and bronchoalveolar lavage yielded PVL\(^+\) MSSA, resistant to penicillin and erythromycin. All other microbiologic assays and cultures were negative. The patient’s respiratory status gradually improved. She was extubated after 15 days and completed a 4-week course of intravenous antibiotic therapy.

**EPISODE 2**

In May 2006, a 2-month-old male infant with CF presented to SLCH in asystole. The child had no previous respiratory illnesses. However, on the morning of admission, he was noted to have tachypnea and cyanosis. Transported to the SLCH Emergency Department, he was found to be in cardiorespiratory arrest, requiring resuscitation. His school-aged sister also has CF and had been found to have pulmonary colonization with CF.

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<table>
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<tr>
<th>CF</th>
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PVL\(^+\) MSSA the previous year. Chest radiographs of the baby demonstrated right middle lobe pneumonia. Arterial blood gas measurement showed hypoxemia and acute respiratory and metabolic acidosis (pH, 6.84, \(P_{CO_2}\), 93 mm Hg, and \(P_{O_2}\), 41 mm Hg; FiO2 1.0). The patient received mechanical ventilator support and intravenous vasopressors for suspected bacterial sepsis. Intravenous ticarcillin–clavulanate, gentamicin, and vancomycin were initiated. The patient’s clinical condition failed to improve, and repeat imaging studies showed necrotizing right middle and lower lobe pneumonia with associated pleural effusion (Figure, B). A thoracoscopy tube was placed, and exudative pleural fluid was evacuated that yielded PVL\(^+\) MSSA. No other organism was identified. Intravenous clindamycin was started, and the patient’s respiratory status improved. He was extubated on hospital day 21, and completed 4 weeks of antibiotic therapy.

**COMPARISON OF S AUREUS ISOLATES**

The presence of the \(lukF\)-PV gene was established with a polymerase chain reaction assay as previously described.\(^4\) Relatedness of \(S\) aureus isolates was examined with repetitive-sequence PCR, a method comparable to pulse field gel electrophoresis in \(S\) aureus typing.\(^5\) Isolates with \(\geq 95\%\) homology were considered related.

In both cases, the PVL\(^+\) \(S\) aureus isolated from the patients during their hospitalization and their siblings were highly homologous. Notably, \(S\) aureus isolates found in the first case and those recovered in the second case were unrelated (Figure, C).

**DISCUSSION**

We describe the apparent transmission of PVL\(^+\) MSSA between patients with CF leading to severe pulmonary infections and acute respiratory failure in 2 young children. Although MSSA is often among the earliest pathogens isolated from the CF lung,\(^1\) its impact on the progression of lung disease is controversial.\(^6\) Recently, highly virulent methicillin-sensitive and resistant strains of \(S\) aureus, expressing the virulence factor PVL, have emerged in association with aggressive, necrotizing infections.\(^2\) In some centers, these strains have been isolated from children more often than hospital-acquired strains.\(^7\) PVL\(^+\) MRSA infections in our CF population caused invasive lung infections, including lung abscesses, which prompted us to test for PVL expression in newly acquired strains of \(S\) aureus when clinically suspected.\(^4\) The pulmonary infections in the 2 young patients reported here are consistent with these observations.

Patient-to-caregiver and patient-to-patient transmission of PVL-expressing MRSA has been reported.\(^8,9\) In this report, we describe transmission of PVL\(^+\) \(S\) aureus between patients with CF. Because patients with CF are often chronically colonized with \(S\) aureus, transmission of a virulent PVL\(^+\) strain could occur at any time. This risk poses new challenges regarding infection control policy for MSSA given that PVL expression may predict enhanced virulence.

Contact isolation has effectively controlled nosocomial spread of MRSA.\(^10\) Similar infection control measures have been implemented at our center to prevent transmission of PVL-expressing MSSA (PVL testing is performed on the basis of clinical suspicion). The ability to detect PVL expression is currently limited to a few laboratories, and further study is required to determine whether PVL testing should be performed routinely.

Although we suspect that the PVL\(^+\) MSSA strains were transmitted between pairs of siblings with CF, the siblings presented may have acquired the organism from a shared source. The parents were well, and the fact that both siblings were not infected simultaneously suggests that the organism was acquired first by the older sibling and then transmitted to the younger, possibly more susceptible, child. Antibiotic susceptibility pattern suggests that in the case of the first sibling pair, transmission occurred within 2 months of initial infection.

In summary, we describe 2 cases of patient-to-patient transmission of PVL\(^+\) \(S\) aureus leading to life-threatening lung infections. Infection control measures may be necessary to prevent spread between patients with CF.

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50 Years Ago in The Journal of Pediatrics

A COMPARISON OF INTRAMUSCULAR AND ORAL BENZATHINE PENICILLIN G IN THE TREATMENT OF STREPTOCOCCAL INFECTIONS IN CHILDREN

Breese BB, Disney FA. J Pediatr 1957;51:157-63

The first patient treated successfully with penicillin was documented in 1942. By the 1950s, a single intramuscular (IM) dose of 600,000 U of benzathine penicillin G had been established to be highly effective in the treatment of β-hemolytic streptococcal infections in children. In a comparative study, the efficacy of the oral form of benzathine penicillin G was compared to its IM regimen, in what is now recognized as a non-blinded, randomized, prospective interventional trial. Children bacteriologically confirmed to have β-hemolytic streptococcal infections (n = 463) were assigned randomly to the IM regimen or to varying dose regimens of oral benzathine penicillin G. All cases were followed up for clinical response, and a repeat bacterial culture was obtained at 3 weeks. Clinical response with bacterial eradication was considered a cure, whereas failure included either recurrent streptococcal infection or throat carriage of β-hemolytic streptococcus at 3 weeks. The initial total oral dose given over an 8-day period was either 3,600,000 U in those <4 years old or 4,800,000 U in those aged ≥4 years. Interim analyses at 2-month intervals indicated statistically significant inferiority with the oral treatment arm that necessitated 2 further dose adjustments to 6,000,000 U and 8,000,000 U given over 10 days.

This study established the treatment duration of 10 days with oral penicillin in streptococcal throat infections. The oral form of benzathine penicillin is a poorly soluble salt of benzylpenicillin and achieves very low plasma concentrations. Oral benzathine penicillin G was quickly replaced by phenoxyethylpenicillin (penicillin V), a chemically improved form that combines acid stability with immediate solubility and rapid absorption.

Most β-hemolytic infections in children are due to Group A streptococci. It is remarkable that even now the recommended drug of choice for such infections remains as penicillin (penicillin V, 800,000 U three times daily) for 10 days. Additionally, Group A streptococcus and other β-hemolytic streptococci remain exquisitely susceptible to penicillin. In terms of efficacy, subsequent studies and meta-analyses of randomized controlled trials in treatment of group A β-hemolytic streptococcal tonsillopharyngitis demonstrated bacteriologic and clinical cure rate significantly favoring cephalosporins compared with penicillin (OR, 1.83; 95% CI, 1.37-2.44, OR for clinical cure rate 2.29, 95% CI, 1.61-3.28, \( P < .00001 \)). However, in the era of concern for antimicrobial resistance, cephalosporins have a wider spectrum of antibacterial activity and are often more costly, thus precluding their routine use in Group A streptococcal infections.

