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ABNORMAL PRODUCTION OF THE TUMOR NECROSIS FACTOR INHIBITOR ETHANERCEPT AND CLINICAL Efficacy of Tumor in a Patient with PAPA Syndome
The Editors’ Perspectives

New methods of sweat testing

Even with the advent of molecular techniques for making the diagnosis of cystic fibrosis (CF), clinicians will continue to need reliable sweat tests to confirm the diagnosis. An evaluation of a new conductivity system is reported in this issue of The Journal by Barben et al from the Children’s Hospital, St. Gallen, Switzerland. This system, the Nanoduct, can be used with samples of sweat as small as 3μL, and it is quick and easy to operate. In this initial study of 111 subjects, the new method performed well compared to the Macroduct sweat collection system with measurement of sweat chloride concentration and osmolality. In an accompanying editorial, Michele Funk and Vicky LeGrys from the University of North Carolina comment on the results of this study. They point out that there is insufficient data to assume that the new method has acceptable performance to replace existing approaches, but they are supportive of performing larger studies to define more precisely the sensitivity and specificity of the new test.

—Robert W. Wilmott, MD

Short gymnasts

Many female gymnasts are short. Among elite gymnasts, stature approximates the 10th percentile for reference standards. Female gymnasts may also be late maturing with a later age at menarche. This raises the question of whether the training necessary for competitive gymnastics is, in part, responsible for slower growth and maturation.

In this issue of The Journal, Thomas et al evaluate the growth spurt in female gymnasts. They find that gymnasts have clearly defined adolescent spurts in height, sitting height, and estimated leg length that occur approximately 1 year later and are slightly less intense than in non-athletic adolescent girls. These results suggest that shorter girls are more successful at gymnastics and are more likely to achieve elite competitive status. It is this selection process, rather than an effect of training, that results in female gymnasts being shorter than other elite athletes.

—Stephen R. Daniels, MD, PhD
Antibiotic prescribing for viral respiratory tract illnesses

Canadian investigators shed additional light on reasons why physicians might prescribe antibiotics when not indicated. Using case scenarios indicating simple viral upper respiratory tract illnesses, predominant variations in case presentations that led to misuse of antibiotics was the child’s degree of fever and lack of wellness. There’s still much work to be done to replace an antibiotic-prescribing attitude “just in case” with knowledge and confidence, as well as with the responsibility to preserve an effective antibiotic armamentarium.

—Sarah S. Long, MD

Puberty and erythropoesis

Puberty in males is associated with an increase in hemoglobin concentration. The mechanism of this increase has not been clear. It has been suggested that this could be an effect of increasing testosterone, but it could also be a result of changes in growth hormone and insulin-like growth factor I. In this issue of The Journal, Hero et al evaluate the regulation of erythropoesis in adolescent boys. Their results support the concept that androgenic effects are the primary ones. This may explain why girls do not have a similar increase in hemoglobin concentration.

—Stephen R. Daniels, MD, PhD

Rituximab for ITP in Children

Rituximab is a chimeric murine/human anti-CD20 monoclonal antibody that depletes B cells. It has been used extensively in B-cell lymphoma and various autoimmune diseases, including idiopathic thrombocytopenic purpura (ITP) in adults. In a paper by Wang et al, the results of treating 24 children with Rituximab are reported. This is the largest pediatric case series to date, and the study showed that 63% achieved a complete response lasting from 4 to 30 months.

—Robert W. Wilmott, MD

TRAPS and PFAPA

In this issue, Saulsbury and Wispelwey report the case of a 22-year-old man with a one year history of fever episodes associated with sore throat, cervical lymphadenopathy, painful oral ulcers, flank pain, photophobia, and conjunctivitis. Some episodes lasted 2 weeks and some also had associated abdominal pain and vomiting and arthralgia. The diagnosis of tumor necrosis factor receptor associated periodic syndrome (TRAPS) was suspected. Mutation of the TNFRSF1A gene was found. Of interest is the patient’s childhood history of fever episodes associated with pharyngitis, mouth ulcers, and adenitis, which episodes resolved following tonsillectomy at age 8 years.

Although the diagnosis of periodic fever with aphthous ulcers, pharyngitis and lymphadenitis (PFAPA) was made in this patient at age 8 years, the child lacked the most characteristic finding: clockwork periodicity of febrile episodes. In retrospect, the early childhood episodes were manifestations of TRAPS. The authors suggest that TRAPS should be considered when evaluating all children with PFAPA. This editor would agree with “considering” TRAPS, as well as Hyperimmunoglobulinemia D (Hyper D), as there may be shared clinical manifestations with PFAPA. Testing for gene mutations is currently the best test for both TRAPS and Hyper D, but is available only at the NIH, is costly, and is not reimbursed by insurance carriers. Unless the PFAPA syndrome is proved in the future to be TRAPS, cases of TRAPS are reported with the typical and restricted features of PFAPA, or genetic testing becomes accessible, one might perform such testing in patients when any one of the typical features of PFAPA is “off” or “extra” features exist. PFAPA typical features are: young age of onset; clockwork periodicity of febrile episodes; symptoms restricted to mouth, throat, and neck; short course of febrile episodes and absent family history of periodic fever syndromes. The single best test for PFAPA awaits discovery of etiology. Meanwhile, clinical criteria very specifically applied are pretty good.

—Sarah S. Long, MD
Finding and helping infants at risk for sequelae from congenital CMV infection

Naessens et al make two points convincingly in their study of detection of congenital CMV infection in Belgium infants: 1) the majority of newborn infants at risk for congenital CMV infection could be identified by maternal antibody screening, and 2) prevention of primary maternal infection in this population would prevent 80% of congenital CMV infection. In the accompanying editorial by Demmler, we learn that what seem like straightforward conclusions, and implementable strategies, still fall short of the goal of detection of infants at risk when the population has high rates of maternal seroprevalence or limited prepregnancy and prenatal medical care. The song has new verses but the chorus is the same — congenital CMV infection is common, yet it is under recognized and often silent. It constitutes the most frequent cause of sensorineural hearing loss, which could be potentially preventable.

— Sarah S. Long, MD

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Electronic monitoring of asthma medication use

The results of electronic monitoring of adherence to long-term controller and quick-relief medication are reported in a study of 75 children with asthma by Walders and colleagues from the National Jewish Medical and Research Center. A classification of adherence patterns was developed that should be useful in future clinical trials. It was demonstrated that adherence to long-term controller medications was predictive of morbidity from asthma in that higher rates of usage were associated with fewer emergency room visits and school absences.

— Robert W. Wilmott, MD

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Invasive Haemophilus influenzae type b disease

After four decades of increase in the incidence of invasive Haemophilus influenzae type b (Hib) disease in the United States, the last two decades have seen more than a 99% decrease in incidence associated with universal use of conjugate Hib vaccine in infants. Recently we have had reminders from the United Kingdom and in Native American and Alaska Native populations that Hib, the pathogen, has not disappeared and that the protection from invasive disease depends every day on availability of immunogenic vaccines and implementation of optimal strategies for their use. We take for granted that these contingencies will be met. This month in The Journal, Saha et al prove that Hib currently is a major cause of bacterial meningitis in infants in Bangladesh, dismissing a prior notion of its rarity as due to inadequate culture techniques. Showing increasing resistance to ampicillin and choloramphenicol, the authors additionally make an immediate case for bringing the more costly third-generation cephalosporins within “reach of the people of Bangladesh” and lay the responsibility of universal immunization on the table of policy makers.

— Sarah S. Long, MD

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Head shape is cultural

Graham et al demonstrate that head shape differs in different cultures as a result of sleeping position. Deformational brachycephaly (flattening in the anterior-posterior dimension) and positional preference occur more frequently with supine sleeping, a price to be paid for the decreased occurrence of SIDS. Although infants with significant brachycephaly were not randomized, a repositioning program was effective at normalizing head shape in young infants with a mean age 5.3 months, and helmet therapy helped normalize head shape in older infants with a mean age of 9 months. In a companion paper, Graham et al report their experience with managing deformational plagiocephaly (head flattened on one side). This head shape problem, resulting primarily from torticollis, was treated with physical therapy, repositioning, and helmets. Helmets were the most effective treatment for plagiocephaly, particularly if used early. Pediatricians need to evaluate head shape and consider early interventions to avoid the need for helmet therapy.
Most large academic clinical departments have regular, usually monthly, faculty meetings to communicate with faculty to provide information, discuss policy and plans, and deal with required university business. In some universities, departmental faculty meetings may be mandatory. However, as the professional lives of academic physicians have grown increasingly complex and productivity-focused, attendance at faculty meetings has decreased or become sporadic. Low and sporadic faculty participation in our faculty meetings caused our department to reexamine the goals and values of our meetings and to develop a new approach to this departmental activity.

METHODS

Before 1997, our departmental faculty meeting was held on the third Tuesday of each month from 4:30 to 6:00 PM. Our Department’s Division and clinical chiefs restated the goals of the departmental faculty meetings as:

- Receiving important professional and financial information,
- Providing faculty input into decisions affecting the department,
- Including faculty in strategic planning,
- Meeting and interacting with departmental faculty outside of one’s own division,
- Building professional skills, and
- Reviewing faculty promotions.

Faculty leadership concluded that the existing structure was effective as an information resource and mechanism to vote on promotions, but it failed to effectively address other key functions. A new plan for faculty meetings was developed by the Chair and Division Chiefs and approved by the faculty.

Beginning in 1997, monthly meetings were discontinued, and three expanded evening meetings were held (fall, winter, and spring). Two faculty meetings for faculty promotion were continued to meet the needs for the senior faculty to discuss mandatory and nonmandatory promotions. Faculty meetings in the new format were scheduled on different evenings of the week to avoid recurrent scheduling conflicts for any particular segment of the faculty.

Each faculty meeting now has a selected focus and is developed by a planning committee. Topics are solicited from the faculty and topic priority is decided by the Division Chiefs. The planning committee selects the speakers and poses two to four questions or critical activities for faculty participation. The general format for the meetings is a buffet dinner from 5:30 to 6:00 PM followed by a 2-hour agenda. The first hour is composed of a series of short presentations (10-30 minutes) by key leaders on the issue. Frequently, the second hour of the program consists of smaller group discussions with each focused on one of the questions identified by the planning committee or on faculty development. Typically the planning committee faculty members facilitate these small group discussions.

To maintain regular communication with the faculty, the Chair’s office began distributing a departmental newsletter one to two times a month. This newsletter lists important deadlines, visiting speakers, faculty news, and publications. The newsletter is sent to full-time faculty and volunteer teaching faculty and residents, as well as to relevant hospital and university administrators.

RESULTS

Topics selected for faculty meetings since 1997 are listed in the Table. Some areas have been repeated as the programs matured or additional input from the faculty was needed. Speakers at the meetings have included departmental faculty, chief residents, faculty from other departments, administrators from Children's Hospital, development officers, the Dean of the School of medicine, and practice plan administrators.

Attendance for each of the topics is shown in the Table. Mean faculty attendance for the eight monthly meetings preceding the change in format was 49.4 (SD = 10). Mean attendance in the 14 newly formatted evening meetings was 78.6 (SD = 11.6) (P < .001). Note that total faculty numbers increased from 135 in September 1997 to 165 in January 2002.

A number of specific programs or initiatives were developed as a direct result of our new faculty meetings. The
The typical monthly departmental faculty meeting has limitations in allowing the faculty to participate in the discussion of key programs or concerns within the department. Our expanded evening format allows for more in-depth faculty-driven presentations and workshops. The dramatically improved attendance at the meetings indicates greater faculty satisfaction with our new system. Furthermore, attendance has been sustained and has trended higher over time.

Important consequences derived from our evening meetings. Although the 2-hour format does not allow for extensive group problem solving, it has many advantages over the previously more passive format. The new format allows new programs to be presented and discussed in the planning phase so faculty have a role in shaping outcomes. Often committee work or survey information was obtained before the department meeting, making the discussion more data driven. The range of topics allowed us to address concerns of all segments of our faculty and make the issues known to all.

In addition, the evening format provides some extended social benefits. Faculty members have the opportunity to converse over dinner with colleagues and new faculty members who they might not see during their busy workdays. The evening schedule avoids most clinical time conflicts and also allows participation of community clinical faculty. They have become a forum for development of teaching skills for the faculty. Continuity clinic and regional clinic faculty were invited to the sessions on education and building teaching skills; a limited number of the volunteer faculty participated. Although the evening format was created, in part, to improve faculty input and increase faculty satisfaction, some faculty expressed displeasure at the obligation for three evenings away from their families each year. This criticism has not been reflected in a low attendance.

One disadvantage of eliminating the monthly meeting is that regular communication with the chair is less frequent. E-mail newsletters may partially offset this deficiency and allow for more rapid communication of time-sensitive matters.

As faculty become busier in their professional and personal lives, innovative programs are required to facilitate communication and ownership of departmental programs. We have utilized a new meeting format for our faculty for more than 5 years and believe that it has produced meaningful improvements in the department and improved faculty involvement and development of new programs.

Table. Topics addressed at the evening faculty meetings

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department made commitments to regional specialty outreach clinics, expanded mentoring programs, developed a new promotion committee and structure, developed a strategic research plan, redesigned inpatient teaching services, expanded programs in minority recruitment and created a committee to address personal barriers to faculty success. In addition, interactive small-group sessions (led by faculty) focused on building specific skills, eg, providing effective feedback, teaching on a busy in-patient service, teaching in the office, preparing a promotion dossier, effective chart documentation for billing, and working with prospective philanthropic donors.

DISCUSSION

The typical monthly departmental faculty meeting has limitations in allowing the faculty to participate in the
NON-ADHERENCE WITH ASTHMA THERAPY: MORE THAN JUST FORGETTING

The ubiquity of non-adherence with medication regimens is well-known to clinicians and has been extensively documented in the literature. Research that has quantified the frequency of pediatric non-adherence across a range of chronic illnesses such as diabetes, rheumatoid arthritis, and cystic fibrosis has found that adherence levels ≤50% of prescribed dosage are common. Studies of children’s adherence with asthma therapy have reported similarly poor average rates of adherence.

Non-adherence with treatment regimens has been repeatedly implicated as a contributor to sub-optimal clinical control in chronic pediatric illnesses. Although the consequences of periodic non-adherence with therapy among children with mild asthma may be insignificant, for some children drug holidays or chronic under-use of controller medications can result in persistent asthma symptoms, increased missed days of school, unnecessary urgent healthcare visits, and increased risk of fatal or near fatal asthma attacks. Understanding those factors that contribute to non-adherence with asthma therapy has the potential to help families more effectively manage their child’s asthma and reduce asthma morbidity.

As reported in this issue of The Journal, Fiese, Wamboldt, and Anbar sought to develop a reliable measure of asthma management routines and to evaluate its association with adherence with therapy, as well as parent and child quality-of-life and healthcare utilization. The investigators based the development of this instrument on their previous work, which suggested that family routines might be instrumental in assisting families manage asthma and adhere with therapy. Factor analysis of the eight-item Asthma Routine Questionnaire resulted in the identification of two factors; the first factor was labeled “Medication Routines” because these four questions dealt primarily with medication-taking activities, and the second factor was labeled “Routine Burden” because these three questions primarily measured whether asthma care was considered a burden.

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ICS Inhaled corticosteroid
routines had children with better medication adherence.\textsuperscript{9} It is important to recognize, however, that the observed correlation between the Medication Routine subscale and adherence behavior is inherently limited by the tautology of using predictor variables that are overlapping or redundant with the outcome variable. Thus, as the investigators note, the correlational study design does not allow a determination of causal association between family reports of established medication routines and children’s adherence with therapy.