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10.1016/j.jpeds.2007.02.007

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Burkitt’s Lymphoma in a Patient with Adenosine Deaminase Deficiency-Severe Combined Immunodeficiency Treated with Polyethylene Glycol-Adenosine Deaminase

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We describe a patient with severe combined immunodeficiency because of aberrations in adenosine deaminase (ADA) who despite adequate replacement with polyethylene glycol-linked ADA (PEG-ADA) for 13 years developed Burkitt’s lymphoma. Although treatment corrected the metabolic abnormalities caused by ADA deficiency, it failed to fully restore cellular immunity. (J Pediatr 2007;151:93-5)

Inherited defects in adenosine deaminase (ADA) activity result in the accumulation of enzyme substrates, such as adenosine and 2’-deoxy-adenosine, which are toxic to T and B lymphocytes. The increased 2’-deoxy-adenosine also inactivates the enzyme S-adenosylhomocysteine hydrolase. The result is profound lymphopenia and increased susceptibility to infections. Hematopoietic stem-cell transplant from a human leukocyte antigen (HLA)-identical sibling remains the treatment of choice for these patients.1 In the absence of such a donor, ADA enzyme replacement was proposed. The use of polyethylene glycol-linked (PEG)-ADA injections results in rapid and significant improvement of the metabolic abnormalities followed by an increase in the number of circulating B and T lymphocytes.1 However, recapitulation of full immune function is not universal.2,3 Few patients have been treated with PEG-ADA for more than 10 years, and the clinical significance of the imperfect reconstitution of the immune system remains unclear.

METHODS

ADA activity and metabolites were assessed as described previously.4 Serum immunoglobulin levels and antibody production were measured using standard methods.5 Lymphocyte cell surface markers and function were assessed as previously reported.6 T-cell receptor variable β-chain expression and measurement of T-lymphocyte receptor excision circles were performed as previously described.6,7

RESULTS

Patient Description

The patient is a 15-year-old female of Somali descent born after a normal pregnancy and delivery to nonconsanguineous parents. She presented with Staphylococcus aureus arthritis of her shoulder at 19 days of age. At 3.5 months she had Listeria monocytogenes meningitis and septicemia. In addition, she suffered from vomiting, diarrhea, and failure to thrive. At 5 months of age, she was diagnosed with staphylococcal pneumonia and septicemia. The patient also was found to have developmental delay and hearing and vision impairment.

Immune Function at Presentation

At presentation, the patient had severe lymphopenia (120 cells/mm³). Flow cytometry analysis of peripheral lymphocytes revealed a markedly reduced number of CD4⁺ T lymphocytes and B lymphocytes (Table). Proliferative responses to phytohemagglutinin

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<tr>
<th>ADA</th>
<th>Adenosine deaminase</th>
<th>PEG-ADA</th>
<th>Polyethylene glycol-linked adenosine deaminase</th>
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also were severely depressed. Levels of serum IgG were extremely low, IgA and IgM were undetectable, and antibody responses to vaccination such as tetanus toxoid were below normal.

Enzymatic Activity and Molecular Analysis

Analysis of the patient's erythrocytes showed undetectable ADA enzyme activity, extremely elevated deoxy-adenosine nucleotides, and reduced S-adenosyl-homocysteine hydrolase activity, consistent with the diagnosis of ADA deficiency. ADA mRNA levels were reduced and sequence analysis of genomic DNA revealed a homozygous Q3X mutation in the ADA gene.

PEG-ADA Treatment

Because no suitable donor could be found, PEG-ADA was initiated at 11 months of age at 30 U/kg intramuscularly twice weekly. Rapid improvement of the metabolic abnormalities was seen, and within 4 months, deoxy-adenosine nucleotides and S-adenosyl-homocysteine hydrolase activity had normalized. Repeated measurements of ADA activity in whole blood throughout the treatment were at least 70% to 80% of normal. Erythrocyte deoxy-adenosine nucleotides measured 15 nmol/mL compared with >300 nmol/mL in patients with ADA-deficiency, confirming continuous activity of PEG-ADA and adequate metabolite clearance.

Effect of PEG-ADA on Immune Function

B lymphocyte numbers remained below normal throughout PEG-ADA treatment, however, IgG, IgA, and IgM levels normalized (Table). This coincided with adequate specific antibody response to tetanus toxoid and poliovirus vaccination. CD4+ and CD8+ T lymphocytes increased to 226 and 247 cells/mm³, respectively, after 10 months of PEG-ADA administration. During the following 13 years of PEG-ADA administration, CD4+ and CD8+ T lymphocytes remained lower than normal controls. Lymphocyte response to phytohemagglutinin mitogen improved initially but remained lower than normal controls. After 12 years of treatment, severely reduced T-lymphocyte receptor excision circles (3 copies/100 ng DNA) were found. However, analysis of T-lymphocyte receptor variable lambda segments showed full representation of all 24 tested families, which were comparable with normal controls (data not shown).

Table. The patient's immune function before and after PEG-ADA treatment

<table>
<thead>
<tr>
<th>Age (Years)</th>
<th>IgG (mg/dL)</th>
<th>IgA (mg/dL)</th>
<th>IgM (mg/dL)</th>
<th>CD4+ (Cells/mm³)</th>
<th>CD8+ (Cells/mm³)</th>
<th>CD19/20+ (Cells/mm³)</th>
<th>CD16/56+ (Cells/mm³)</th>
<th>Mitogen response to phytohemagglutinin</th>
</tr>
</thead>
<tbody>
<tr>
<td>&gt;1‡</td>
<td>60</td>
<td>&lt;7</td>
<td>&lt;7</td>
<td>11/1400</td>
<td>2/500</td>
<td>5/610</td>
<td>NA</td>
<td>4/127†</td>
</tr>
<tr>
<td>2-3</td>
<td>370</td>
<td>150</td>
<td>60</td>
<td>226/1300</td>
<td>247/620</td>
<td>280/720</td>
<td>250/180</td>
<td>12/60‡</td>
</tr>
<tr>
<td>4-5</td>
<td>630</td>
<td>100</td>
<td>90</td>
<td>446/700</td>
<td>128/490</td>
<td>54/390</td>
<td>32/130</td>
<td>6/60‡</td>
</tr>
<tr>
<td>6-7</td>
<td>1040</td>
<td>110</td>
<td>70</td>
<td>84/650</td>
<td>17/370</td>
<td>150/270</td>
<td>242/100</td>
<td>3/111†</td>
</tr>
<tr>
<td>8-9</td>
<td>830</td>
<td>100</td>
<td>130</td>
<td>37/650</td>
<td>4/370</td>
<td>155/270</td>
<td>185/100</td>
<td>2/32†</td>
</tr>
<tr>
<td>10-11</td>
<td>1410</td>
<td>140</td>
<td>90</td>
<td>177/650</td>
<td>26/370</td>
<td>82/270</td>
<td>172/100</td>
<td>56/861†</td>
</tr>
<tr>
<td>12-14</td>
<td>1560</td>
<td>160</td>
<td>100</td>
<td>34/530</td>
<td>8/330</td>
<td>66/110</td>
<td>188/70</td>
<td>4/190†</td>
</tr>
</tbody>
</table>