Yet even without clear evidence of a causal association the potential benefit of established family routines to effective asthma management practices is intuitively obvious. A recent study of adherence with antiretroviral therapy further supports this premise. Wagner et al found that the extent to which a patient’s daily activities (eg, eating breakfast, watching favorite television programs) were routinized was the single best predictor of adherence with therapy.\textsuperscript{11} And Irvine et al has reported that parents of children with well-controlled asthma and good refill adherence were more likely to report organized morning and evening routines related to inhaler administration, compared with parents of poorly controlled children and low refill adherence.\textsuperscript{12} It makes sense that families that function in an orderly, structured, and predictable fashion can provide the every day “bones” on which regular medication routines can be hung. Family routines can not only facilitate regular adherence, they also can model and reinforce the skills and values necessary for children to develop effective disease self-management when they transition to adolescence. However, the reality for many families today is that day-to-day life is anything but predictable or routine. Between day care, soccer practice, weekends at Dad’s house, and car pooling, household schedules can change on a daily basis. And for low-income families or families struggling with other stressors such as marital discord, substance abuse, or other health problems, the challenges of daily life may overwhelm family routines. In fact, chaotic family life has not only been associated with poor adherence but also with increased asthma morbidity.\textsuperscript{8,12}

Although some portion of children’s poor adherence with asthma therapy may be attributable to irregular family medication routines, it also is important to recognize that not all non-adherence is the result of forgetting or disordered routines. Considerable research suggests that pediatric non-adherence is multidimensional and dynamic. Factors such as comprehension of the therapy and health beliefs each may influence parents’ ability and willingness to follow prescribed therapy. Parents may be inadvertently non-adherent with the prescribed asthma therapy if they fail to fully understand what medications their child should take or how to take them. In addition, if parents do not understand the importance of regular daily adherence with controller medications they may discontinue use when their child’s symptoms improve. Research suggests that parents’ knowledge of their child’s prescribed asthma regimen is often inaccurate, with parents frequently misunderstanding the role or value of controller therapies.\textsuperscript{13,14} Studies in adults suggest that patients forget or misunderstand close to half of the information provided by their doctor during an office visit, and for some patients low health literacy may be a particular barrier to understanding and adhering to therapy.\textsuperscript{15,16} Parents’ opinions about the value and role of asthma medications for their children do not necessarily match those of the treating physician. Parental concerns about giving an otherwise healthy child a daily medication may lead parents to use as little medication as possible. Reikert et al found that mothers who expressed greater concerns about the safety or value of asthma controller medications were more likely to be discordant with their child’s physician about whether the child was prescribed daily ICS therapy for asthma.\textsuperscript{17} Parent’s well-being also can influence their ability to adhere with their child’s prescribed therapy. For example, Bartlett et al found that mothers with more depressive symptoms reported greater non-adherence with their child’s asthma therapy.\textsuperscript{18} This is consistent with a growing body of research that suggests that depression may be one of the most important red flags for non-adherence in both adults and children.\textsuperscript{19}

Finally, it’s worth remembering that non-adherence with therapy does not necessarily result in worsening asthma. Parents may find some variation of the prescribed therapy that they believe works better for their child than the doctor-prescribed regimen. Parents may be hesitant to candidly tell their child’s pediatrician that they have not followed “doctor’s order.” Regardless of the child’s level of adherence or asthma control, pediatricians will need to use sensitive interviewing and active listening in order for parents and children to feel comfortable discussing actual patterns of medication use.

Solutions to adherence problems in asthma management will be as varied and idiosyncratic as each family being treated. As Fiese et al suggest, most families will benefit from concrete behavioral advice on establishing regular medication routines such as schedules for refills, reminders, or linking medication use to existing daily routines.\textsuperscript{9} Some families also will require additional patient education and review to fully understand and endorse the value of daily prophylactic asthma therapy. And a few high-need families will require more extensive social or psychological services and support in order to create a stable home life compatible with effective asthma adherence and management. The one essential adherence intervention appropriate for all families will be open-ended, non-judgmental provider-patient communication about the families’ knowledge, concerns, barriers and motivation to adhere with the prescribed therapy.

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TESTING DIAGNOSTIC TESTS: WHY SIZE MATTERS

For more than 50 years, quantitative determination of sweat chloride has been the gold standard in the diagnosis of cystic fibrosis (CF). Currently, the sweat chloride test is used to confirm or rule out the diagnosis of CF in two populations: neonates identified by newborn screening programs and patients presenting with clinical features suggestive of the disease. The sweat chloride test, when performed correctly, is accurate and reliable but labor intensive. In an effort to simplify the test, sweat conductivity methods have been developed. Conductivity represents the nonspecific measurement of the total anion activity in a solution and therefore has a higher concentration in sweat than chloride. Sweat collected in Macroduct coils and transferred to the Sweat-Chek conductivity analyzer (Wescor Inc., Logan, Utah) has been used in some settings as a screening test for CF. Individuals with a conductivity result above a prescribed cut-point are then referred for a confirmatory quantitative chloride measurement at a CF care center. Some have suggested that sweat conductivity performs as well as sweat chloride in diagnosing CF and could be used alone as a confirmatory test.

Recently, a new point of care conductivity analyzer, Nanoduct (referred to as the "new system"), has been developed for use especially in the neonatal population. As described in the accompanying article by Barben et al, the new system combines sweat collection and analysis into a single disposable conductivity sensor, using 3 μL of sample. The sensor and readout provide conductivity results within 30 minutes. The potential advantages over the traditional sweat chloride test are ease of use and availability of results within a short period of time. The important question remaining to be answered is whether the new system is as diagnostically accurate as the quantitative sweat chloride test in discriminating between CF and healthy individuals. The Barben article is the first published paper evaluating this new instrument.

In their study of 20 patients with classic CF, 73 patients referred for sweat testing, and 1 patient with nonclassic or borderline CF, Barben et al reported 100% sensitivity (95% CI, 83% to 100%) and 100% specificity (95% CI, 95% to 100%) for the new system compared with sweat chloride testing. We cannot know the true sensitivity or specificity of a diagnostic test. We can only observe the results from studies with relatively small numbers of individuals. From this imperfect information, we conclude that the chances...
SCRENNING FOR CONGENITAL CYTOMEGALOVIRUS INFECTION: A TAPESTRY OF CONTROVERSIES

C ongenital infection with cytomegalovirus (CMV) is an under-appreciated endemic public health problem that was first heralded by Weller in 1971 as an issue worthy of our attention and concern. Since then, numerous large cohort studies of mother-infant pairs conducted around the world have documented congenital infection rates between 0.3% and 2.2%. These studies also have documented that most (85%–90%) infections with CMV are silent at birth, yet longitudinal studies of identified newborns have shown that late sequelae, especially progressive hearing loss and possibly also learning or behavioral differences, emerge months and even years later, in up to 15% of these children. A smaller proportion, 5% to 15%, of congenitally infected newborns have recognizable symptoms and signs at birth, and, unfortunately, many of these children will experience neurosensory sequelae that will significantly impact their quality-of-life. Although preconceptional antibody in the mother appears to protect the fetus against transplacental infection and severe disease, the protection is incomplete because both symptomatic congenital infections and neurosensory sequelae, including hearing loss, have been documented in children born congenitally infected with CMV as a result of their mother’s recurrent CMV infection. The article by Naessens et al published in this issue of The Journal provides the latest information on the prevalence of congenital CMV infection in Brussels, Belgium. In accordance with other studies, the authors showed that 54% of 7140 pregnant women had serologic evidence of past CMV infection, and 4.1% of pregnant women experienced a primary CMV infection documented by seroconversion during pregnancy or a recent primary or recurrent CMV infection documented by immunoglobulin (Ig)M antibody on the first prenatal visit. They also documented congenital CMV infection in 44 (0.62%) of the newborns studied, with most (36; 82%) born to women with primary or recent primary infections. Based on their findings, the authors proposed a strategy for screening pregnant women that would identify >80% of newborns at risk for congenital infection and neurosensory sequelae. Unfortunately, rather than being closer to solving the problem, we have yet another weave for the tapestry of controversies that covers rather than confronts the multitude of issues surrounding congenital CMV infection.

The controversy surrounding screening for congenital CMV infection is not new. In fact, the idea to screen newborns for congenital CMV infection was first proposed in the early 1970s, when the link between congenital CMV infection and hearing loss, first realized by Medearis in 1964,8 was strengthened by several investigators who conducted longitudinal studies on the outcome of congenitally infected newborns. In 1982, Hanshaw wrote an editorial that accompanied one such study conducted by Saigel et al in which he emphasized the importance of congenital CMV infection as a major cause of nonhereditary sensorineural hearing loss and posed that the cost of a CMV screening program may be justified when considering the price for delayed diagnosis, unnecessary tests, and proven value of early intervention in hearing-impaired children. He concluded his editorial with the opinion, “We have more reason to consider screening newborns than we have ever had before.” Now, in 2005, the controversy surrounding screening newborns for congenital CMV infection continues, recently fueled by the realization that universal newborn hearing screening, endorsed as a standard of care in 2000, may miss more than two thirds of children who develop hearing loss from congenital CMV infection.11,12 Given decades of understanding about the large numbers of affected children and the proven benefit of early intervention, the issue should not be whether we should screen but rather which approach is best to screen and monitor all congenitally infected newborns.

To date, a variety of approaches have been evaluated to diagnose congenital CMV infection in the newborn. These methods include

See related article, p 194.

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detection of CMV-specific IgM antibodies in cord or neonatal blood; cytology on urinary epithelial cells; visualization of viral particles in urine by electron microscopy; cultivation of virus by cell culture or by centrifugation and fluorescence-enhanced cell culture methods in urine or saliva; detection of viral antigens in urine; detection of viral DNA of urine, saliva, or blood; and, most recently, detection of viral DNA in stored blood filter paper or Guthrie cards used to screen newborns for other diseases, which, ironically, are less prevalent and impact fewer numbers of families than does congenital CMV infection.2,13,14

Rather than universal newborn screening, Naessens, et al proposed to limit neonatal testing for congenital CMV infection to those babies who are born to pregnant women who are serologically selected to have experienced a primary or recent primary infection. However, this approach would miss most babies born to women from groups with higher (>80%) seroprevalence for CMV, and because congenital CMV infections from maternal recurrent infections also may produce neurosensory sequelae, this approach does not offer a solution that is adaptable to all populations.6,6 Also, women who do not seek or cannot readily access routine prenatal care, such as adolescents and women of nonwhite race from low income groups, would be excluded from this strategy. Because the offspring of these groups appear to have the greatest risk for congenital CMV infections, especially in the United States, this strategy, once again, falls short.15,16

Another controversy surrounding congenital CMV infection is management of the pregnancy in a woman who is shown to have experienced a primary or near primary infection. Practicing physicians, as well as experts, are conflicted in their opinions; some advocating testing all women of childbearing age and others preferring not to screen women for CMV antibody because of the lack of consensus on how to, or even whether to, diagnose intrauterine infection in the fetus. Invasive procedures, such as amniocentesis and fetal blood sampling, can diagnose CMV infection in the fetus, and ultrasonography may correlate infection with some, but not all, manifestations of fetal CMV disease.17 Because prenatal treatment options are not yet available, as they are, eg, for congenital toxoplasmosis, choices about what then to do with the knowledge that a woman is carrying a baby with intrauterine infection or disease as a result of CMV are limited to elective termination of the pregnancy or expectant, usually anxious, observation until delivery. However, on the other hand, prenatal testing also affords an opportunity to educate women about behaviors in the home and workplace that are high risk for transmission of CMV and to provide recommendations, such as avoiding intimate contact with salivary secretions and urine from young children and careful handwashing after changing diapers and wiping secretions.18

A screening program that is contingent on identification of the serostatus of a woman at the beginning of her pregnancy should therefore confront and address these controversies and provide a plan of action for practitioners. However, because our currently available, incomplete understanding of this issue has left a void of consensus, the decision-making process on how to proceed with the clinical information obtained from these procedures must be individualized to each patient, her partner, and her doctor.

A newborn screening strategy also must address the issue of cost effectiveness and cost benefit; and it is unclear whether the strategy proposed by Naessens et al is more advantageous in this regard than universal testing of all newborns for congenital CMV. Furthermore, a plan for notification of the healthcare workers and family caring for the newborn identified as congenitally infected, and guidelines for acute and long-term management are necessary. For example, should asymptomatic, as well as symptomatic, newborns be evaluated for evidence of somatic and neurosensory disease? Because the most common sequelae of congenital CMV infection is progressive hearing loss, screening programs designed to identify CMV-infected newborns may be linked to universal newborn hearing screening programs for optimal efficiency and provide a plan for long-term audiologic follow-up for those newborns who are congenitally infected with CMV.12

In summary, the controversies surrounding identification of newborns who have this common, and ironically under appreciated, congenital infection have continued now for more than 3 decades.19

We should acknowledge Naessens et al for their effort to seek a solution to this challenging problem, but we also much encourage other scientific investigators to be courageous and confront the problem of congenital CMV infection and disease with studies that will produce potentially meaningful and productive solutions.

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**HELICOBACTER PYLORI DIAGNOSTIC TOOLS: IS IT IN THE STOOL?**

*Helicobacter pylori* *(Hp)* infection has been recognized as one of the most common infections around the world, affecting almost half of the world population. The infection has a cohort effect, and most infected adults acquire this disease during their childhood. Thus, pediatricians and primary care physicians are placed at the forefront of the ongoing battle to eradicate this infection. To date, the most reliable method to diagnose *Hp* in children, adopted as the gold standard by several *Hp* committee experts from Europe and North America, is histology. The invasiveness of this method has prompted the search for an alternative, noninvasive, diagnostic method to detect *Hp* infection in children. Antibody tests (ELISA) to detect *Hp* infection in various body fluids were the first to appear, but they have failed to provide adequate accuracy to replace gastric biopsy as the gold standard, especially in young children. The 13C urea breath test (UBT) was developed to detect *Hp* organisms by measuring the radiolabel carbon in the breath of infected children. Studies from Europe and Canada reported a very good accuracy rate, and expert guidelines have suggested this method for monitoring bacterial eradication after therapy. Despite these results, UBT is limited by being technically cumbersome, time-consuming, expensive, unavailable in certain countries, and of low reliability in very young children (<2 years of age). A new noninvasive test has been developed: the stool antigen test (EIA; Premier Platinum HpSA; Meridian Diagnostics Inc, Cincinnati, Ohio). This polyclonal test relies on the detection of *Hp* antigens shed in the stool of infected children. The test was enthusiastically accepted by clinical researchers and by community physicians, as it provides an easy method for epidemiologic data as well as for clinical diagnosis.

Initial reports of the HpSA were very encouraging, and a sensitivity of up to 100% was reported; however, later studies demonstrated a wide range of accuracy rate, especially in young children. Moreover, it was suggested that the accuracy of HpSA in young children was not evident. The current impression among experts is that polyclonal HpSA test is yet to reach the adequate accuracy level to be considered as the gold standard for detection of *Hp* infection in children, and that studies with larger number of *Hp* infected children will be needed before the final verdict is reached.

In this issue of *The Journal*, Megraud et al report a large, prospective, multicountry, multicenter study that compared the accuracy of four *Hp* diagnostic tests (UBT, serum antibody, urine antibody, and stool antibody tests) in European children. Positive infection was defined as a positive culture and/or positive histology with positive rapid urease test (RUT). A total of 503 children were recruited, 30 were dropped for missing data or the inability to decide on their infection status (positive/negative), and 157 were further dropped for incompleteness of all four diagnostic tests assigned for the study. At the end, a total of 316 (63%) children completed the study, of whom 132 (42%) were *Hp* positive and 184 (58%) were *Hp* negative. Overall, UBT was found to be the most accurate method, followed by serology. Urine and stool tests were the least accurate. Changing the recommended wavelength cutoff level to define a positive result for the urine and stool tests improved sensitivity but
INVITED COMMENTARY

DOES NEWBORN SCREENING SAVE MONEY? THE DIFFERENCE BETWEEN COST-EFFECTIVE AND COST-SAVING INTERVENTIONS

SCOTT D. GROSE, PhD*

Benjamin Franklin famously said that an ounce of prevention is worth a pound of cure, but he didn’t say that it is necessarily less expensive. A recent review from the Partnership for Prevention concluded that most recommended clinical preventive services are cost-effective but not cost-saving.1 A cost-saving intervention results in both better health outcomes and less total spending by payers, including medical care and other direct costs of care, as well as costs associated with the intervention. From a societal perspective, economic benefits also include averted indirect costs or productivity losses from premature mortality or disability, although these are not included in cost-savings from the payer perspective.

The identification of cost-savings with cost-effectiveness remains a common misconception.2 Cost-effectiveness is defined in terms of low cost to achieve a given outcome, which can be calculated relative to other interventions or to a fixed cutoff. Cost-effectiveness is not primarily about saving dollars; rather, it involves seeking to maximize health improvements with limited resources, that is, getting good value from investments that save lives or improve health and development. For example, newborn screening has been demonstrated to prevent intellectual disability and reduce the need for special education services3 and appears to be highly cost-effective.1

Even if an intervention is found to be cost-saving in a specific place and time, additional expenditures will not necessarily also be cost-saving. For example, although childhood immunizations are reported to be cost-saving on average, new immunizations for additional diseases are not necessarily cost-saving, nor are efforts aimed at increasing immunization coverage in hard-to-reach populations.1,4 Similarly, it is hazardous to generalize from the balance of costs and benefits of screening for one disorder to other disorders or from one screening technology to another.

The argument that public health programs save taxpayers money can be persuasive, but arguments advanced on the basis of incomplete data can also be misleading. For example, the expansion of Medicaid coverage of prenatal care in the late 1980s and early 1990s was driven by claims such as, “For each additional $1 spent on prenatal care, $2.57 in medical care costs would be saved.”5,6 Such statements were based on data linking birth weight with prenatal care usage; subsequent evidence did not show reduced rates of low birth weight and medical expenditures.6,7 That the cost-saving rationale was not confirmed does not mean that this was not a worthwhile or cost-effective policy.

Each state or territory in the United States has a program to screen newborn infants for metabolic and other diseases through analysis of dried blood spot specimens. Early treatment for many of these diseases can reduce the risk of death or severe disability. Estimates of the potential cost-savings from the prevention of symptomatic disease and disability should be based on reliable data. A recent newspaper article on expanded newborn screening for metabolic disorders incorrectly stated, “The cost of treating and caring for children who suffer from these rare diseases can range from $500,000 to $1 million per year, according to the US Centers for Disease Control and Prevention.”8 In reality, the Centers for Disease Control reported that the present value of lifetime costs of developmental disabilities that can be prevented by newborn screening ranges from $500,000 to $1 million.9,10 Furthermore, these estimates include both direct costs of care and productivity losses, with direct costs accounting for less than half the total.

Based on outdated information, certain advocates claim that newborn screening programs more than pay for themselves through reduced costs of care. For example, it was recently stated that “For every dollar you spend on screening, you’ll save $10 to $20 in long-term … costs.”11 Another statement that has been used in support of newborn screening is, “Estimates are that for every dollar spent on newborn screening, $9 in medical care and treatment costs are saved, resulting in a national savings of $36 million every year.”12 These statements, which appear loosely based on a quarter-century-old estimate of the benefits of screening for congenital hypothyroidism,13 are inaccurate.
ECONOMIC EVALUATIONS OF NEWBORN SCREENING

Reductions in medical costs with screening are most likely for disorders that are disabling but not lethal. For example, phenylketonuria (PKU) and congenital hypothyroidism (CH) cause irreversible intellectual disability requiring expensive, life-long treatment but are not life-threatening. In contrast, disorders such as galactosemia and maple syrup urine disease (MSUD) can be lethal if untreated, but treatment does not necessarily prevent disability nor reduce costs.14 Screening for lethal disorders can be a cost-effective means to reduce infant deaths.

When screening of all newborn infants for PKU was introduced in the 1960s, a rationale for public funding was that most individuals with PKU ended up in publicly funded institutions, costing tens of thousands of dollars per year per patient. A relatively recent economic evaluation of screening for PKU, which assumed that all individuals with mental retardation would be institutionalized, estimated averted costs of $1.60 for each $1.00 in costs incurred.15 Because less than 8% of people with mental retardation in the United States now reside in institutions or group homes,12 it is not clear that PKU screening is still cost saving.

The addition of CH to screening panels beginning in the late 1970s was a highly cost-effective, indeed cost-saving, policy for the prevention of mental retardation. CH has a higher incidence than PKU and a lower cost of treatment; in addition, the fixed costs of screening were already covered. One economic analysis that accompanied the introduction of screening in 1979 calculated that screening for CH with the same dried blood spot specimen collected to test for PKU would result in $7.80 in averted costs of care per $1.00 in incremental screening and treatment costs.10 A 1988 analysis concluded that a strategy of screening for both PKU and CH using a single specimen per infant would be cost-saving on average, with an average ratio of $2.90 in averted costs per $1.00 in screening and treatment costs.16

On the other hand, more intensive newborn screening efforts have not been found to be cost-saving. For example, routine collection of a second specimen to screen for PKU and CH has been found to raise screening costs by a greater amount than the associated reduction in costs of care.16 The same report found no evidence that screening for galactosemia, MSUD, or homocystinuria would reduce net costs.16 Likewise, studies of newborn screening for sickle cell disease (SCD) and other hemoglobinopathies have not found this to be cost saving. One analysis calculated that universal screening for SCD in Alaska would save only $0.11 per $1.00 in incremental cost.17 On the other hand, this is a cost-effective policy for the prevention of childhood deaths in the United States, with an average cost per life-year saved of $12,000.18

Four economic evaluations of expanded newborn screening using tandem mass spectrometry (MS/MS) have been published, three from the United States and one from the United Kingdom.19-22 One analysis evaluated the use of MS/MS to screen for just one metabolic disorder, medium-chain co-A dehydrogenase deficiency (MCADD).19 A second analysis assessed using MS/MS to screen for a number of disorders, including PKU.20 A third analysis calculated the additional costs and benefits of using MS/MS to screen for MCADD, on the assumption that the costs of operating MS/MS were already covered by using it to screen for PKU.21 Finally, the UK analysis analyzed using MS/MS to screen for both PKU and MCADD as well as for 12 additional disorders.

The two analyses from the United States that calculated the costs of adopting MS/MS both concluded that using this technology would not be cost-saving, with averted costs of only $0.20 to $0.27 for each $1.00 spent on screening and workup.16,20 A third US analysis concluded that using MS/MS to screen for MCADD might be cost-saving, but only if the fixed costs of setting up and operating MS/MS are excluded.21

A recent health technology assessment study from the United Kingdom has concluded that using MS/MS to screen for both PKU and MCADD would be cost-saving.22 The study assumed a relatively low MS/MS screening cost, less than $3 per infant, and projected that the majority of the cost of implementing MS/MS would be offset by eliminating separate testing for PKU and reducing follow-up from false-positive screens for PKU.22 This latter assumption is debatable, since depending on the current screening method, switching to MS/MS could result in little reduction in follow-up costs for PKU.20 The UK report also considered screening for 11 additional disorders or groups of disorders detectable through MS/MS. The study concluded that screening for 9 sets of disorders probably would be cost-effective but not cost-saving, with incremental cost-effectiveness ratios up to $25,000 per life-year saved. The disorders ranked in terms of incremental cost-effectiveness ratios are long-chain fatty acid defects, glutaric academia type I, urea cycle disorders, branched-chain acyl-CoA metabolism defects, methylmalonic academia, isovaleric academia, propionic academia, MSUD, and tyrosinemia.

DISCUSSION

As with other health interventions, one should not assume that expansion of newborn screening will be cost-saving. Screening for PKU and congenital hypothyroidism were found to be cost-saving when introduced, but the health care context in the United States has subsequently changed in a way likely to reduce averted costs of care. Screening for other disorders has not yet been demonstrated to be cost-saving, although rigorous research, including collection of better data on costs and outcomes, is needed to better assess the health and economic implications of newborn screening. Conflicting assessments as to whether screening using MS/MS is cost-saving have been published. The wide range in estimates reflects lack of agreement regarding costs and outcomes as well as different ways in which the technology can be implemented.

A challenge to public health advocates is to use economic concepts and findings in making the case for newborn screening. Claims that every dollar spent on a public...
health program will save taxpayers $2.00 or more in averted costs often turn out to be misleading. Public health is about saving lives, preventing disability, and improving health-related quality of life, not about financial savings, although economics plays an important role because of the need to use scarce resources wisely.

Newborn screening has long been accepted as cost effective, based primarily on evidence for a subset of disorders: PKU, CH, and SCD.\(^1\) Newborn screening has been demonstrated to save lives and prevent serious disability\(^3\) and represents an exemplary public health success for which continued funding is essential. Whether expanded screening using MS/MS is cost-saving is not the important policy question. The available evidence suggests that expanded screening for MCADD using MS/MS is also cost-effective as a public health strategy, assuming an additional laboratory cost of $15 or less per infant.\(^{19,21}\) This does not take into account the benefits and follow-up costs from screening for additional disorders through MS/MS, as modeled in two other studies.\(^{20,22}\)

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FAMILY ASTHMA MANAGEMENT ROUTINES: CONNECTIONS TO MEDICAL ADHERENCE AND QUALITY OF LIFE
BARBARA H. FIESE, PHD, FREDERICK S. WAMBOLDT, MD, AND RAND D. ANBAR, MD

Objectives To develop a reliable measure of asthma management routines and examine its association with health care utilization, medical adherence, and quality of life.

Study design Families (n = 153) with a child with asthma, drawn from two sites, participated in the study. Parents completed the Asthma Routines Questionnaire, Adherence to Clinical Trials interview, Functional Severity of Asthma Questionnaire, and Caregiver Quality of Life. Children completed the Pediatric Quality of Life. Electronic monitoring of medication use over a period of 12 months was available for children at one study site.

Results A principal component factor analysis revealed two dimensions to the Asthma Routines Questionnaire: Medication Routines and Routine Burden. Medication Routines were related to medical adherence and to health care utilization. Routine Burden was related to caregiver and child quality of life.

Conclusions The Asthma Routine Questionnaire holds promise as a reliable assessment of family practices related to medication use. The emotional burden of daily care can be distinguished from medication use, which is more closely linked to adherence issues. Targeted questions during regular care may reveal family routine practices amenable to intervention. (J Pediatr 2005;146:171-6)

Current asthma practice guidelines emphasize the importance of daily and regular monitoring of asthma symptoms and detailed action plans in the event of an attack. Many of the recommendations are framed as part of the family’s daily or weekly routines such as vacuuming the house once per week, monthly cleaning of duct systems, and monitoring peak flows. Accordingly, asthma management becomes part of ongoing family life, and those families who are more capable of the organization of family routines are expected to have more effective management strategies. The purpose of this study was to develop a reliable measurement of family-based asthma management routines and to examine their relation to medication adherence, health care utilization, and quality of life.

Regular family routines are reportedly related to shorter bouts of respiratory illness in infants. The emotional investment in family rituals has been found to be associated with reduced anxiety levels in children with asthma. We developed an 8-item questionnaire that ranged from specific aspects of medication routines to more global affective investment in asthma care. Previous research has found that routines cluster in two factors: practices and emotional connections. On the basis of previous research on family routines, we expected to identify a routine practice factor and a factor more closely associated with emotion and affect. We were also interested in the degree to which asthma routines were associated with medication adherence, health care utilization, and asthma-related quality of life. We expected that predictable and regular asthma routines would be associated with greater medication adherence, less emergency health care utilization, and more positive quality of life.

METHODS

Participants
Families (n = 133) with a child with asthma participated in the study; the children were on average 9 years old, with a range of 5 to 18 years of age. The families were relatively

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diverse across ethnic background (65% white, 20% black, 8% Latino, 3% Asian, 1% Native American, 2% unspecified) and socioeconomic status (SES), with a mean Hollingshead score of 39.31 (SD = 15.99) All classes of SES were represented (ranging from low to high: class I, 19%; class II, 35%; class III, 20%; class IV, 11%; class V, 15%). Slightly more than half of the parents were first-married (60%), with the remainder single (12%), remarried (13%), separated/divorced (14%), or widow/widower (2%). Disease severity (as determined through parent report of child symptoms) was relatively equally distributed (mild, 27%; mild persistent, 25%; moderate persistent, 26%; severe, 22%).

Procedure
Families were recruited from two study sites. Families from study site 1 (n = 72) were participants in a larger longitudinal study focusing on family factors and medical adherence. The families were originally recruited through area public schools and a health maintenance organization and resided in a large western city. Families from study site 2 (n = 61) were recruited from two university-based pediatric clinics (pulmonary and general ambulatory care) in a mid-sized northeastern city. At both sites, families completed questionnaires in a laboratory setting. Parents and children completed questionnaires independently. Institutional review board approval was granted at both sites. Informed consent was obtained from parents and assent from children.

Measures
**Asthma Routines Questionnaire.** The Asthma Routines Questionnaire was developed as an 8-item questionnaire, using a forced-choice format. Item selection was developed in a 3-step fashion. First, current practice guidelines were evaluated for the presence of routines associated with good disease management. Second, an initial pool of items was developed to reflect current practice guidelines and to correspond to central aspects of routines such as role assignment and feelings toward carrying out the routine. The initial pool of items was presented to a panel of experts (primarily physicians and psychologists) knowledgeable in families and asthma research. On the basis of comments made by the panel, the items were rewritten to decrease the likelihood of socially desirable responses. The 8 items that made up the questionnaire include questions pertaining to role assignment, burden, house cleaning, taking medications, timing of medications, medical visits, filling prescriptions, and personal family growth as the result of the disease (Figure; available online at www.us.elsevierhealth.com/jpeds). Item scores range from 1 to 4. Some items are reverse-scored to reflect higher routinization.

**Medical Adherence**

For study site 1, electronic monitoring of medication use was conducted in 2-month blocks over a 12-month period, using the Doser-Clinical Trials Version. Adherence rates were calculated by dividing recorded use by prescribed dose averaged across the 12-month period. At both study sites, a 24-hour recall method was used. Parents were questioned about medication use by means of the Adherence in Clinical Trials (ACT) interview. This measure has been validated against objective criteria of medication adherence, including medication measurement and electronic monitors across diverse patient populations. However, the self-report measure typically overestimates adherence when compared with electronic monitoring.

**Functional Severity of Asthma**

The Functional Severity of Asthma scale is a 6-item scale (wheezing, night wakening, speech limitations, activity limitations) completed by parents. The scale has proven to be reliable, with an internal consistency estimate of 0.89. Validity was demonstrated through correlations between severity and school days missed and medical visits.

**Health Care Utilization**

The number of times the child had been hospitalized, sought emergency room care, and visited the doctor for asthma was collected through parent interview.

**Caregiver Quality of Life**

Parents completed the Pediatric Asthma Caregiver’s Quality of Life Questionnaire (CQoL). The CQoL consists of 13 questions, on a 7-point scale, pertaining to how the child’s asthma has affected the caregiver’s quality of life. Two dimensions have been identified: Emotion (9 items) and Activity Limitations. A total score may also be generated. This measure has been validated by using other health-related quality-of-life measures. Changes in quality of life are reported to be related to perceived burden and asthma severity. Internal consistencies for this study were found to be 0.91, 0.87, and 0.92 for Activity, Emotion, and Total, respectively.