*Patient/lower 10th percentile in normal age matched children.
†Mitogenic response are expressed as stimulation index.
‡Before PEG-ADA therapy.
At 14 years of age, she began complaining of right hip pain and limping. Serum lactate dehydrogenase level was elevated. Computed tomography and gallium scan suggested a single lesion in the right hip without involvement of other sites. Magnetic resonance imaging showed bony destruction of the right iliac wing, acetabulum, and ischium. Consequently, a bone biopsy was performed, which revealed cell morphology typical of Burkitt’s lymphoma. Tumor cells were positive for B-lymphocyte markers CD20 and CD79a, and showed aberrant co-expression of T-lymphocyte marker CD43. DNA analysis demonstrated B-lymphocyte clonality. In situ hybridization of the lymphoma cells for Epstein-Barr virus RNA was negative.

The patient tolerated chemotherapy well, with no organ toxicity, and remains in clinical remission 20 months after diagnosis with complete resolution of the hip lesions by magnetic resonance imaging.

**DISCUSSION**

We report a patient with ADA deficiency who received PEG-ADA for 13 years. Reduction of deoxy-adenosine nucleotides and rise of S-adenosylhomocysteine hydrolase concentration in the blood were evident rapidly after the initiation of treatment and were practically normalized 4 months later. The most significant clinical improvement following treatment was a sharp reduction in severe invasive bacterial infections. This was likely because of the complete recovery of the patient’s humoral immunity. In contrast, the recovery of T-lymphocyte function was limited, possibly contributing to the patient’s susceptibility to viral infections. Repeated evaluations of the immune system showed that the number as well as the in vitro function of T lymphocytes, which initially had improved somewhat, had gradually declined.

It remains unclear why PEG-ADA fails to promote a complete recovery of the immune system in some patients. It can be argued that although PEG-ADA might be effective in reducing the levels of purine metabolites in the blood, it may be less effective in removing toxic metabolites from highly sensitive thymocytes. Alternatively, the immune dysfunction could stem from the inability of PEG-ADA to enter the cells.

At 14 years of age, the patient developed extra-nodal Burkitt’s lymphoma of the right iliac–ischial bone. The morphologic and immunohistochemical features of her lymphoma met the diagnostic criteria of Burkitt’s lymphoma as defined by the 2001 World Health Organization lymphoma classification. Lymphoma has been reported in four other patients with ADA deficiency, including two who received prolonged PEG-ADA replacement therapy, one patient who received repeated red blood cell transfusions, and another patient after bone marrow transplantation. It is therefore plausible to assume that the lymphoma developed in our patient as a result of incomplete reconstitution of the immune system with PEG-ADA.

This case illustrates the challenge in the long-term management of patients with ADA-deficient severe combined immunodeficiency. PEG-ADA replacement may be ineffective in completely correcting the immune function and preventing the development of lymphoma in ADA deficiency.

REFERENCES

Who Provides Physicians with Advice over the Internet? A Study of a Pediatric Subspecialty Discussion Group

THOMAS R. WELCH, MD

Internet-based bulletin boards have become one method by which physicians access patient management advice from colleagues. The reliability of this advice has never been examined. “PEDNEPH,” a bulletin board/discussion group for pediatric nephrologists was studied over a 6-month period. One hundred thirteen responses were provided to 50 questions by 56 respondents. Thirty-one (27%) of these 113 responses were provided by respondents with a recent peer-reviewed publication pertinent to the question posted. The 8 most frequent providers of advice on this bulletin board included only 3 with current board certification in pediatric nephrology. These individuals had a wide range of citation frequencies; the most frequent provider of information had no indexed publications or citations. Participation as a respondent in this bulletin board does not ensure that an individual has formal qualification. (J Pediatr 2007;151:96-7)

The internet has clearly revolutionized the dissemination of medical information. The ability to search online databases has made it possible for physicians anywhere to obtain instant access to current, peer-reviewed material pertinent to a clinical problem.

Another model of internet use by health care professionals is the bulletin board or discussion group. In these systems, members of the group post questions relating to patient management issues for review by the rest of the group. Members then reply to these queries, with these replies often stimulating further commentary. Many of these replies provide very specific management advice.

Information obtained on the internet from sources such as Medline or the Cochrane database has been subject to the typical peer review process inherent in scholarly publication. On the other hand, information provided in discussion groups is obviously unreviewed. Furthermore, physicians posting queries to such groups may know little about the background or qualifications of respondents.

A recent study1 examined such a discussion group: Physicians Online. The study addressed demographic features of the board participants, not looking into the academic background of respondents. In this study, I monitored another physician discussion group and used other online databases to determine additional information about the providers of responses in the group.

METHODS

Databases

“PEDNEPH” is a pediatric nephrology discussion group (PEDNEPH@listhost.uchicago.edu). The online Science Citation Index is a subscription-only service of the Institute for Scientific Information (isinet.com). Medline is the National Library of Medicine database, accessed in a variety of ways such as through PubMed (www4.ncbi.nlm.nih.gov/pubmed/). The American Board of Pediatrics (abp.org) maintains an online database of current subspecialty certification. The American Board of Internal Medicine maintains a similar database of certification in internal medicine (http://www.abim.org/who/index.shtm). Although some of these databases require a subscription for access, all are in the public domain.

Search Strategy

All original postings to PEDNEPH over a 6-month period were reviewed. Those that did not pose questions involving patient management (eg, postings of job openings, meeting announcements) were excluded.

Next, all the replies to these patient management postings were reviewed. A 10-year Medline search was conducted for each respondent, and the titles and abstracts of all publications were examined. A determination was made as to whether the respondent had a Medline-indexed publication over the 10-year period that was pertinent to the question

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0022-3476/$ - see front matter
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posed. “Pertinent” was interpreted very broadly. For example, any publication dealing with kidney transplantation would be considered pertinent to a specific question about dosing immunosuppression in a transplant recipient.

After the total numbers of responses were enumerated, the 8 respondents with the greatest number of total responses were examined in more detail. The individuals accounted for 50% of the total responses during the study period. The American Board of Pediatrics site was queried to determine whether they had current pediatric nephrology board certification. If this were not the case, the American Board of Internal Medicine site was queried to determine whether they had certification by that entity. The Science Citation Index was used to determine the total number of published citations to their work occurring over the past 3 years.

**RESULTS**

Of the 72 patient management questions posed during the period of study, 50 received at least 1 response and form the basis of this report. There were a total of 113 responses to these 50 questions by 56 respondents. Of these 113 responses, 31 (27%) were provided by respondents with at least 1 publication to their work occurring over the past 3 years. Interestingly, the most frequent provider of patient management suggestions (Respondent 8) was not board certified and had no indexed publications or citations.

**DISCUSSION**

There are a wide variety of ways in which the Internet has become involved in health care. The public can access a nearly limitless number of sites and discussion groups that provide unreviewed advice. On the other hand, there are more sophisticated sites through which consumers are able to request information from a physician–moderated website. Toub and Dunkel, for example, described a “virtual physician office” to which women could post obstetric- or gynecologic-related questions. The authors considered such interactions to be an “extension” of the in-person consultation. Many state medical boards and subspecialty boards have open public access to their licensure and certification databases.

Medical students are exposed to a wide variety of internet-based resources, ranging from course information to databases. Management of such information is becoming an important part of the contemporary medical school curriculum.

Practicing physicians, similarly, can access a variety of resources to aid in the management of specific clinical problems. The ability to retrieve such information, even including hard copies of journal articles, from remote sites has nearly eliminated geographic barriers to the availability of this information.

Discussion groups and bulletin boards have grown in popularity along with these other resources. Although these have the advantage of bringing specific clinical problems to the attention of a widespread audience, participation in such programs is generally open to all. Consequently, the casual user has no immediate way to ascertain that the provider of information has any particular expertise.

“Expertise” is a difficult quality to define with precision. In academic medicine, one’s peer-reviewed publications and citations to such publications are frequently used as objective measures of expertise. Certification by a recognized specialty board is another widely acknowledged criterion of expertise. Because this was a bulletin board focused on pediatric nephrology, only board certification in nephrology was specifically queried. In fact, one of the frequent responders was a transplant surgeon, who obviously would be considered “expert” in the area of kidney transplantation in spite of the absence of nephrology board certification. Nonetheless, this highlights the fact that the casual user of such a bulletin board has no real information pertaining to the background of the respondents.

This small study suggests that participation as a respondent in this pediatric nephrology bulletin board is no guarantee of recognized expertise. Although the advice provided may well be appropriate, users of such services must exercise caution not to consider such opinions as necessarily “expert” or evidence-based.

**REFERENCES**

Fetal Hemophagocytic Lymphohistiocytosis in a Premature Infant

Hemophagocytic lymphohistiocytosis (HLH) is a rare disease resulting from abnormal proliferation of histiocytes in tissues and organs. Hydrops, hepatosplenomegaly, thrombocytopenia, and fetal distress are manifestations of fetal HLH. We encountered a case of fetal HLH in a premature infant with oligohydramnios, placental hemophagocytosis, fetal ascites, thrombocytopenia, and hypoproteinemia.

The mother was a 29-year-old woman with a birth history of gravida 1 para 1. Fetal MRI showed oligohydramnios and fetal ascites at 30 weeks' gestation (Figure, A). Maternal screening tests for toxoplasma, rubella, cytomegalovirus, herpes simplex virus, human immunodeficiency virus, and human parvovirus were normal. A male infant was born at 31 weeks' gestation with a birth weight of 1990 g. Apgar scores were 2 and 5 at 1 and 5 minutes. He had respiratory distress and required mechanical ventilation. Thoracoabdominal radiography showed pulmonary hypoplasia and abdominal distension (Figure, B). Laboratory findings showed thrombocytopenia (platelet count: 8000/μL), hypoproteinemia (total protein: 1.8 g/dL), hyperferritinemia (2070 ng/mL), and a high level of soluble interleukin-2-receptor (2630 U/mL). Hemophagocytosis was found in his bone marrow aspirate (Figure, C) and within placental villous capillaries (Figure, D); thus, we diagnosed fetal HLH. Genetic tests for familial hemophagocytic lymphohistiocytosis including FHL2 (PRF1) and FHL3 (MUNC13-4) were normal. At 42 days of age, the infant died of multiple organ failure. Autopsy findings showed hemophagocytosis in the liver, spleen, lymph nodes, and bone marrow (Figure, E).

Fetal HLH with oligohydramnios has not been described previously. We speculate that fetal circulatory insufficiency due to hypoproteinemia led to the manifestations of oligohydramnios and fetal ascites. Our experience suggests that fetal HLH should be considered when oligohydramnios and fetal ascites are found perinatally and placental hemophagocytosis, thrombocytopenia, and hypoproteinemia are present at birth.

REFERENCES

Clinical Research Abstracts for Pediatricians


—John G. Frohna, MD, MPH

Bacterial meningitis score accurately predicts which children are at low risk


Question Given the widespread use of pneumococcal conjugate vaccine, does the Bacterial Meningitis Score predict which children are at very low risk of bacterial meningitis?

Context Children with cerebrospinal fluid (CSF) pleocytosis are routinely admitted to the hospital and treated with parenteral antibiotics, although few have bacterial meningitis.

Design Multicenter, retrospective cohort study. The authors previously developed a clinical prediction rule, the Bacterial Meningitis Score, based on five predictors: positive CSF Gram stain, CSF absolute neutrophil count (ANC) of at least 1,000 cells/µL, CSF protein of at least 80 mg/dL, peripheral blood ANC of at least 10,000 cells/µL, and a history of seizure before or at the time of presentation. In the current study, patients lacking any of these predictors are classified as very low risk.

Setting 20 US academic medical centers through the Pediatric Emergency Medicine Collaborative Research Committee of the American Academy of Pediatrics.

Participants All children aged 29 days to 19 years who presented to participating emergency departments between January 1, 2001, and June 30, 2004, with CSF pleocytosis (CSF white blood cells ≥10 cells/µL) and who had not received antibiotic treatment before lumbar puncture.

Outcome The sensitivity and negative predictive value of the Bacterial Meningitis Score.

Main Results Among 3295 patients with CSF pleocytosis, 121 (3.7%; 95% confidence interval [CI], 3.1%-4.4%) had bacterial meningitis and 3174 (96.3%; 95% CI, 95.5%-96.9%) had aseptic meningitis. Of the 1714 patients categorized as very low risk for bacterial meningitis by the Bacterial Meningitis Score, only 2 had bacterial meningitis (sensitivity, 98.3%; 95% CI, 94.2%-99.8%; negative predictive value, 99.9%; 95% CI, 99.6%-100%), and both were younger than 2 months old. A total of 2518 patients (80%) with aseptic meningitis were hospitalized.