**Pediatric Quality of Life**

Children 8 years of age and older completed the Pediatric Asthma Quality of Life Questionnaire (PAQLQ), which is a 28-item questionnaire specifically designed for children and adolescents. Three scores are generated that pertain to how asthma interferes with activities, presence of asthma symptoms, and emotional reactions to asthma. Internal consistencies for this study were found to be 0.88, 0.74, 0.88, and 0.93 for Symptoms, Activities, Emotions, and Total, respectively.

**Statistical Procedures**

Analyses began with descriptive statistics and an examination of characteristic differences between sites. A principal component factor analysis with Varimax rotation was conducted to determine the factor structure of the Asthma Routine Questionnaire. Correlations were generated between the resulting factors and demographic characteristics to rule out possible confounds. Correlations were generated between the Asthma Routine scores and measures of medical adherence,
health care utilization, and quality of life. Analyses were conducting with the use of JMP (SAS; Cary, NC). Individual item analysis was conducted by means of analysis of variance, using Levene tests to rule out the possible effects of heterogeneity of variances.

RESULTS
Site Comparison and Factor Analysis

There were some significant differences between the two study sites on demographic characteristics. The sample from study site 1 was overall of a higher socioeconomic background ($t [125] = 2.74$, $P < .01$) and more likely to be married ($\chi^2 = 11.55$, $P < .02$). Children in the site 1 sample were also somewhat older, with a mean age of 10 years compared with 8.6 years in the site 2 sample ($t [130] = 3.39$, $P < .001$).

All response points were used on all items on the Asthma Routine Questionnaire, ranging from a low of 1 to a high of 4. The responses were somewhat positively skewed, with the lowest mean score within the item being 2.64 (SD = 0.97) and the highest mean score being 3.43 (SD = 0.78). Shapiro–Wilks tests conducted on each item indicated that a fitted normal curve was the best fit for the distribution; thus, no transformations were warranted. A principal components factor analysis with Varimax rotation was conducted. By following conventional standards, we considered an item to load on a factor when it exceeded a value of 0.3.12,13 We note that retained items all loaded above 0.5; thus, 0.3 appears to be a conservative decision rule for factor loadings in this instance. At the first pass of the data, we identified 3 factors with eigen values greater than 1.0 (2.29, 1.47, and 1.06) accounting for 58% of the variance. Upon closer examination, it was determined that one item loaded equally on two factors and that the resulting $\alpha$ value of the third factor was unacceptably low (<0.5). The factor analysis was rerun, forcing two factors that resulted in acceptable eigen values and accounted for 47% of the variance. Summary scores were generated by taking the average score across items for each factor, and internal consistency was examined. For the first factor, dropping items did not increase the consistency estimate. For the second factor, one item was dropped to improve the $\alpha$ Level. Factor weights by item are presented in Table I. We labeled the first factor Medication Routines because most of the items pertained to when medications were taken and prescriptions filled. We labeled the second factor Routine Burden because many of the items pertained to whether asthma care was viewed as a chore.

We generated correlations between the summary scores and demographic characteristics and found that Medication Routines were negatively related to child age ($r = -0.24$, $P < .01$) but not significantly related to SES or functional severity of asthma. There were no significant differences on the Medication Routines scale according to marital status. Routine Burden was also positively related to functional severity of asthma ($r = 0.3$, $P < .01$) and was distinguishable across severity groupings, with parents of children with more severe asthma reporting the greatest Routine Burden ($F [3,99] = 4.22$, $P < .01$). In further analyses, we used child age as a covariate when examining significant findings for the Medication Routine scale and SES and functional severity when examining significant findings for the Routine Burden scale.

We present the nonparametric correlations of primary interest in Table II. Because of concerns about the possible effects of age on Medication Routine scores and SES on Routine Burden scores, we reran the analyses by partialling for the effects of age and SES and found the same pattern of results. The relation between Routine Burden and number of physician visits was not significant when partialled for the effects of severity ($r = 0.14$). Parents whose children had received asthma care in the emergency department ($n = 29$) reported significantly ($F [1,99] = 4.03$, $P < .05$) more Routine Burden ($M = 2.89$ [0.13]) than those who did not receive such care ($M = 2.51$ [0.08]). However, this effect did not hold when considering functional severity ($\chi^2 [2.88] = 1.33$; not significant). The correlations between school days missed and Medication Routines and Routine Burden were not significant. The relation between Routine Burden and most number of rescue puffs taken in a row remained statistically significant when partialled for the effects of functional severity ($r [100] = 0.31$, $P < .01$).

The relation between Routine Burden and Pediatric Quality of Life remained statistically significant for Symptoms ($r [98] = -0.24$, $P < .05$), Emotions ($r [98] = -0.33$, $P < .001$) and Total ($r [98] = -0.27$, $P < .01$) when partialled for the effects of functional severity. Table III presents analyses of variance on the ACT items that call for categoric responses (ie, yes/no). Levene tests for heterodasticity of variances were not significant across all items (range of Levene tests, 0.11 to 1.01).

DISCUSSION

We sought to examine the relation between family routines and pediatric asthma management. Overall, we found that routines connected to medication use were related to medical adherence and that the burden associated with management routines was negatively related to quality of life. We frame our discussion around the 2-fold nature of asthma management routines, study limitations, and clinical implications.

Consistent with previous studies examining family routines outside the context of chronic childhood illness, two aspects were identified. The first factor we labeled medication routines. This aspect of asthma management centers around regularity, predictability, and planning for medication use. All of the children in this study were prescribed daily controller medications that must be refilled on a regular basis, taken at regular times of the day, and, more often than not, involve a routine reminder. Parents who
reported better medication routines had children with better medication adherence as determined both by electronic monitoring and parent report. The predictability of family routines assists parents in adapting to their role as caregiver and is associated with feelings of competence and fewer childhood respiratory infections. Parents who are able to organize their lives to include routine monitoring of medications find it easier to fold medication use into their daily patterns. Indeed, when we considered individual responses to the ACT adherence interview, we found that parents who reported more medication routines had less trouble reminding their child to take their medications and that overall, their child rarely or never forgot to take their medications. It is also plausible that parents who can create regular routines are also better monitors of medication use. This probably is a transactional process because children who are raised in more predictable and routine environments may be better equipped to follow instructions and are more likely to be responsive to structure. Engaging children in regular medication practices may decrease conflict over adherence often noted in the literature. We noted that medication routines were negatively related to child age, suggesting that as children reach adolescence there may be a decrease in family-based routines. This is consistent with the literature on child development suggesting that the influences outside the family (eg, peers) play a larger role in structuring behavioral practices in adolescents. During this transition period, it may be important to consider routines that are initiated by the adolescent rather than overseen by the parent.

The second factor that we identified was labeled Routine Burden. This factor reflected the degree to which asthma management was perceived as a chore and hassle. This factor was not related to medication adherence but was related to caregiver and child quality of life. Caregiver report of quality of life is more closely tied to negative affect than it is to health care use. Caregiver report of quality of life was not related to medication adherence but was related to utilization and disease burden. In this study, we found that parents who perceived asthma care as a burden also reported that they had more trouble reminding their child to take their medications and overall reported poorer quality of life. This second factor appears to tap into the emotional weight of routine care. The emotional connections made during family gatherings have been found to be associated with more optimal mental health and well-being.

We also found that the child’s perceived quality of life was associated with the parent’s report of routine burden. This provides partial support that our findings are not solely due to response bias on the part of the caregivers. We speculate that
in family environments in which parents are overwhelmed or burdened by care that there is a cost to the child as well. When we consider the child’s response to the emotional aspects of quality of life, we note that there was more perceived burden. Children’s reports of quality of life are also subject to affective variation and have been found to be closely related to child anxiety. However, what is new about our present findings is that when parents and children report poorer quality of life, there is less of an emotional investment in asthma care routines and more of an emotional drain. It is important to recognize that routine burden was also associated with SES. It may be that with fewer resources and more limited access to care that maintaining regular routines is more of a struggle and challenge. The multiple pathways that lead to poorer economic circumstances cannot be ignored. Sameroff et al. have found that multiple personal and social stressors adversely influence child and family development in an additive manner.

There are several limitations to this study. Although the overall internal consistencies of the factor summary scores were within acceptable parameters, they were nonetheless relatively low. Hence, both additional work on the Asthma Routine Questionnaire and replication with a larger sample are clearly warranted. Test-retest reliability also needs to be established. It will also be important to further consider age differences and whether this approach is relevant to families with older adolescents. Because our data analysis was correlational, we cannot state with any certainty that more regular medication routines cause better adherence. However, prior intervention research aimed at improving medication adherence has successfully done so by pairing medication taking with existing family routines and creating a routine to which medication taking can be linked. Still, until this direction of effect is established, it is important to remain mindful that better adherence may make it easier to follow regular medication routines.

In a previous report, we proposed that interventions aimed at creating new or reinforcing existing routines could result in better medication adherence. With the findings of this study, we suggest that particular attention be paid to the type of routines that families create around medication use. Does the family have a plan for tracking supplies of medication and anticipating the need to fill prescriptions? Is medication use tied to specific daily routines such as tooth brushing, bedtime stories, or meal times? Is the whole family involved in care (and aware of routines) so that when one member forgets or is otherwise absent, there is a backup? These may be relatively straightforward questions that could be addressed.

### Table III. Analysis of variance of Adherence Questions in relation to Asthma Routines Questionnaire

<table>
<thead>
<tr>
<th>Question</th>
<th>Response group</th>
<th>Asthma Routines Questionnaire</th>
<th></th>
<th>Adjustment t</th>
<th>P value</th>
<th>Tukey HSD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Does child test inhaler before using?</td>
<td>0 = no, 1 = yes, 2 = sometimes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ever have trouble reminding your child to take meds as prescribed?</td>
<td>0 = no, 1 = yes, 2 = sometimes</td>
<td>0.43 (2.96) NS</td>
<td>6.15 (2.96) .01</td>
<td>1 &gt; 2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>In past 2 wk child forgot to take medication</td>
<td>0 = never took, 1 = 1 to 3 times, 2 = rarely, 3 = never forgot</td>
<td>2.80 (4,100) .03 4 &gt; 0</td>
<td>1.34 (4,100) NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall, would you say in the last week your child</td>
<td>0 = never took inhaler, 1 = often forgot, 2 = sometimes forgot, 3 = rarely forgot, 4 = never forgot</td>
<td>4.93 (4,100) .001 1 &lt; 3, 4</td>
<td>1.11 (4,100) NS</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Would you say that your child took his/her inhaler</td>
<td>0 = just as told, 1 = not as told, but as needed for health, 2 = not as told and not as much as needed for health</td>
<td>12.43 (2,103) &lt; 0.001 0 &gt; 1.2</td>
<td>0.38 (2, 103) NS</td>
<td></td>
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</table>
during a routine visit. Indeed, in reviewing current outreach educational programs, we note that effort to individualize programs to meet the needs of families is considered a central element of intervention effectiveness.27,28 Programs that incorporate telephone contact with families may add questions pertaining to medication routines such as, when did the child take his last medication and what routines were involved?

The second factor that should be considered is whether the family feels overwhelmed or burdened by daily demands. If so, a different type of intervention is called for that addresses the family’s level of emotional stress and coping abilities. In the context of educational outreach programs, families who appear unduly burdened by routine care may benefit from a referral to professionals who can address resource and mental health needs.

Pediatric asthma management is a multifaceted activity. When families are able to create predictable routines around daily care, their children may be better equipped to follow doctor’s orders, may be less likely to have anxiety-related symptoms, and have a better overall quality of life. Pediatricians can play a valued role in assisting families to recognize their daily routines and use them to promote health in their patients.

REFERENCES

PATTERNS OF QUICK-RELIEF AND LONG-TERM CONTROLLER MEDICATION USE IN PEDIATRIC ASTHMA

NATALIE WALDERS, PHD, SHERYL J. KOPEL, MSc, DAPHNE KOINS-MITCHELL, PHD, AND ELIZABETH L. McQUAID, PHD

Objectives  To simultaneously examine adherence to long-term controller and quick-relief medications and to contrast patterns of medication use in children with asthma.

Study design  Cross-sectional, 1-month follow-up study conducted with 75 children ages 8 to 16 years diagnosed with persistent asthma and prescribed quick-relief and long-term controller medications by metered dose inhaler. Participants were a subsample of a larger adherence study. The primary outcome measure was adherence to both medications as measured by electronic monitoring devices. A classification framework for contrasting adherence patterns between medication classes was developed to identify cases for individual analysis.

Results  High levels of nonadherence to long-term controller medications (median = 46% of prescribed doses taken) and variable patterns of quick-relief medication use (range = 0 to 251 doses over the month) were documented, whereas consistent relationships between patterns of medication use across both classes were not found. Individual cases identified by the classification scheme illustrated the complexity and clinical utility of contrasting adherence patterns.

Conclusions  Monitoring long-term controller medication adherence may be more predictive of morbidity than quick-relief medication use, except in outlier cases in which monitoring both medication types may be valuable for clinical and empirical purposes. (J Pediatr 2005;146:177-82)

Asthma is the most common chronic illness in childhood and represents a leading cause of hospitalizations, emergency department visits, and functional limitation.1,2 Emphasis has been placed on attempting to identify patients at the highest risk for detrimental asthma outcomes.3 Medication adherence has been identified as a key factor in asthma outcome,4 and adherence assessment is important for research and clinical applications. A variety of assessment methods have been developed, including self-report,5 canister weighing,6 pharmacy records,7,8 and electronic monitoring.9 Although self-report and canister weighing are inexpensive, they tend to overestimate actual adherence.10 Analysis of pharmacy refill records is flawed by the inability to measure actual medication use and the need to access claims data8 or managed care organization records.11 Although electronic monitoring is a costly and technologically complex approach,12 it is generally accepted as the most accurate way to monitor adherence and has been widely used in both adult13,14 and pediatric research.10

Despite national guidelines designating the instrumental role of long-term controller medications in managing persistent asthma,9 actual medication adherence does not meet standard of care.16-18 Inadequate daily medication adherence (DMA) has been widely documented in pediatric9 and adult samples13,14 in contrast to the sparse data available on patterns of quick-relief, or PRN (as needed) medication use. Poor adherence to controller medications has been linked to morbidity,19 and increased health care costs.20 Research assessing potential determinants of medication adherence suggests that variables such as income,13 minority status,21 family dysfunction,22 and parental attitudes23 are associated with lower DMA. More remains to be learned in this area, such as how DMA relates to reliance on PRN medications.

Widespread nonadherence with controller medications contributes to overuse of PRN medications.24-26 However, PRN medication use is understudied. Several studies in the adult literature examined both classes of medications and reported an association between overuse of PRN medications and underuse of inhaled corticosteroids.27,28 A study

<table>
<thead>
<tr>
<th>DMA</th>
<th>Daily medication adherence</th>
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<tr>
<td>MDI</td>
<td>Metered-dose inhaler</td>
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From National Jewish Medical and Research Center, Denver, Colorado; and Bradley/Hasbro Research Center, Brown Medical School, Providence, Rhode Island.
Supported by grant funding from the American Lung Association (CG-002 and CI-002-N) and the National Institutes of Health (HD-37023).
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The current study used objective electronic monitoring techniques to simultaneously assess DMA and reliance on PRN medications. Based on previous studies, we expected to find insufficient DMA, and we predicted an inverse relationship between daily medication adherence and reliance on PRN medications. Moreover, we expected significant relationships between adherence patterns and asthma-related morbidity. In addition, a classification scheme for categorizing associations between DMA and reliance on PRN medications was developed to identify adherence pattern profiles for both medication classes.