Conclusions This large multicenter study validates the Bacterial Meningitis Score prediction rule in the era of conjugate pneumococcal vaccine as an accurate decision support tool. The risk of bacterial meningitis is very low (0.1%) in patients with none of the criteria. The Bacterial Meningitis Score may be helpful to guide clinical decision making for the management of children presenting to emergency departments with CSF pleocytosis.

Commentary Meningitis is the most feared infectious entity among febrile infants and children presenting to emergency departments. Studies of clinical diagnosis have not identified reliable criteria for distinguishing between bacterial and viral etiologies.1 The decline in incidence of bacterial meningitis with the advent of effective vaccines against S. pneumoniae and H. influenzae b has heightened the potential value of such criteria in avoiding unnecessary admissions and treatment. Nigrovic, et al’s validation of their previously derived prediction rule is a welcome adjunct to clinical decision making. Their new retrospective study involves an acceptable simplification of the original rule which required calculation of a point score.2 A prospective clinical validation, although desirable, would be problematic in the post-vaccine era. In addition, this limitation is substantially mitigated by the objective nature of the predictors, with seizure being the only non-laboratory parameter. The sensitivity for detecting bacterial meningitis is 98%, identical to that observed originally. Given the low likelihood of bacterial meningitis prior to application of the rule, a patient with none of the predictors should have well less than a 1% probability of a positive CSF culture. Clinicians may be advised to be judicious in applying
this rule to very young infants. In both studies, cases of missed bacterial meningitis were less than one year of age. Although Nigrovic, et al found a peripheral ANC ≥ 10³ to be an independent predictor of bacterial source, a study of febrile infants less than 90 days old found an inverse relationship between peripheral WBC count and likelihood of bacterial meningitis, suggesting that young infants’ physiological responses to CNS infection may differ. 3

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REFERENCES

School-based influenza vaccination program reduces influenza-related outcomes among household members


Question How effective is a school-based influenza vaccination program at decreasing the incidence of influenza-like illness?

Design Prospective study of households of elementary school students.

Setting Demographically similar clusters of elementary schools in four states.

Participants 11 clusters of schools. Intervention schools were assigned to participate in a vaccination program. 1-2 control schools were selected for each cluster.

Intervention During a predicted week of peak influenza activity in each state, all households with children in intervention and control schools were surveyed.

Outcomes Influenza vaccination rates and outcomes of influenza-like illness during the previous 7 days.

Main Results In all, 47% of students in intervention schools received live attenuated influenza vaccine (LAIV). As compared with control-school households, intervention-school households reported significantly fewer influenza-like symptoms and office visits during the recall week; rates of emergency department use did not vary between the two groups. Paradoxically, intervention-school households (both children and adults) had higher rates of hospitalization per 100 persons than did control-school households. However, there was no difference in the overall hospitalization rates for children or adults in households with vaccinated children, as compared with those with unvaccinated children, regardless of study-group assignment. Rates of school absenteeism for any cause (based on school records) were not significantly different between intervention and control schools.

Conclusions Most outcomes related to influenza-like illness were significantly lower in intervention school households than in control-school households.

Commentary Influenza vaccination rates among children have historically been low, especially among children with high-risk conditions. 2,3 King, et al illustrate the potential of a population-based program to reduce the burden of influenza-like illness among elementary school children; they do so through an influenza vaccination intervention focused solely on healthy children. Although the findings presented are encouraging, there are several important aspects to bear in mind while interpreting these results. This intervention was restricted to administration of FluMist (MedImmune) live attenuated influenza vaccine (LAIV). Since children with underlying medical conditions such as a chronic disease are not eligible for LAIV, they were excluded from receiving influenza vaccine through the intervention. 4 Additionally, this study did not assess the reasons why children chose not to be vaccinated through the school-based program, nor does it control for the degree to which students may have been vaccinated in other settings. These aspects of the study design confound interpretation of the findings presented, such as increased absenteeism among unvaccinated students – a group that presumably includes the entirety of the student population with underlying medical conditions. As a consequence, one cannot determine which characteristics of schools prior to the intervention were associated with the greatest incremental improvements in influenza-related outcomes. In addition, the influenza-like illness outcomes assessed by King et al are based on self-reported data from households, the accuracy of which may be questionable. While the analysis presented does provide an understanding of the overall effect of a school-based LAIV program, it falls short of illustrating the full potential that might be realized by a more comprehensive school-based effort that also includes the option of trivalent inactivated influenza vaccine (TIV). This is an important consideration given the prevalence of chronic conditions among children and the relative expense of LAIV, which is not covered by many insurance programs.

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REFERENCES

100 The Journal of Pediatrics • July 2007
Early placement of tympanostomy tubes does not improve developmental outcomes in normal children


Question Among children with persistent middle ear effusion, does prompt insertion of tympanostomy tubes improve developmental outcomes?

Design Prospective study.

Setting Children were recruited from two Pittsburgh area hospitals and six group practices.

Participants 6350 infants were enrolled soon after birth. 429 children were followed over the course of the study.

Intervention Before 3 years of age, 429 children with persistent effusion were randomly assigned to undergo the insertion of tympanostomy tubes either promptly or up to 9 months later if effusion persisted.

Outcomes Literacy, attention, social skills, and academic achievement at 9 to 11 years of age.

Main Results Mean (±SD) scores on 48 developmental measures in the group of children who were assigned to undergo early insertion of tympanostomy tubes did not differ significantly from the scores in the group that was assigned to undergo delayed insertion. These measures included the Passage Comprehension subtest of the Woodcock Reading Mastery Tests (mean score, 98±12 in the early-treatment group and 99±12 in the delayed-treatment group); the Spelling, Writing Samples, and Calculation subtests of the Woodcock-Johnson III Tests of Achievement (96±13 and 97±16; 104±14 and 105±15; and 99±13 and 99±13, respectively); and inattention ratings on visual and auditory continuous performance tests.

Conclusions In otherwise healthy young children who have persistent middle-ear effusion, as defined in this study, prompt insertion of tympanostomy tubes does not improve developmental outcomes up to 9 to 11 years of age.

Commentary Recognizing the deficiencies of prior retrospective studies, Paradise and colleagues designed a prospective study to determine if there is a causal relationship between early otitis media with effusion (OME) and later impairments of speech, language, and cognitive development. The study findings failed to identify an association between early otitis media and later impairments, even after 9-11 years. These new findings are important because they provide reassurance that developmental impairments that are not identified at an earlier age do not come into play later in the setting of greater academic challenges. When the analyses from the observational cohort at the follow-up periods were adjusted for demographic variables including socioeconomic status, there were either no significant correlations between effusion duration and outcome test scores or the effect size (percent of variance) related to the effusion duration was clinically meaningless. Since a hearing loss of 40 decibels or higher was quite uncommon among subjects in the study of Paradise et al, it could not address whether this level of hearing loss also leads to impairments. Likewise, the study does not address the question of whether ventilating tubes will help children with preexisting language or other developmental delays. The 2004 OME guideline recommends the following approach to persistent middle ear effusion: Determine during visits the laterality and duration of the effusion and the child’s non-otitis related risk for a speech, language or learning problem. In otherwise normal children, perform a hearing test when the effusion has been present for 3 months and continue with “watchful waiting” at 3 to 6 month intervals until the effusion clears. Refer for ventilating tubes when there is a bilateral hearing loss of 40 decibels or higher, a speech or language delay, or structural abnormalities of the eardrum develop.

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REFERENCE


Pulsed corticosteroids of no benefit in Kawasaki disease


Question Among children with acute Kawasaki disease, does the use of corticosteroids, in addition to standard therapy, decrease the risk of coronary artery abnormalities?

Design Double-blind, placebo-controlled, randomized trial.

Setting Eight centers in North America.

Participants 199 children with 10 or fewer days of fever.

Intervention Patients were randomly assigned to intravenous methylprednisolone, 30 mg per kilogram of body weight or placebo. All patients then received conventional therapy with intravenous immune globulin, 2 g per kilogram, as well as aspirin, 80 to 100 mg per kilogram per day until they were afebrile for 48 hours and 3 to 5 mg per kilogram per day thereafter.

Outcomes The primary outcome was coronary artery dimensions. Secondary outcomes included adverse events, days in the hospital, and days with fever.
Main Results At week 1 and week 5 after randomization, patients in the two study groups had similar coronary dimensions, expressed as z scores adjusted for body-surface area, absolute dimensions, and changes in dimensions. As compared with patients receiving placebo, patients receiving intravenous methylprednisolone had a somewhat shorter initial period of hospitalization \((P = 0.05)\) and, at week 1, a lower erythrocyte sedimentation rate \((P = 0.02)\) and a tendency toward a lower C-reactive protein level \((P = 0.07)\). However, the two groups had similar numbers of days spent in the hospital, numbers of days of fever, rates of treatment with intravenous immune globulin, and numbers of adverse events.

Conclusions The data do not provide support for the addition of a single pulsed dose of intravenous methylprednisolone to conventional intravenous immune globulin therapy for the routine primary treatment of children with Kawasaki disease.

Commentary The clinical trial reported by Newburger on behalf of the Pediatric Heart Network sets a new standard for clinical trials in Kawasaki disease (KD) that should be emulated by other researchers. Of the 3,528 references listed in Pub Med for “Kawasaki disease,” there are 104 studies reporting data on the use of steroids in acute KD. Despite these numerous publications, the controversy on the role of steroids in the treatment of acute KD has raged on for 40 years. The reason for this is simple: most published clinical trials in KD patients have failed to meet the requirements of adequate sample size, randomized multicenter study design, use of placebos, and blinded third party reading of all echocardiograms. While this optimal study design is expensive and complex, it is also the only way to definitively answer questions regarding optimal therapy. The days of underpowered, single-center clinical trials in KD should be over. The Newburger study now provides a long-awaited answer regarding the role of steroids in conjunction with intravenous gamma globulin in primary therapy of acute KD: there isn’t one. The lack of efficacy of steroids in mitigating coronary artery damage must be telling us something important about the nature of this vasculitis and how it differs from other vasculitides for which steroids are the mainstay of treatment. It is time to do “reverse translational medicine” and take this observation back to the laboratory to learn something fundamental about the immune response in acute KD.

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Intranasal influenza vaccine may be a safe, effective option for many children


Question Among children 6 to 59 months of age, is live attenuated influenza vaccine safe and effective?

Design Randomized, controlled trial.

Setting Physician’s offices and primary care clinics in 16 countries (United States, Europe, Middle East, Asia).

Participants Children 6 to 59 months of age.

Intervention Live attenuated (intranasal) influenza vaccine was compared with inactivated (intramuscular) influenza vaccine.

Outcomes Number of culture-confirmed cases of influenza-like illness, medically significant wheezing, and other serious adverse events.

Main Results Live attenuated vaccine was more effective than inactivated vaccine in preventing influenza \((153 vs 338 cases, p<0.001)\). The superior efficacy of live attenuated vaccine, as compared with inactivated vaccine, was observed for both antigenically well-matched and drifted viruses. Overall, there was no significant difference in medically significant wheezing between the two groups. However, several subgroups of children who had not previously received vaccine in the live attenuated group had more wheezing after the first dose. This trend was notable in previously unvaccinated children 6-11 months old \((3.8% vs 2.1%, p=0.076)\). With respect to hospitalization for any cause, rates were higher in recipients of live attenuated vaccine who were 6-11 months old \((6.1% vs 2.6%, p=0.002)\). There was a trend towards more hospitalizations in children 6-47 months old with a prior history of wheezing who received live attenuated vaccine. Conversely, in children 12-59 months old with no prior history of wheezing who received live attenuated vaccine, there was a trend towards fewer hospitalizations.

Conclusions In children over one year of age without a prior history of wheezing, live attenuated influenza vaccine was safe and more effective alternative to inactivated vaccine.

Commentary The option of an intranasal influenza vaccine is quite attractive in light of patient aversion to shots and the often limited availability of inactivated influenza vaccine doses. However, concern about the safety of live attenuated influenza vaccine has thus far prevented its widespread use in children. This multicenter study reports efficacy and safety data on a large number of children, including a subpopulation of children with a previous history of wheezing. The broad scope of this study allows it to address the concern over whether live attenuated vaccine is associated with wheezing in previously healthy children. The answer appears to be “no,” except perhaps in 6-11 month olds. However, it also reveals more questions about safety in children less than 12 months of age, and in children with a past history of wheezing. The overall incidence of adverse events was not significantly different between the two groups in this study; it was only in post-hoc secondary analysis that a few differences emerged. Further studies are needed to determine the true risk of live attenuated vaccine in children 6-11 months of age and children with a past history of wheezing. For those children 12-59 months of age without a past history of wheezing, this
study adds convincing efficacy and safety data in support of offering the live attenuated vaccine.

Should clinics stock the intranasal vaccine during the upcoming influenza season? In light of data from this study, live attenuated influenza vaccine would be a good option for children 12-59 months of age without a past history of wheezing. Perhaps the more practical answer to this question lies in whether insurance companies are willing to cover the intranasal form. Some parents may also be willing to pay for the intranasal vaccine in order to spare their child another intramuscular injection. The authors suggest that live attenuated vaccine may also be a reasonable option in children 6-11 months of age after they have received a first dose of inactivated vaccine, because increased wheezing in this age group was only seen after the first dose of live attenuated vaccine.