**METHODS**

Use of DMA/PRN medications was examined within the context of a larger study of adherence to asthma medications across the transition to adolescence. This paper combines results on adherence to long-term controller medications from a subset of the data with an original series of analyses on patterns of quick-relief medication use. The methods of the larger study have been described in detail elsewhere. In brief, families were recruited from primary care clinics, by advertisements, and through a local asthma camp. Eligible children had physician-diagnosed asthma for at least 6 months before participation and had been prescribe one or more controller medications for daily use. Specifically, all eligible children had a form of persistent asthma and a prescription for at least one preventive medication delivered by metered-dose inhaler (MDI). Additionally, all children were prescribed PRN medications (eg, Albuterol), also delivered by MDI, for as-needed symptom control. Children with mild intermittent asthma were not included because these patients would not be prescribed a daily controller and would only necessitate PRN medication.

**Procedures**

Written parent consent and child assent were obtained in accordance with institutional review board guidelines. Data collection took place over a 1-month period. During an initial visit, children and parents completed questionnaire packets and were given a brief orientation to the MDILOG devices. Questionnaire packets assessed asthma-related morbidity in the previous year, including report of emergency room visits, hospitalizations, school absences, and physician visits.

Although all families were told that medication use was being recorded, they were not aware of the specific features of the MDILOG device (ie, the date stamp of inhalations and the capacity to detect actuations without inhalation). PRN use and DMA were recorded following the first study visit. The initial 3 days of data were excluded to minimize potential changes in medication use patterns due to device novelty. Participants received financial compensation for their time and for returning MDILOG devices.

**Measures**

**MDILOG data collection: DMA and PRN medication use.** The MDILOG electronic asthma medication monitor (Westmed Technologies Inc, Englewood, Colo) is a small device that is easily attached to inhalers and records the date and time of each MDI actuation, using a computer chip. It further indicates whether medication is actually inhaled by use of a temperature-sensitive thermistor. The MDILOG device performs a self-check and battery test nightly to help ensure data integrity. Procedures to quantify actual medication use through MDILOG data collection are described in detail elsewhere. In the current study, “dumping” was operationally defined as 10 or more actuations without inhalation in a 1-minute time span. Five cases were identified with medication dumping patterns and those events were eliminated from individual case files. Across the larger sample, more than 250 devices were assigned to study participants. The majority of devices were returned to the laboratory in working order; however, 9% were unrecoverable because of device failure, loss, or damage. No differences were found between cases of dumping or device malfunction and the remaining sample in terms of sex, asthma severity, race, or age.

**Daily medication adherence.** Daily medication adherence was calculated as total doses taken per day divided by prescribed doses per day. Throughout this paper, the term “dose” reflects a single “puff” of either class of medication. A “dose” was defined as an actuation that was recorded as an actual inhalation by the MDILOG. Days that reflected greater than 100% adherence, due to additional doses recorded, were truncated to 100%. Average daily adherence was computed across the entire study period. Mean adherence was computed across medications if a child had more than one prescribed daily medication.

**PRN medication use.** PRN data were summarized to provide information regarding episodic and typical patterns of use in three ways: (1) total number of doses across the study period, (2) number of days during which any PRN medication was taken, and (3) maximum number of PRN doses on any day during the study period.

**Data analysis.** Distributions of all variables were examined and probit transformations were conducted when appropriate to normalize distributions of proportional variables, such as DMA. Probit transformation involved converting all proportions to their corresponding z scores and then adding a constant to ensure that resulting values were positive. The first set of hypothesis testing examined associations between DMA and PRN, using both correlational analysis and logistic regression. Second, descriptive analyses were conducted to examine medication use patterns by determining...
the frequency of using daily medication alone, PRN medication alone, neither medication, or both medications on a daily basis across the full sample. Relationships between asthma morbidity and DMA and PRN use were also evaluated. Finally, a framework for categorizing the relationship between DMA and PRN was developed and used to select and describe representative case examples.

**RESULTS**

**Participants**

Eligible participants included children receiving concurrent monitoring of PRN use and DMA while participating in a larger study of asthma management. Detailed information concerning recruitment and participation rates for the full sample may be found elsewhere. In brief, 52% of those approached to participate were eligible, provided consent and completed the protocol, 37% were ineligible, and 11% were eligible yet uninterested. The most common reason for ineligibility was mild asthma or the lack of appropriate asthma medication for purposes of adherence monitoring by MDIlog devices.

The present subsample included 75 children with asthma, ages 8 to 16 (median = 11.57, SD = 2.09), 45% female. The current sample represents 72% of the original sample, with no significant differences found between the samples in terms of sex, age, race, socioeconomic status (SES), asthma severity, or asthma functional morbidity. The ethnic composition of the subsample included 68% white, 27% black, 4% biracial, and 1% Latino. All SES levels were represented within the sample, from unskilled laborers (level 1) to major professionals (level V). The average SES across families (median = 41.43, SD = 11.93) fell within Hollingshead level IV, corresponding to technical workers and minor professionals. Asthma severity ratings were assessed by a pediatric specialist in accordance with NIH guidelines, using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale. The range of persistent asthma severity using a series of questions adapted from an empirically validated scale.

Daily Medication Adherence

Variability in DMA was documented across the sample, ranging from taking prescribed medications none of the time to 98% of the time over the course of the month. On average, daily medications were taken 46% of the time (median = 0.46, SD = .28). Consistent with previous analyses for the full sample, an inverse relation was found between age and DMA in the present sample (r = −0.23, P < .05). No differences in DMA were found by sex, race, SES, or asthma severity. Additionally, adherence did not vary as a function of prescribed dosing patterns (eg, number of puffs prescribed per day).

**PRN Medication Use**

Reliance on PRN medication over the course of the month varied considerably, ranging from no doses to 251 total doses. PRN data were not normally distributed. On average, 38 doses of PRN medication were used in the month period (median = 30, SD = 50.22). Across the sample, 85% used their PRN medication at least once over the month, and 23% were frequent users with 2 or more PRN doses per day. Daily dosing of PRN ranged from no puffs to 18 puffs, with the average number of puffs per day at 1.41 (SD = 2.44) and the average maximum number of doses in a day at 5 puffs (SD = 4.07). Although rates of DMA varied according to age, no association was found between age and reliance on PRN use. Furthermore, no differences in PRN use patterns were found for race, SES, or asthma severity.

**Relation Between Patterns of DMA and PRN Use**

Contrary to the hypotheses that PRN and DMA use would demonstrate an inverse relationship (eg, higher DMA corresponding to lower reliance on PRN), a statistically significant relationship between medication classes did not emerge (Spearman ρ = 0.06, not significant). A logistic regression was conducted to further examine the day-to-day relation between PRN and DMA. The logistic regression model examined the probability that a child would not require PRN medication on a daily basis based on the extent of adherence to daily medication. The model accounted for the fact that the dataset contained multiple correlated observations of daily medication use over the course of the month. The regression was conducted on a subset of the full sample, excluding those subjects with one or more incomplete data points in the other class of medication use over the month period. No statistically significant results emerged from this analysis, indicating the lack of a coherent and consistent relation between PRN use and DMA across the full sample on a day-by-day basis.

**Daily Medication Use Patterns**

Records of daily medication use were subsequently examined in detail for the full sample. Days with accurate recordings of both PRN and DMA data were included in this analysis, excluding any instances of dumping or device failure, reflecting 97% of days in the sample. A total of 2042 days of data (from 2100 monitored days) were examined. Days with...
missing data from either one or both MDILogs caused by device flaw or recording error were excluded to allow for direct comparisons between DMA and PRN use. Across the sample, both medication types were taken on 30% of days and neither medication was taken on 27% of days. On 36% of days, DMA medication was taken, whereas PRN medication was not used. In contrast, on 7% of days PRN medication was used, whereas DMA medication was not taken. As a result, the most frequent medication pattern involved use of DMA medication without reliance on PRN medication; however, this only represented approximately one third of all monitored days.

Medication Adherence and Morbidity

Relationships between morbidity indicators in the previous year (ie, hospitalizations, emergency room visits, and school absences) and medication adherence were examined. In the year before adherence monitoring, 37% of the sample had required an emergency room visit and 17% had been hospitalized. On average, 7 school days had been missed in the previous year (range, 0 to 45 days; SD = 8.6) because of asthma complications. Although a negative relation emerged between level of DMA and emergency room visits \( (r = -0.25, \ P < .05) \) and school absences \( (r = -0.25, \ P < .05) \), no associations were found between any morbidity indicators and PRN use.

Classification Scheme for Categorizing DMA and PRN Relationships

Because of the lack of a linear relation between DMA and PRN use, we considered alternative exploratory approaches for investigating the interplay between medication use variables. A 2 \( \times \) 2 model was designed to represent “high” and “low” levels of DMA and PRN use, and case examples using this methodology were identified to characterize these relationships (Figure 1). Interquartile ranges were generated for PRN and DMA values, and the lowest and highest quartiles were used to identify group membership. From this classification approach, categories of “frequent medication users,” “ineffective adherence pattern,” “optimized adherence pattern,” as well as “infrequent medication users” were generated (Figure 1). Because we did not develop this model in an a priori fashion, it was not used for hypothesis testing purposes. Rather, we used this model to guide in the selection of case examples to illustrate patterns of medication use and to provide a framework for future research. This methodology provided a systematic approach for identifying informative case examples and conceptualizing extreme parameters of adherence patterns. The classification framework based on interquartile ranges classified 24% of the sample into 1 of the 4 domains. A total of 7 children were classified as frequent medication users, 3 were classified as ineffective adherence, 5 were identified as optimized adherence, and 5 were in the infrequent medication use domain.

The medication profiles of two subjects are displayed in Figures 2 and 3. Both examples reflect adherence patterns in patients with mild persistent asthma. The first example of a 16-year-old boy illustrates “ineffective adherence,” with a total of 107 PRN doses over the month, including 14 doses in a single day, and minimal adherence to his long-term controller medication at 11% of prescribed doses. This patient had a history of an emergency room visit and hospitalization in the previous year. In contrast, the second case example reflects “optimized adherence,” as evidenced by a 9-year-old boy with no doses of PRN medication and near perfect adherence with long-term controller medication at 97% of prescribed doses. This patient had no history of medical use in the past year.

**DISCUSSION**

Consistent with previous research,\cite{10,33} nonadherence to long-term controller medication was common in our sample, with less than half of all prescribed doses taken. Moreover,
patterns of PRN use were highly variable, ranging from no doses over the month to more than 250 doses. Based on the intended purpose of controller medications to prevent asthma symptoms, and the assumption that overreliance on PRN medications in the context of nonadherence to controller medications would serve as a proxy for poorly controlled asthma, an inverse relationship was anticipated between these medication classes. Contrary to expectation, no significant associations were identified. Additionally, although DMA did relate to emergency room visits and missed school days, no relation between PRN use and morbidity was found.

The lack of statistically significant relationships between DMA and PRN use and between PRN use and morbidity may be interpreted in several ways. First, the non-normal distribution evidenced by the highly variable patterns of PRN use limited our ability to detect linear associations between variables. Moreover, our data emphasize the complexity of PRN use and suggest that PRN use may not serve as an appropriate overall indicator for the quality of asthma management. For example, high PRN use may reflect frequent pretreatment for exercise, use of the same MDI by multiple family members, or high sensitivity to mild symptoms. In contrast, monitoring DMA may provide a less ambiguous assessment of asthma self-management in most situations. Important exceptions include case examples identified by our classification framework, which highlighted extreme medication use patterns.

Our study provides important descriptive data on adherence patterns that extend beyond the typical presentation of the extent to which DMA doses are taken as prescribed. Through the simultaneous monitoring and analysis of PRN and DMA patterns of medication use, several important results emerged. First, the fact that only one third of days involved what is considered to be "ideal" medication use (ie, compliance with daily medication and lack of reliance on PRN medication) and nearly one third of days involved no medication use whatsoever emphasizes that clinicians should attend closely to patient report of barriers to compliance. Additionally, the finding that only 7% of days involved reliance on PRN medication without daily medication use questions whether a one-to-one relationship generally exists between insufficient DMA compliance and intensified PRN reliance. The lack of a significant logistic regression result examining the relation between adherence to daily medications and the probability of being able to go without PRN medication also suggests that overreliance on PRN medication may not be a direct proxy for poor DMA. Examination of the association between DMA and PRN use may be most informative and instructive in extreme circumstances. The presented classification scheme based on interquartile ranges provides a new methodology for examining such instances.

The generalizability of our findings should be interpreted with the following limitations in mind. First, when monitoring PRN use, it is important to consider that patients may be prescribed PRN medications as pretreatment before physical exertion. In this regard, PRN medication can also be prescribed for preventive reasons, rather than in the typical reliever fashion, and regular use may be indicative of appropriate management rather than a response to symptoms. Our study was limited by the lack of a systematic assessment of physician recommendations for the use of PRN medication before objective monitoring. Additionally, our inability to objectively monitor PRN use by nebulizer in the small subset of children with both MDI and nebulizer medication delivery devices was another limitation. Our sample was limited to children with persistent asthma with access to treatment plans that incorporated anti-inflammatory inhalers. Monitoring PRN use patterns in patient samples without access to preventive medications may also reveal important findings. Finally, we were also limited by the unavailability of prospective morbidity data over a sustained period after the adherence monitoring phase and by the lack of simultaneous morbidity data over the monitoring period. Instead, we relied on retrospective parent report data from the month before study entry, which provided a less accurate characterization of morbidity, and adherence behaviors could have been influenced by previous morbidity events. The optimal approach would have involved collection of objective morbidity data (eg, medical chart review) over a long-term period incorporating retrospective, concurrent, and prospective time periods.

Adherence research presents a series of methodologic and measurement challenges, and these challenges appear even more complex when investigating medications prescribed on a PRN basis. To obtain the most systematic assessment of PRN use, the following are recommended. First, objective measurement by electronic monitoring is an essential component of a rigorously designed study, in contrast to less methodologically sound approaches, such as self-report. In addition, researchers should obtain detailed information concerning prescribed PRN use from physicians (eg, when symptomatic, before exercise, and according to peak flow readings). Moreover, the use of daily diaries recording reasons for each PRN use, in accordance with electronic monitoring, may shed light on patterns of PRN medication use. Finally, analysis of time-stamping data available on MDILog devices...
and monitoring for longer than a single month would provide more detailed analysis and interpretation of medication usage patterns beyond those examined in the current study.

Our results suggest that the utility of concurrent analysis of PRN use and DMA may yield findings with limited interpretability. An alternative approach stemming from the classification framework presented involves examining PRN and DMA at extreme ends of distribution ranges to serve as critical markers for identifying children with problematic adherence patterns. Further study is necessary to determine whether the framework serves to identify patients at increased risk of detrimental asthma morbidity and associated outcomes. Efforts to more closely examine patients evidencing the “ineffective adherence” pattern may be a particularly useful next step to increase our understanding of the complex and sometimes counterintuitive relationship between adherence behavior and asthma outcomes.

Effective asthma management is composed of a range of complex behaviors, including strategies for trigger avoidance, acute symptom assessment and management, and effective collaboration with a health care team. Appropriate use of DMA and PRN medications should be seen as elements of a more comprehensive asthma management plan. Novel strategies to enhance appropriate medication use, such as tracking the use of long-term controller medications and providing feedback regarding actual use, show some promise for effecting change in adherence. Patients evidencing problematic patterns of overreliance on PRN medications in the context of nonadherence with controller medications may benefit from such behavioral approaches to enhance illness management.