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50 Years Ago in The Journal of Pediatrics

CLINICAL AND PATHOLOGICAL OBSERVATIONS IN INFANTS WITH COARCTATION OF THE AORTA AND PATENT DUCTUS ARTERIOSUS

AND

THE DUCTUS ARTERIOSUS IN THE NEWBORN PERIOD
Mitchell SC. J Pediatr 1957;51:12-17

Fifty years ago, coarctation of the aorta was rarely diagnosed in the nursery. Thus, a few days after discharge, these infants presented in extremis with a history of poor feeding and marked decrease in urine output with metabolic acidosis. Initial physical examination was often not helpful. Chest radiography would reveal massive cardiomegaly. Cardiac catheterization was often performed, and the diagnosis of a coarctation of the aorta was established. Primary treatment was medical and included digoxin and diuretics, and, if the infant survived, surgical correction was performed months to years later. The mortality rate was high.

The diagnosis and management of coarctation of the aorta has changed immensely in the past 50 years. The article by Mitchell was one of the first to evaluate the timing of the ductal closure in the newborn. This research predated the use of cardiac ultrasonography. The article by Goldring et al acknowledged the difficulty in establishing the diagnosis of coarctation of the aorta. They report the use of “the flush technique” to measure the blood pressure in these very sick infants. The establishment of the diagnosis, however, did not markedly improve the ultimate outcome because surgery was not performed immediately.

Fifty years after the publication of these articles, newborns have their femoral pulses palpated and their blood pressures recorded by Doppler scanning. If there is any suspicion of coarctation, a cardiology consult is obtained, and cardiac ultrasound scanning is performed. The patency of the ductus arteriosus and the aortic arch is evaluated. If there is a coarctation of the aorta, prostaglandins are started immediately, reestablishing blood flow to the lower body. The metabolic acidosis is cleared, and urine output is reestablished.

The newborn is taken to the operating room, and, if the coarctation is a simple one, a complete repair is performed. In some centers, balloon angioplasty is the treatment of choice. If associated cardiac anomalies are present, they are dealt with soon after this initial intervention. Even newborns with complex cardiac anatomy do much better than infants with a simple coarctation of the aorta did 50 years ago.

There continues to be room for improvement, however. Prenatal diagnosis of a coarctation is now common, and in utero treatment is not far off. One can only speculate what the state of the art will be 50 years from now.

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10.1016/j.jpeds.2007.02.006
Neonatal hypoglycemia and occipital cerebral injury

To the Editor:

We read with interest the case series published in The Journal by Filan et al. The authors stated that there is no good explanation for the increased occipital lobe vulnerability in neonates with hypoglycemia. We recently had a neonate with hypoglycemia and occipital lobe lesions with characteristic MRA findings that may shed some light on the cause of increased occipital lobe vulnerability and hypoglycemia.

A 39-day-old boy having periods of unresponsiveness and shallow breathing was admitted. He was the product of an uncomplicated pregnancy and delivery. He had been admitted at 5 days of age because of a similar episode. At the time, a full septic workup revealed no infection, blood glucose of 4 mg/dL, and CSF glucose of 5 mg/dL; a CT of the brain showed bilateral occipital swelling. The low glucose blood level was corrected. An MRI of the brain done at 16 days of age showed bilateral occipital parenchymal loss more pronounced on the right than on the left. At 39 days of age, a repeat MRI showed similar findings and an MRA showed an anatomic variant of the circle of Willis (Figure).

We consider the possibility of a causal relation between anatomic variant of the circle of Willis and hypoglycemia-induced occipital lobe lesions because of their association in our patient. This consideration was reinforced by a finding that the side of the larger occipital infarct and the aberrant origin of the posterior cerebral artery was the same (see Figures). We searched the literature to see if any of the previously reported cases with occipital lobe infarcts and hypoglycemia had similar findings in the circle of Willis. We were unable to find any instance of MRA, CT angiogram, or conventional angiography in any of the previously reported cases.

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Andrea Rerecich, ARNP

Figure. A, Bilateral occipital cystic encephalomalacia more prominent on the right side. B, Hypoplastic right A1 portion of the anterior cerebral artery. C, Origin of the right posterior cerebral artery from the right internal carotid artery.
distinguish vasogenic edema from cytotoxic edema. In our se-
able interest in the ability of diffusion-weighted MR imaging to
hypoglycemic newborn infant is speculative. There is consider-
could initiate cerebrovascular autoregulatory dysfunction in the
cerebral injury and anatomic variants of the Circle of Willis.
Of course the question is whether the observed anatomy is
causative or an incidental finding, given that variation in Circle
of Willis anatomy is known to occur in up to one third of the
population. The significance of internal carotid artery origin of
the posterior cerebral artery is that internal carotid artery disease
may result in injury to posterior cerebral regions. In this infant,
the authors have not described MR evidence of middle cerebral
artery infarction. In addition, bilateral occipital injury is difficult
to reconcile with a unilateral anatomic variant. However, we have
been considering a vascular pathophysiology for the pattern of
injury we reported. The posterior distribution of cerebral injury
has anatomic characteristics similar to the syndrome of reversible
posterior leukoencephalopathy (RPLS). RPLS is believed to be
due to loss of cerebral autoregulation, vascular endothelial cell
dysfunction, and subsequent vasogenic edema. Although origi-
nally thought to be a reversible pathology, cytotoxic edema and
permanent brain injury can result. Neonatal hypoglycemia is
associated with increased cerebral blood flow. Whether this
could initiate cerebrovascular autoregulatory dysfunction in the
hypoglycemic newborn infant is speculative. There is consid-
erable interest in the ability of diffusion-weighted MR imaging to
distinguish vasogenic edema from cytotoxic edema. In our se-
ries, we did not observe increased water diffusion coefficients to
support vasogenic edema. However, diffusion measurements are
time-dependent, and the mean age at first MR scan was 4.5
days.

In conclusion, our opinion is that the anatomic variation
reported is more likely to be incidental. However, further
vascular studies in infants with hypoglycemia and occipital
injury would assist in delineating any role that such vascular
anatomy may have in this disorder.

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REFERENCE
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Reply

To the Editor:

We thank the authors for reporting their interesting
observation in this infant. We are also unaware of any previ-
ous reports of an association between neonatal occipital cere-
bral injury and anatomic variants of the Circle of Willis.

Of course the question is whether the observed anatomy is
causative or an incidental finding, given that variation in Circle
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Swaddling and excessive crying

To the Editor:

van Sleuwen et al1 state that swaddling is “yet another
new intervention without scientific proof of efficacy.” Swad-
dling is an ancient intervention. Although its ability to lessen
colicky crying (>3 hours per day) has not yet been proven, it
has been demonstrated to benefit both infant sleep and non-
colic crying.

The applicability of this study’s finding that swaddling
yielded only trivial relief to colicky infants is limited by two
design flaws: 1) swaddling was only used during sleep, 2)
other calming measures (eg, feeding) were actively discour-
aged.

Nighttime swaddling may improve infant sleep, but
to diminish colicky crying, the authors should have emulated
other studies and used it during episodes of irritability.4-7

Swaddling is just one of several known and effective
infant-soothing interventions. Noncolicky crying can be less-
ened by swaddling as well as by rocking, prone or upright
positioning, white noise, and sucking.8-14 Further, there is
evidence that multiple, simultaneous interventions (including
wrapping) have an additive calming effect.15 Brazelton and Nu-
gen10 observed that upset newborn infants often require a lay-
ering on of multiple soothing inputs (from arm restriction to
holding to rocking) to reduce agitation. An innovative multimodal
approach for colicky crying17,18 is currently under study.

Parents of fussy babies desperately need help. Over
recent years, evidence has mounted that infant crying can
cause significant morbidity and mortality (eg, nursing failure,
marital discord, postpartum depression, sudden infant death
syndrome, child abuse, excessive evaluation/treatment of gas-
troesophageal reflux, and disordered bonding).17 Overfeeding
in response to excessive infant crying has been suggested as a
risk factor in childhood obesity. The design flaws in this
particular study are significant, and physicians should not be
persuaded to eliminate swaddling from their lists of infant
soothing methods for excessive criers.

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Souza AS. Studying the evolution of reversible posterior leucoencephalopathy in chil-
DISCLOSURE
Dr Karp is the author of the book/DVD, The Happiest Baby on the Block.

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Reply
To the Editor:
We appreciate the comments regarding our article. Swaddling is indeed a very ancient practice. However, it is only recently being used as an intervention to soothe excessively crying infants, as we stated in our article.1 Our study is the first to try to establish evidence for its efficacy in this group of infants.

Dr. Karp states that our study design is seriously flawed. We strongly disagree. The study was designed to establish whether swaddling gave an added benefit to a pattern of stimulus reduction and regularity. We established that in fact it did not.1 The study design was well suited to the research question. Dr. Karp would have wanted us to ask a different question: namely whether swaddling during excessive crying is effective and whether swaddling as part of multiple interventions is effective. To answer these questions, the design is not suitable, but that was not our intention.

Is our study relevant, or would Dr. Karp’s research questions be more elucidating? The evidence for behavioral modification of baby care supporting regularity and stimulus reduction as an effective intervention for excessive crying was partly based on the study by Wolke et al,2 who proved behavioral management counseling to be more effective in reducing excessive crying than empathy or waiting for spontaneous remission. A somewhat similar approach with emphasis on regularity has been described by Hofacker et al.3 Furthermore, carrying an infant as a reaction to excessive crying has been shown to be ineffective in a randomized controlled trial by Barr.4

There was more evidence for this approach when conceiving the study than for applying multiple interventions to curb crying as Dr. Karp advocates. However, he states that a trial is currently being carried out, and we are of course very interested in the results. On the other hand, our study showed that excessive crying was reduced by 41.9% after the first intervention week and by 49.5% after 2 weeks of applying stimulus reduction and regularity, and 75% after 8 weeks.1 Furthermore, at follow-up of a small group at age 3 to 5 years, 87.3% of the parents (n = 197) experienced our behavioral management counseling as helpful.

The approach described in our study was easy to implement at well-care baby clinics. Therefore we believe our study is very applicable and clinically relevant to tackle this major problem.

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On the pathogenesis of recipient twin limb ischemia

To the Editor:

We read with great interest the article on recipient twin limb ischemia (RTLI). However, we are concerned about several statements, in particular those concerning the pathogenesis of RTLI.

Contrary to what is suggested in the report, perinatal limb ischemia in recipients with twin-to-twin transfusion syndrome (TTTS) has previously been recognized as a distinct entity. Several cases of RTLI have been reported, linking specifically recipient-status and limb ischemia.

Although the pathogenesis of RTLI remains to be elucidated, several authors suggest an association between polycythemia and RTLI. These findings are in contrast to the lack of association with polycythemia reported by Broadbent. Several factors may explain this discrepancy. First, as correctly suggested by the author, limb ischemia in the 3 reported cases was probably caused by umbilical artery catheterization rather than the fact that these were (non-polycythemic) recipients. Second, of the other 6 cases of postnatal RTLI reported in the literature, severe polycythemia was present in 5. In the sixth case, hemoglobin or hematocrit values were not mentioned. Severe polycythemia can cause thrombosis and limb ischemia. Because recipient twins may have polycythemia at birth, this pathogenetic link between RTLI and polycythemia should not be disregarded. Other mechanisms such as paradoxically elevated angiotensin levels in recipients may also cause peripheral vasoconstriction and ischemia.

Broadbent does not mention whether RTLI in TTTS is a rare phenomenon or not. In our experience, the prevalence of limb injury is extremely low. In a consecutive series of almost 200 cases of TTTS presented at our national fetal treatment center in recent years, we have encountered only 1 case of prenatal RTLI and no cases of postnatal RTLI. The treatment of choice for TTTS at our institution is fetoscopic laser surgery, whereas the 3 cases presented in the article were treated with amnioreduction. Amnioreduction is not a curative treatment and allows inter-twin blood transfusion to persist. Whether laser treatment reduces the prevalence of RTLI remains to be elucidated.

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Reply

To the Editor:

Lopriore et al comment on recipient twin limb ischemia (RTLI) as a distinct entity and discuss its incidence and etiology.

Fox et al emphasized the association of limb ischemia with recipient status in an article published between the final submission and publication of our article, and this was not picked up and included in our discussion. They and other authors should have their contribution acknowledged, as indicated by Lopriore et al. The question of whether recipient status is essential to the condition needs mention because of reports of 2 affected donors, a conundrum which could be explained by reversal of donor and recipient status that occurs in 5% of cases of twin-to-twin transfusion syndrome (TTTS).

Regarding incidence, Dickinson et al reported 3 cases of RTLI in 112 cases of TTTS, Mari et al reported 2 cases of RTLI in 223 cases of TTTS, and Cincotta et al reported 1 case of RTLI in 14 cases of TTTS. De Lia (who used laser therapy) reported 1 case of limb necrosis in 67 cases of TTTS. The overall risk in moderate to severe TTTS appears to be 1% to 3%. We hope the lower rate reported by Lopriore et al will be confirmed by other studies.

In antenatal-onset RTLI, hemoglobin levels at the onset of ischemia have not been reported. In 1 case, a fetal blood sample taken when ischemia was established showed a hemoglobin level of 170 g/L. We documented the hemoglobin or hematocrit level at onset of postnatal limb ischemia in 10 twins, 6 of whom were definite cases of RTLI; 2 of the 6 had severe polycythemia at onset, and 4 did not. There appears to be a heterogeneous etiology in which recipient twins are prone to limb ischemia in the variable presence of polycythemia, thrombophilia, vasoactive hormones, arterial catheters, and perhaps drugs. The risk of limb ischemia in recipient twins is not over when the hemoglobin level is in reference range.
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