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CONDUCTIVITY DETERMINED BY A NEW SWEAT ANALYZER COMPARED WITH CHLORIDE CONCENTRATIONS FOR THE DIAGNOSIS OF CYSTIC FIBROSIS

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Objectives The aim of the study was to determine if a new conductivity measuring sweat test system (Nanoduct) could reliably identify patients with cystic fibrosis (CF) and differentiate them from healthy subjects.

Study design On the same day and in the same patient, the new system was tested in comparison with the Macroduct sweat collection system measuring chloride concentration and osmolality.

Results Subjects (n = 111) 3 weeks to 60 years of age were investigated. Three children had no sweat production, and in 14 children, only conductivity could be measured. In the remaining 94 subjects, the new system identified all patients with classic CF (mean conductivity, 115 mmol/L; range, 92 to 137) and differentiated them from healthy subjects (mean conductivity, 36 mmol/L; range, 17 to 59) within a mean time of 20 minutes.

Conclusions Measuring sweat conductivity using the new test system reliably differentiated between patients with and those without CF. This suggests that the new system could be used as a diagnostic test in addition to its suggested screening value. (J Pediatr 2005;146:183-8)

Making the diagnosis of cystic fibrosis (CF) is not always simple, but determining sweat chloride concentration is still the gold standard test. Since the inception of the sweat test 50 years ago, the pad method, using quantitative pilocarpine iontophoresis (QPI), is believed to be the most accurate test. This method is well established but time-consuming and open to many sources of error. It has been shown to be reliable even for infants in laboratories with trained, experienced and skilled technicians, but in our hands, difficulties arose in newborn infants during the first month of life because at least 75 mg of sweat should be obtained on gauze or filter paper in 30 minutes for an accurate result.

In the past, there have been many improvements in the method for sweat collection. These days, the Macroduct coil system (referred to as the macro-collection system throughout the text) is widely used and internationally accepted. This system collects sweat within a coil of fine plastic tubing, from which it can be taken and analyzed immediately for ionic composition or conductivity. This minimizes evaporation and condensation. However, as with traditional QPI, the collection of a sufficient amount of sweat (15 μL in 30 minutes) can still be difficult, especially in very young children. In addition, measuring quantitative chloride concentration is time-consuming, and the result is often not available immediately. Many studies have been performed to evaluate the use of osmometry and conductivity in sweat, which suggested that both methods are as effective as QPI in their ability to discriminate for diagnosis of CF. These methods are simpler and their results are available within a shorter time.

Nanoduct is a novel, simple, and practical sweat analysis system to measure sweat conductivity in situ. It requires only 3 μL of sweat, and results are available within 30
minutes. In this study, we wanted to determine if the new system could reliably identify patients with CF and differentiate them from healthy subjects.

METHODS

On the same day and in the same patient, the new sweat analysis system (Wescor Inc, Logan, Utah) measuring sweat conductivity was tested in comparison with the macro-collection system (Wescor) and colorimetric chloride and osmolality determination. The tests were performed on the left and right forearm, respectively. All were carried out in the respiratory outpatient clinic at the Children’s Hospital in St Gallen, Switzerland, by two specially trained assistants between June 2002 and July 2003. The Children’s Hospital in St Gallen is a tertiary hospital for the eastern area of Switzerland, with a population of almost 1 million.

Study Population

Subjects who were tested belonged to two groups: The first group included 21 patients (age, 2 to 24 years) known to have CF and who were willing to have a sweat test during their routine visit to the CF outpatient clinic. There were no exclusion criteria. The diagnosis of CF was made earlier on the basis of positive sweat test results in duplicate (chloride concentration >60 mmol/L and osmolality >200 mmol/kg) and/or a positive result on gene mutation analysis, plus typical clinical symptoms according to current international consensus.1,5,13,15,16,21 Twenty patients had classic CF with chronic lung disease and pancreatic insufficiency: 17 were homozygous for ΔF508 and 3 were heterozygous for ΔF508 and an additional CF allele (1717-1G, 3905insT, or R553X). One child had a nonclassic form of CF21 with chronic rhinosinusitis and nasal polyps (heterozygous ΔF508 and a new deletion 3271 + 39delG with 4 single nucleotide polymorphisms).

The second group (control group) included children and adult patients (n = 85) recruited from the respiratory outpatient clinic who were referred for investigation of a variety of pulmonary symptoms or for excluding CF. We did sweat testing on the request of the referring doctors or based on the indication for excluding CF by the study coordinator. An alternative diagnosis to CF was made in every case. In addition, 5 healthy volunteers (2 students, 2 residents, and 1 physiotherapist) were tested. At each test procedure, subjects and parents, respectively, were asked about the discomfort of the method and which test method they preferred without using a structured questionnaire. Informed consent was obtained from all subjects and/or their parents, and the study was approved by the local ethics committee.

Sweat Collection and Chloride Determination

The macro-collection system includes an iontophoretic unit with a battery-operated current source, which, during a 5-minute period, raises, keeps constant, and then lowers the current. The maximum current during the procedure is 3 mA for 5 to 7 minutes (Webster Sweat Collection System 3500, Wescor). The electrodes of the unit are made to hold pilocarpine-containing gel disks with a diameter of 2.8 cm (Pilogel 0.5%). After iontophoretic stimulation, the area of the forearm of the patient was cleaned with deionized water, then dried, and the collector system was attached with straps. This disposable collector consists of a slightly concave plastic disk with a hole in the center. This hole is connected to a small, plastic tube (internal diameter, 0.65 mm) coiled over the top of the disk. Sweat, secreted under pressure after pilocarpine stimulation, is forced through the hole into the tubing with minimal dead space. A small amount of a water-soluble dye (10 μL) on the concave surface of the disk allows easy visualization of the sweat collected. The amount of sweat is sufficient when the capillary is filled, which usually takes approximately 30 minutes. However, it varies considerably from patient to patient. In case of slow sweat production, the collection was—according to the manufacturer’s recommendation—continued for as long as 1 hour to maximize the sweat yield. Sweat chloride concentration was then measured coulometrically, by using the Chloride Analyzer 925 (Ciba Corning Diagnostics, Switzerland), and osmolality was measured by using the Vapor Pressure Osmometer 5520 (Wescor). For determination of both chloride and osmolality, a minimum of 30 μL sweat was required, which is equivalent to approximately 30 mg sweat. Sweat chloride concentration of >60 mmol/L and osmolality of >200 mmol/kg were regarded as positive for CF, and sweat chloride of <40 mmol/L and osmolality of <170 mmol/kg were taken as normal.1,3,15,16,21

New Sweat Test Analysis System

The new micro-flow conductometric device measures sweat conductivity.20 Briefly, the system incorporates the classic method of inducing sweat by pilocarpine iontophoresis. The pilocarpine is absorbed into the skin of the patient from pilocarpine iontophoretic disks by a controlled DC electric current source supplied by the new inducer/analyzer. The maximum current flow during the procedure is 0.5 mA for 2 to 3 minutes. The iontophoresis is followed by a continuous-flow analysis of sweat conductivity through the use of a newly developed conductivity sensor. After pilocarpine stimulation, the electrode-gel assembly at the anode is removed from its holder and the stimulated skin within the ring of the holder is washed free of salt with a cotton swab and deionized water and dried with fresh swabs. The disposable conductivity sensor cell is immediately inserted into this holder at the anode. The cathode electrode remains in place to provide an electrical contact with the skin, essential for the measurements. Electrodes and the sensor are connected to the inducer/analyzer by a single control cable. The continuous-flow principle allows the initial sweating rate to be displayed in grams per square meter of skin surface per minute, which is important in accepting sweat test results. Its continuous sweat flow sensor requires only 3 μL of sweat. Measured sweat conductivity is approximately 15 mmol/L higher than the sweat chloride concentration because of additional anions such
as lactate and bicarbonate, therefore a value of >80 mmol/L is regarded as consistent with CF.\textsuperscript{17}

### Statistical Analysis

Descriptive statistics, graphical analysis, and analysis of differences between the two sweat tests by 2-sided paired \( t \) tests were all performed with S-PLUS 6.0 (Insightful Corp, Seattle, Wash). The Fisher exact test was used for comparison of failure rates. The exact Wilcoxon-Mann-Whitney test was used for comparison of collection times in patients with CF and those without, by means of the macro-collection system; 95\% confidence intervals were calculated for the sensitivity and specificity of the new system with StatXact 5.0.3 (Cytel, Cambridge, Mass).

### RESULTS

Subjects (\( n = 111 \)) 3 weeks to 60 years of age underwent sweat testing; 64 (58\%) were male. In 17 subjects (15\%) with a median age of 1.3 years (range, 1 months to 7 years), all belonging to the control group, one or both tests could not be performed: In 3 children, neither the chloride determination nor the new sweat test system could be performed because of absent or insufficient sweat production (<3 \( \mu L \)). In 14 children, only conductivity determined by the new system could be measured (mean value, 32 mmol/L; range, 5 to 57). Ten of these 14 children had insufficient sweat production (<15 \( \mu L \)) to measure osmolality or chloride concentration, and only conductivity using the new system could be measured. In 4 cases, sweat leaked out of the macro-collector either on transport to the laboratory or while processing in the laboratory. The failure rate of the macro-collection system was significantly higher than that of the new system (\( P = .003 \)).

In the remaining 94 subjects (85\%), the new sweat test reliably identified all patients with CF and differentiated them from healthy subjects (\textit{Table} and \textit{Figure 1}). Sensitivity and specificity both were 100\%, with an exact 95\% CI of 83\% to 100\% and 95\% to 100\%, respectively. The regression line between conductivity and chloride concentration had an intercept of 23, slope of 0.85 (95\% CI, 0.80 to 0.90), \( R^2 = 0.94 \), and \( S_E = 112,579 \). The two sweat test methods (old versus new system) gave significantly different values: The mean difference was 7 mmol/L in classic CF (\( t = 2.87, P = .010 \)) and 21 mmol/L in healthy subjects (\( t = 19.6, P < .001 \)) (\textit{Figures 2 and 3}). The mean difference over both groups was 17 mmol/L (SD, 11; 95\% CI, 15 to 20). The mean difference of the two methods was significantly higher in healthy subjects than in patients with classic CF (\( t = 5.90, P < .001 \)).

Two children had normal conductivity (59 and 31 mmol/L, respectively) and normal chloride (19 and 13 mmol/L, respectively), but their osmolality values were abnormal (205 and 290 mmol/kg, respectively).

The mean sweat amount with the macro-collection system was 44 \( \mu L \) (range, 8 to 105). Seventy-seven (70\%) subjects had enough sweat (>30 \( \mu L \)) to measure both chloride and osmolality. However, 17 healthy subjects (median age, 1.2 years; range, 2 months to 23 years) had insufficient sweat production (<15 \( \mu L \)) with the macro-collection system, and only osmolality could be measured (mean value, 114 mmol/kg; range, 83 to 156). The mean sweat collection rate with the new system was 3.3 g/m\(^2\) per minute. Only two healthy children had a sweat rate below 1 g/m\(^2\) per minute; they were not included in the comparison of chloride versus conductivity.

The duration of the new sweat test (from beginning of induction until the receipt of the result) took 20 minutes on average (range, 13 to 31). In contrast, the mean induction time (including preparation) for iontophoresis with the macro-collection system was 13 minutes (range, 6 to 20) and the mean duration of the sweat collection was 33 minutes (range, 13 to 49). Macro-collection times did not differ significantly between patients with CF and healthy control subjects (median, 30 and 31.5 minutes, respectively; standardized Wilcoxon Mann-Whitney statistic, 0.13; \( P = .90 \)). The average time until the receipt of the final result from the laboratory was several hours; in most cases the results were not available before next day.

Overall, 97\% (108 of 111) of the subjects and parents, respectively, preferred the new sweat test mainly because the result was available quickly; only 3 subjects had no opinion. Forty-nine (44\%) subjects indicated that there was less discomfort with the new sweat test and 33 (30\%) had equally uncomfortable sensations. Only 2 subjects indicated that they

### Table. Conductivity versus chloride and osmolarity

<table>
<thead>
<tr>
<th>Conductivity (new system) mmol/L</th>
<th>Chloride concentration (macro-collection system) mmol/L</th>
<th>Osmolality (macro-collection system) mmol/kg</th>
</tr>
</thead>
<tbody>
<tr>
<td>Healthy subjects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(n = 73)</td>
<td>36 ± 1.0</td>
<td>15 ± 0.6</td>
</tr>
<tr>
<td>Classic CF</td>
<td>9 (17–59)</td>
<td>5 (7–34)</td>
</tr>
<tr>
<td>(n = 20)</td>
<td>115 ± 2.8</td>
<td>108 ± 2.3</td>
</tr>
<tr>
<td>Nonclassic CF</td>
<td>12 (92–137)</td>
<td>10 (90–130)</td>
</tr>
<tr>
<td>(n = 1)</td>
<td>60</td>
<td>54</td>
</tr>
</tbody>
</table>

Values are shown as mean ± SEM, SD, and ranges in parentheses.
had a little more discomfort with the new sweat test; 27 (24%) subjects sensed no discomfort at all.

**DISCUSSION**

This study compared conductivity measurements by using the new sweat analyzing system to sweat chloride measured quantitatively by obtaining sweat with the macro-collection system. The study has shown that measuring conductivity with the new sweat test reliably differentiated between patients with CF and those without CF. Using the manufacturers recommended cutoff value of 80 mmol/L, there were no false positive or negative results, which suggests that the new sweat test system can be used as a diagnostic test in addition to its putative fast screening value in an outpatient application.

Measuring conductivity was first described more than 50 years ago by Licht and Shwachman, and this method was put forward as a simple and practical diagnostic test in children. Hammond et al have demonstrated that conductivity measured with the macro-collection system and Sweat-Check analyzer (which is the predecessor of the new system) is as effective as chloride in its ability to discriminate diagnostically between patients with CF and those without CF. They compared the macro-collection system and conductivity analysis with QPI in 1090 patients over a period of 10 years. The main disadvantage with the macro-collection system was the insufficient sweat amount in 6.1% of the patients, compared with 0.7% with the QPI. Heeley et al confirmed their findings that sweat conductivity is as effective as chloride measurement. The mean conductivity for CF was 110 mmol/L (67 to 141) and for healthy subjects, 37 mmol/L (18 to 71). However, because the authors did not provide sufficient data of method comparison studies (eg, bias studies, regression plots), the validity of the study has been questioned. Mastella et al found a good sensitivity and specificity for the conductivity analyzing systems: No patient detected by the classical QPI technique was considered negative by conductivity. However, 9.1% of all patients had less than 15 μL sweat, which is necessary for the measurement of conductivity. Most of the inadequate collections occurred in the age group younger than 4 months. The largest study was performed by Lezana et al, who used the Sweat-Chek analyzer in 3834 subjects (age, 1 month to 54 years; median, 1.8 years) over a period of 10 years. They found a median conductivity of 111 mmol/L (82 to 148) for patients with CF.
(n = 294) and 36 mmol/L (12 to 89) for healthy subjects. Their calculated best cutoff value to diagnose CF was ≥90 mmol/L, with a sensitivity of 99.7% and a specificity of 100%; the best conductivity cutoff value to exclude CF was <75 mmol/L. This study was also hampered by the lack of regression or bias studies.

Despite these studies, the American National Committee for Clinical Laboratory Standards does not accept conductivity as a definitive diagnostic tool, and the American CF Foundation recommends conductivity only as a screening test. According to the Foundation, a patient having a sweat conductivity ≥50 mmol/L should be referred to an accredited CF care center for a QPI. In general, a conductivity value <50 mmol/L is considered normal by the CF Foundation. Taking a cutoff level of 90 mmol/L, as chosen by Lezana et al., conductivity could also be used as a diagnostic test in addition to its screening value. In our study, we chose the manufacturer’s recommended cutoff value of 80 mmol/L, which had a sensitivity and specificity of 100% in our study population. On the basis of our study results, the upper limit of normal conductivity is 54 mmol/L (+2 SD ≥97th percentile) and the lower limit being diagnostic for CF is 91 mmol/L (−2 SD ≥3rd percentile). Therefore, we considered conductivity values between 60 and 80 mmol/L as borderline values (gray area in Figure 1). The only child with nonclassic CF in our study had a conductivity of 60 mmol/L and a chloride concentration of 54 mmol/L, which are considered borderline values in both tests.

So far, only one study comparing the new system with QPI has been published in abstract form. Losty et al investigated 86 patients, of whom 34 had CF. With an upper limit of normal of 60 mmol/L, conductivity had a specificity of 96% in ruling out CF. Losty et al observed an excessive false-negative rate, which was explained later by a defective batch of sensors.

Evaporation can influence the results of sweat testing. The American National Committee for Clinical Laboratory Standards recommends that the sweat collection time should not exceed 30 minutes; extending the collection time will not significantly increase the sweat yield and may lead to sample evaporation. Our average sweat collection time with the macro-collection system was 33 minutes (range, 13 to 49). Because there was no significant difference in collection time between the patients with CF and the healthy control subjects, we assume that evaporation during sweat collection can be neglected. However, 2 children in our study population had normal conductivity (59 and 31 mmol/L, respectively) and normal chloride (19 and 13 mmol/L, respectively), but their osmolality collected by the macro-collection system was abnormal (205 and 290 mmol/kg, respectively). Both measurements were made on a very hot day with unusually high inside and outside temperatures. Although the measurement of chloride was done immediately, the measurement of osmolality was delayed for a few hours.

Sweat conductivity is a reflection of all anions in sweat; therefore, on average, sweat conductivity is approximately 15 mmol/L higher than chloride. We observed 3 children and 2 adults with CF in whom conductivity was lower than chloride (Figure 2). This observation and the fact that the slope of the regression line in Figure 1 is significantly below 1.0 and that the mean difference of the two sweat test methods is significantly lower in patients with CF than in healthy subjects can be explained by two possible mechanisms: In sweat probes with high concentrations of ions, either chloride is falsely measured too high or conductivity is falsely measured too low. Our study cannot explain which of the two explanations is correct.

The new pilocarpine disks of the new system allow maximal gland stimulation after only 2 to 3 minutes of iontophoresis at 0.5 mA total current, which results in less discomfort, reduced investigation time, and better safety. The small dimensions of the conductivity sensor allows a reading to be obtained with a minimum sweat production of only 3 µL, which can be particularly valuable in small children. In our study, the failure rate of the new system caused by insufficient sweat was only 2.7% (3 of 111), which was significantly lower (P = .003) compared with the macro-collection system (15.3%). Although the new system is designed for neonates, it was equally effective in patients of any age. The quick receipt of the results minimized the waiting time for the parents and patients, so it was not surprising that nearly all participants and parents preferred the new sweat test.

In summary, the new sweat analyzing system is simple and quick, and the results are reliable. Our study suggests that the new system can be used as a diagnostic test in addition to screening. A positive result should always be confirmed with another sweat test by measuring conductivity or chloride concentration or by gene mutation analysis.

REFERENCES

Objective  To assess the relationship between pancreatic enzyme therapy (PET) and the clinical outcomes of growth, abdominal pain, constipation, gassiness, and number of stools in cystic fibrosis (CF).

Study design  Patients (n = 1215) >4 weeks of age from 33 Cystic Fibrosis Foundation accredited sites who had a sweat chloride >60 mmol/L or two CF-causing mutations were enrolled using a proportionate sampling strategy in a nonblinded study. Patients submitted a stool sample and completed a questionnaire. The study coordinator also completed a questionnaire for each patient. Enzyme dosing and growth, abdominal pain, gassiness, constipation, and number of stools were compared.

Results  Of the 1215 enrolled patients, 1131 (93.1%) were prescribed PET. Only 14.9% had pancreatic function assessed before enrolling in this study. Stool elastase-1 analysis identified 1074 (89%) patients as pancreatic insufficient (PI). There was no association between PET and the outcomes: growth, abdominal pain, gassiness, constipation, and number of stools.

Conclusion  PET dose is not correlated with growth or gastrointestinal symptoms. More sensitive outcome measures of the effectiveness of PET in patients with CF are needed to guide treatment of PI. (J Pediatr 2005;146:189-93)

Pancreatic insufficiency (PI) occurs in 89% of patients with cystic fibrosis (CF).1 Malabsorption occurs in PI and can compromise nutritional status and health. Replacement of pancreatic enzymes is an important treatment in patients with CF. However, information is lacking on the effectiveness of pancreatic enzyme therapy (PET) on growth. Additionally, information on PET dosing and on whether a clinically identifiable dose response relationship exists for PET and common gastrointestinal symptoms in CF are lacking.

Patients are classified in the Cystic Fibrosis Foundation (CFF) Registry as PI or pancreatic sufficient (PS) based solely on whether or not PET was prescribed.2 Recently, we showed that misclassification of patients as PI or PS occurs.1 For patients with PI, PET dosing is based on whether signs and symptoms of malabsorption are present or persist on a given dose of enzymes.3 Signs and symptoms of malabsorption are self-reported and include frequent or loose bowel movements, excessive gas, and stomach pain.4 Care provider observation of poor growth also is used to adjust PET doses, and in some cases, additional studies such as 72-hour fecal fat may be used.4

We hypothesized that PET is associated with improved growth, ie, patients taking a higher dose of enzymes would have better growth than those taking a lower dose. We also hypothesized that PET has a positive effect on abdominal pain, gassiness, and diarrhea.

METHODS

Inclusion Criteria

Criteria included: patients with CF from CFF-accredited centers, sweat chloride >60 mmol/L or two CF-causing mutations, age >1 month, and willingness to fill out a questionnaire and to collect a small, random stool specimen without stopping or changing PET. Exclusion criteria: acute diarrhea, surgically created enterostomy (excluding gastrostomy), or short bowel syndrome. The local coordinator completed a questionnaire.
Data was blinded at the coordinating center. Institutional review boards at the coordinating center and local sites approved the protocol.

Based on the 2000 CFF Patient Registry, there were 21,000 patients with CF. The patients were stratified according to age groupings: 4 weeks to 5 years 12 months, 6 years to 13 years 12 months, and 14 years and older. An analysis after 1000 patients allowed termination based on the trend analysis and results from the interim reports.1

Fecal elastase-1 was quantitated using a human monoclonal enzyme-linked immunosorbent assay test (ScheBo Biotech Pancreatic Elastase kit, Marietta, Ga); PI was defined as fecal elastase-1 ≤200 μg/g of stool.

The patient questionnaire asked if PET was used. If yes, the patient recorded the brand name and amount of PET with snacks and meals. The respondent was asked: “Do you have stomachaches at least once a week, are you constipated at least once a week, are you very gassy and how many stools do you have each day, 0-1, 2-3, or >4?”

The local coordinators provided the weight and height at the most recent clinic visit, sweat chloride level, genotype, whether PET was prescribed, symptoms present at the time of diagnosis, whether the patient had ever had pancreatitis, and which if any pancreatic function tests had been performed.

Sample size and power calculations were determined using a proportionate sampling strategy stratified by site size, gender, and age distribution of patients. Data were computerized using standard nomenclature and source codes in Microsoft Access (Microsoft Corporation, Seattle, Wash). Quality measures were performed on 100% of the sample.

<table>
<thead>
<tr>
<th>Age</th>
<th>CF database</th>
<th>Planned enrollment</th>
<th>Actual enrollment</th>
<th>Study</th>
<th>CF database</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
<td>Number</td>
<td>%</td>
</tr>
<tr>
<td>4 wk-5 y</td>
<td>4520</td>
<td>22</td>
<td>550</td>
<td>313</td>
<td>26</td>
</tr>
<tr>
<td>6 yr-14 y</td>
<td>7230</td>
<td>34</td>
<td>850</td>
<td>477</td>
<td>39</td>
</tr>
<tr>
<td>&gt;14 y</td>
<td>9250</td>
<td>44</td>
<td>1100</td>
<td>425</td>
<td>35</td>
</tr>
<tr>
<td>Total</td>
<td>21,000</td>
<td>100</td>
<td>2500</td>
<td>1215</td>
<td>100</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Age</th>
<th>Number PI enrolled</th>
<th>% PI enrolled</th>
<th>Number PS enrolled</th>
<th>% PS enrolled</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 wk-5 y</td>
<td>284</td>
<td>90.7</td>
<td>29</td>
<td>9.3</td>
</tr>
<tr>
<td>6 yr-14 y</td>
<td>427</td>
<td>89.5</td>
<td>50</td>
<td>10.5</td>
</tr>
<tr>
<td>&gt;14 y</td>
<td>363</td>
<td>85.4</td>
<td>62</td>
<td>14.6</td>
</tr>
</tbody>
</table>

Table I. Proportion of patients with CF enrolled in study compared with the 2000 Cystic Foundation database

Table II. Percent of PI and PS in patients enrolled in study

Figure 1. Growth in children with CF. A, Patients ≤ 2 yrs. B, Patients 3-20 yrs. C, Patients > 20 yrs.
during data collection. Prevalence rates of PI versus PS for the sampled population were compared with PET using parametric and nonparametric test procedures. We examined differences in proportion and skewed distributions in the data using bivariate analyses (Fisher’s exact test). A Bonferroni correction was applied before completion of statistical testing. All statistical analyses for combination 2

3

2 comparative testing used reference standards (eg, age group >14 years; # stools/day >4). The Statistical Analysis Systems software package (SAS Institute Inc, Cary, NC) was used.

This is the second report of findings from a large national study on stool elastase.

**RESULTS**

We enrolled 1215 subjects at 33 sites (50.1% male) over 6 months. Our sample was not significantly different from the CFF registry for age and gender. Approximately 10% of the sample was PS. There were significantly (Fisher’s exact test = 0.032) more patients with PS in the older age group than in the younger groups (Tables I,II).

Of the 1215 enrolled patients, 1131 (93.1%) were prescribed PET and 1110 (98.1% of those prescribed PET) reported taking the prescribed PET daily. PET was prescribed despite the fact that only 14.9% of the patients had ever had any specific test of pancreatic function performed. There was no association between the incidence of pancreatitis or liver disease reported by the study coordinator and the dose of PET.

Table III. Gassiness, constipation, and stomachaches in patients with CF

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Total patients</th>
<th>&lt;6 years</th>
<th>6-14 years</th>
<th>&gt;14 years</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PI n (%)</td>
<td>PS n (%)</td>
<td>PI n (%)</td>
<td>PS n (%)</td>
</tr>
<tr>
<td>Gassy</td>
<td>574 (53.6)</td>
<td>49 (34.8)</td>
<td>139 (49.5)</td>
<td>6 (20.7)</td>
</tr>
<tr>
<td>Constipation</td>
<td>568 (53.1)</td>
<td>48 (34.0)</td>
<td>139 (49.5)</td>
<td>6 (20.7)</td>
</tr>
<tr>
<td>Stomachaches</td>
<td>117 (10.9)</td>
<td>18 (12.8)</td>
<td>21 (7.4)</td>
<td>2 (6.9)</td>
</tr>
</tbody>
</table>

*Fisher's exact test = .013; χ² = P < .012.
*Fisher's exact test = .014; χ² = P < .02.

Table IV. Pancreatic enzyme intake and gassiness, constipation, and stomachaches in PI patients with CF

<table>
<thead>
<tr>
<th>Enzyme intake (units/kg/meal)</th>
<th>Number reported being very gassy n (%)</th>
<th>Number reported being constipated n (%)</th>
<th>Number reported having stomachaches n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>&lt;500</td>
<td>58 (10.1)</td>
<td>57 (10.0%)</td>
<td>16 (13.7)</td>
</tr>
<tr>
<td>500-999</td>
<td>91 (15.9)</td>
<td>91 (16.0%)</td>
<td>21 (17.9)</td>
</tr>
<tr>
<td>1000-1499</td>
<td>130 (22.6)</td>
<td>130 (22.9%)</td>
<td>28 (23.9)</td>
</tr>
<tr>
<td>1500-1999</td>
<td>121 (21.1)</td>
<td>120 (21.1%)</td>
<td>24 (20.5)</td>
</tr>
<tr>
<td>2000-2499</td>
<td>84 (14.6)</td>
<td>83 (14.6%)</td>
<td>12 (10.3)</td>
</tr>
<tr>
<td>2500 and &gt;</td>
<td>90 (15.7)</td>
<td>87 (15.3%)</td>
<td>16 (13.7)</td>
</tr>
</tbody>
</table>

There were 164 patients ≤2 years of age, of whom 16 were PS. Four patients with PS (25%) had a weight/height ratio <5th percentile. In contrast, 8.8% (13 children) of patients with PI had a weight/height ratio <5th percentile (Figure 1A). Compared with the normal population, twice as many patients with PI and 5 times as many patients with PS
were <5th percentile. From 3 to 20 years of age (Figure 1B; 84 PS and 725 PI), there was no difference in the distribution of body mass index (BMI) percentiles between PS and PI. There were 241 patients >20 years of age (41 PS, 200 PI). Approximately 41% of patients with PS >20 years of age were within the healthy range of BMI compared with 74% of the patients with PI (Figure 1C). Approximately the same proportion of patients with PS and PI were malnourished using Centers for Disease Control criteria.6 We found no relationship between the dose of PET and weight/height ratio, BMI percentile, or BMI.

Tables III and IV show the relationship between being gassy, having constipation, or having stomachaches and PET. There was no significant difference between patients with PS and PI in the number of daily stools, and the dose of PET had no effect on the number of stools (Figure 2, Table V). Gassiness and constipation were present in significantly more patients with PI than PS (P <.012 and P <.02, respectively). However, higher PET dose was not associated with a decrease in these symptoms.

### DISCUSSION

The importance of nutrition for CF patients cannot be overestimated, and optimal nutrition may be difficult to achieve when repeated infections, declining pulmonary function, and malabsorption occur. Our hypothesis that the dose of PET is associated with improved growth and fewer symptoms is not supported by the findings of this study. Replacement PET is an important treatment for patients with CF, but information on how to dose PET and whether a clinically identifiable dose response relationship exists is lacking. Patients are described as PI or PS in the CFF Registry based solely on whether or not PET is prescribed.1 We reported that 93.1% of patients with CF were prescribed PET despite the fact that only 14.9% had ever had any specific test of pancreatic function performed.1

To compare growth in patients with PS and PI, we used the Centers for Disease Control growth curves that include weight/height ratio for children ≤2 years of age, BMI percentiles for 2 to 20 years of age, and BMI for >20 years of age.5,6 As a group, patients with PI had better growth, and, when growth was completed, they had healthier BMIs than patients with PS. The high proportion of poorly growing patients with PS ≥2 years of age suggests these children should be carefully evaluated. Patients with PS may have gastrointestinal dysfunctions other than PI such as impaired small bowel or liver function that result in malabsorption; they may eat less, have more illnesses, or have another coexisting disease. Any one of these conditions could negatively affect growth and could be overlooked if the focus is limited to PI.

Infants and young children who have failure to thrive when they are diagnosed with CF should have an objective measure of pancreatic function.

Patients with PS and PI 3 to 20 years of age had similar growth. It may be that 3 to 20 years of age is a nutritionally less demanding time, overall health is better, or it may be that PI is a more serious disease and that the sickest and least healthy children had died. Our data also could be interpreted to indicate that the prescription of PET was appropriate and sufficient and supported growth at the same rate as occurs in patients with PS. However, we found no relationship between PET dose and BMI percentile.

In this study, 9.6% of patients with CF reported abdominal pain at least once a week, approximately the same incidence as the general population.7-9 There was no difference between patients with PI or PS. This incidence is far less than the 27% reported by Durno et al10 in their retrospective chart review. The chart review would allow for cumulative data over 30 years and would identify those patients who in our study had not yet experienced abdominal pain. The data reported in this paper is cross sectional but was directly reported by the family/patient.

In the general population, the incidence of constipation varies from about 15% in adults11 to 34% to 37% in children from birth to 12 years of age.12 In a chart review of 168 patients with CF >5 and <30 years of age,13 the incidence of constipation was 32% for PS and PI combined. We observed 1.5 times the incidence of constipation in patients with PI compared with patients with PS. Further, there was no correlation between constipation and PET dose, suggesting constipation cannot be used as a marker of appropriate PET dosing. The commonly held belief that constipation is a consequence of high enzyme doses is not supported by this data.

About 1.6 times more patients with PI reported gassiness compared with patients with PS. Our results suggest that increasing PET dose does not improve the patients’ subjective sense of gassiness. The prevalence of gassiness was
similar to that of constipation and suggests that treatment for constipation be offered for gassy patients.

In the non-CF population, mean bowel movements are reported to be 1.8, 1.4, and 1.0 per day for children 6 to 12 months of age, 1 to 3 years of age, and >3 years of age, respectively. Both patients with PI and patients with PS reported more stools per day than the non-CF population. Because diarrhea is the main clinical expression of malabsorption, we expected to find more patients with PI reporting a higher number of stools per day compared with those with PS and that increasing the PET would be associated with fewer stools. In fact, we found no difference between the patients with PI and the patients with PS in the reported frequency of stools, and PET dosing had no effect on the reported number of stools. This suggests the number of stools per day is not a good clinical discriminator for making decisions about PET dosing.

In conclusion, in this multi-center study of 1215 patients with CF, we found no association with reported PET dose and abdominal symptoms or growth. These findings suggest that underlying conditions and more sensitive combined indicators of the effectiveness of PET warrant further study.

REFERENCES


CORRECTION

The article “Abnormal production of the tumor necrosis factor inhibitor etanercept and clinical efficacy of tumor in a patient with PAPA syndrome,” by Cortis et al, which appeared in the December 2004 issue of The Journal (volume 145, pages 851-5) should have been entitled “Abnormal production of tumor necrosis factor (TNF) – α and clinical efficacy of the TNF inhibitor etanercept in a patient with PAPA syndrome.” The unit in the table (p. 852) under “SF leukocyte,” (X103/mm3), should have been (103/mm3).
A SEROLOGIC STRATEGY FOR DETECTING NEONATES AT RISK FOR CONGENITAL CYTOMEGALOVIRUS INFECTION

Anne Naessens, MD, PhD, Anne Casteels, MD, Luc Decatte, MD, PhD, and Walter Foulon, MD, PhD

Objectives To evaluate the feasibility of a serologic screening program in pregnant women to detect neonates at risk for a congenital cytomegalovirus infection.

Study design Unselected mother-infant pairs (n = 7140) were studied. In the mother, serologic screening consisted of the testing for cytomegalovirus antibodies at the first prenatal visit and at birth. In the neonate, cytomegalovirus urine culture was performed to diagnose congenital infection.

Results Serologic screening showed evidence of past infection in 3850 women (53.9%); 192 (2.7%) women had both immunoglobulin (Ig)G and IgM antibodies when first tested during pregnancy. Seroconversion was detected in 44 seronegative women (1.4%). Forty-four congenital infections were diagnosed (0.62%): 8 in women with past infections, 22 in women who seroconverted, and 14 in women who initially had positive IgM antibodies.

Conclusions Screening at the first prenatal visit and at birth defines two major risk groups for congenital cytomegalovirus infection: women with seroconversion during pregnancy and women with IgM antibodies in their first prenatal serum sample (0.6% and 2.7%, respectively, of the pregnant population). In these selected babies (3.3% of the study group), cytomegalovirus urine culture should be performed. This type of screening allows the detection of 82% of all congenital cytomegalovirus infections. (J Pediatr 2005;146:194-7)

Congenital cytomegalovirus infection, the most common fetal viral infection, occurs in 0.2% to 2.2% of live-born infants. Of the infected infants, only 10% are born with symptomatic disease.1,2 Because of the importance of congenital cytomegalovirus infection as a health problem, it would be of medical importance to identify children with congenital cytomegalovirus infection soon after birth. Congenital cytomegalovirus infection is a common cause of sensorineural hearing loss,3,4 and unrecognized hearing loss has an adverse effect on the development of language and communication skills.5,6 Detecting hearing loss before it becomes clinically apparent enables rapid intervention, which could increase the chance of normal development.7,8 A neonatal screening program to detect congenital cytomegalovirus infection is difficult to implement. Specific cytomegalovirus-immunoglobulin (Ig)M detection on cord blood is not sensitive in detecting congenital cytomegalovirus-infected children9 and therefore cannot be used as a neonatal screening program. The best test to diagnose congenital infection is virus isolation in tissue cultures or demonstrating of cytomegalovirus DNA by polymerase chain reaction, accomplished with urine or saliva. Isolation must be attempted in the first 2 to 3 weeks of life, because virus excretion after that time may represent an infection acquired at birth. Diagnosis of congenital infection is also possible by polymerase chain reaction on blood stored on filter paper.10 However, screening with these techniques (culture, polymerase chain reaction) are time-consuming and need a structured sampling system that cannot be introduced in every hospital.

In this study, we evaluated the effectiveness of a serologic screening program during pregnancy to detect a small group of women to whom the majority of infants with congenital cytomegalovirus infection are born.

METHODS

Pregnant Women

From June 1996 until February 2003, 10,383 women delivered at the Brussels University Hospital. From these women, 7140 pregnant women were followed in prenatal
consultation at the University hospital and were included in the study. The Committee of Medical Ethics of the hospital approved this epidemiologic survey.

Median age and parity of these women were 29.3 years and 1.8 children. Eighty percent of the women were between 20 and 34 years of age.

Each pregnant woman was tested twice for cytomegalovirus IgG and IgM antibodies: a first time during the first prenatal consultation and a second time at delivery on cord blood. Antibodies were performed with an enzyme immunoassay method, using ETI-cytok-M or G plus (DiaSorin, Saluggia, Italy).

According to the results of the serologic profile, we subdivided the women into the following groups. Group 1 comprised women without IgG antibodies in the first prenatal serum sample. These women were considered susceptible to primary cytomegalovirus infection. When IgG antibodies, with or without IgM antibodies, were detected in these women at birth (seroconversion), the diagnosis of primary infection during pregnancy was made. Group 2 comprised women with positive IgG antibodies in the absence of specific IgM antibodies. These women were considered immune; their primary infection with the virus was assumed to have taken place before the current pregnancy. Group 3 comprised women with both IgG and IgM antibodies present in the first serum sample. These women were considered to be possibly infected with cytomegalovirus during the current pregnancy. This infection could be either a primary or a recurrent infection.

### Diagnosis of Congenital Cytomegalovirus Infection

In live-born infants, the diagnosis of congenital cytomegalovirus infection was based on the isolation of cytomegalovirus from urine obtained within 7 days after birth. The diagnosis of congenital infections in second-trimester abortions or death in utero was based on the isolation of cytomegalovirus from either amniotic fluid or internal fetal organs or on the presence of typical cytomegalic cells in different fetal organs on histologic examination.

Cytomegalovirus cultures were performed in duplicate on MRC5 cells. One tube was processed after 24 hours of incubation with a monoclonal anti-cytomegalovirus antibody (clone E13, Biosoft, Varilhes, France, detecting the immediate early antigen). The other tube was further incubated and assessed for the appearance of cytopathogenic effect.

### RESULTS

#### Results of Serologic Screening

The results of the serologic screening are summarized in the Table. From the 7140 women included in the study, 3098 (43.4%) had no IgG or IgM antibodies in the first serum sample and were considered not immune. From these 3098 initially seronegative patients, 44 (1.4%) seroconverted during pregnancy. Three thousand eight hundred fifty women (53.9%) had only IgG antibody in their first prenatal serum sample. One hundred ninety-two (2.7%) of the pregnant women had IgG as well as IgM antibodies in their first prenatal serum sample.

#### Results of Neonatal Investigation

The results of the virologic data are summarized in the Table. Of the 7140 mothers investigated, virologic data were available in 5599 of the neonates. Urine cultures were available in 2948 (76.6%) neonates with mothers with prior immunity; in 2473 (79.8%) born to seronegative mothers, including the 44 mothers with seroconversion; and in 178 (92.7%) neonates whose mothers had positive IgM antibodies in the initially serum sample.

Congenital cytomegalovirus infections were found in 44 instances: 42 live-born neonates with a positive urine culture and 2 second-trimester abortions, with a positive cytomegalovirus culture of the amniotic fluid.

Serologic data on cord blood samples were available in 41 of the 44 congenitally infected infants. Cytomegalovirus IgM antibody was found in 18 (44%) of these cord blood specimens. In 3 patients, no cord blood was available because of second-trimester abortions (2 cases) and a technical error (1 case).

#### Congenital Infection According to Mother’s Immune Status

The number of congenitally infected children according to the immune status of the mother is summarized in the Table. Eight congenital infections were found among the women with a serologic profile suggesting prior immunity.
that a serum sample for cytomegalovirus antibodies is taken infected children born to mothers with prior immunity. In our study (44%), it increases the detection rate of congenitally screening, cord blood should be the preferred delivery sample. recurrences are less likely to be detected during serologic recurrences than that observed in our study. Since women with antibodies will have more congenital infections caused by infections. A population with a high prevalence of IgG design. These variations must be taken into consideration the seroprevalence rate in the population, and the study was 82.5%, with an overall congenital infection rate of 1.3%.14 The rate of congenital infections varies widely among different countries.15 Stagno et al reported an incidence of congenital cytomegalovirus infections of 0.55%, from which 63% occurred after a primary infection, 23% after recurrent infection, and 13% after an unclassifiable infection. The rate of congenital infections in women with prior immunity in his study was 0.40%. Fowler et al found a higher rate of congenital infection in women with previous immunity. In their retrospective cohort study performed in multiparous women, congenital cytomegalovirus infection was found in 1.0% of 2857 infants born to immune women. The prevalence of women with cytomegalovirus IgG antibody in their study was 82.5%, with an overall congenital infection rate of 1.3%.14 The variations observed depends on the population studied, the seroprevalence rate in the population, and the study design. These variations must be taken into consideration when introducing a serologic screening for cytomegalovirus infections. A population with a high prevalence of IgG antibodies will have more congenital infections caused by recurrences than that observed in our study. Since women with recurrences are less likely to be detected during serologic screening, cord blood should be the preferred delivery sample. Although the sensitivity of IgM on cord blood was low in our study (44%), it increases the detection rate of congenitally infected children born to mothers with prior immunity. The application of such a serologic survey implicates that a serum sample for cytomegalovirus antibodies is taken in early pregnancy and that the result of this first sample is available to the pediatrician at delivery. Moreover, the results from the delivery sample must be available at the infant’s discharge from the hospital. Good communication between obstetricians, pediatricians, and the laboratory are a prerequisite for the success of such a screening. The use of electronic medical records with fields for the serologic data could facilitate the efficiency of such a screening program.

Screening for infections during pregnancy implies that abnormal test results such as a positive IgM result in the beginning of pregnancy will create anxiety in the patient. Good counseling and the use of confirmatory procedures are necessary to limit the number of invasive procedures. A valuable laboratory technique that can be used to discriminate between primary and nonprimary infections is the IgG avidity. IgG avidity is always low in primary infections and increases with time. Presence of high avidity antibody excludes a recent primary infection. Unfortunately, low avidity is not synonymous with recent infection, since low avidity can be detected in 18% of the patients with past cytomegalovirus infection.17 We conclude that serologic screening with IgG and IgM antibodies in the beginning of pregnancy and on cord blood is able to select a small group of children with a high risk of congenital cytomegalovirus infection. Urine from these infants should be collected for cytomegalovirus culture. The sensitivity of such a screening for detecting children with congenital cytomegalovirus infection is at least 82%.

DISCUSSION

In our population, an incidence of 0.62% congenital cytomegalovirus infections was found. Since we did not obtain urine cultures from all infants, the 0.62% incidence of congenital infection found in this study is probably slightly underestimated. Of all congenital infections diagnosed in the present study, 50% occurred after a proven primary maternal cytomegalovirus infection (seroconversion), 18% in patients with previous immunity, and 32% among patients with initial IgM-positive serum samples. Two high-risk groups for delivering a baby with congenital cytomegalovirus infection are seen in our population: the patients with IgG seroconversion (50% risk of transmission) and the patients with IgM positivity in their first serum sample (7.3% risk of transmission). These patients represent 3.3% of the total study population. Virologic screening of these high-risk babies would enable the detection of 82% of all the congenital cytomegalovirus infections. Moreover, congenital infections not found by this screening method would originate from recurrent and not from primary infections. Although recurrences can occasionally cause major sequelae in the newborn infant,11,12 the presence of maternal antibodies will provide some protection and the risk for severe damage will be lower.13,14 The rate of congenital infections varies widely among different countries.15 Stagno et al reported an incidence of congenital cytomegalovirus infections of 0.55%, from which 63% occurred after a primary infection, 23% after recurrent infection, and 13% after an unclassifiable infection. The rate of congenital infections in women with prior immunity in his study was 0.40%. Fowler et al found a higher rate of congenital infection in women with previous immunity. In their retrospective cohort study performed in multiparous women, congenital cytomegalovirus infection was found in 1.0% of 2857 infants born to immune women. The prevalence of women with cytomegalovirus IgG antibody in their study was 82.5%, with an overall congenital infection rate of 1.3%.14 The variations observed depends on the population studied, the seroprevalence rate in the population, and the study design. These variations must be taken into consideration when introducing a serologic screening for cytomegalovirus infections. A population with a high prevalence of IgG antibodies will have more congenital infections caused by recurrences than that observed in our study. Since women with recurrences are less likely to be detected during serologic screening, cord blood should be the preferred delivery sample. Although the sensitivity of IgM on cord blood was low in our study (44%), it increases the detection rate of congenitally infected children born to mothers with prior immunity. The application of such a serologic survey implicates that a serum sample for cytomegalovirus antibodies is taken in early pregnancy and that the result of this first sample is available to the pediatrician at delivery. Moreover, the results from the delivery sample must be available at the infant’s discharge from the hospital. Good communication between obstetricians, pediatricians, and the laboratory are a prerequisite for the success of such a screening. The use of electronic medical records with fields for the serologic data could facilitate the efficiency of such a screening program.

Screening for infections during pregnancy implies that abnormal test results such as a positive IgM result in the beginning of pregnancy will create anxiety in the patient. Good counseling and the use of confirmatory procedures are necessary to limit the number of invasive procedures. A valuable laboratory technique that can be used to discriminate between primary and nonprimary infections is the IgG avidity. IgG avidity is always low in primary infections and increases with time. Presence of high avidity antibody excludes a recent primary infection. Unfortunately, low avidity is not synonymous with recent infection, since low avidity can be detected in 18% of the patients with past cytomegalovirus infection.17 We conclude that serologic screening with IgG and IgM antibodies in the beginning of pregnancy and on cord blood is able to select a small group of children with a high risk of congenital cytomegalovirus infection. Urine from these infants should be collected for cytomegalovirus culture. The sensitivity of such a screening for detecting children with congenital cytomegalovirus infection is at least 82%.

REFERENCES

COMPARISON OF NON-INVASIVE TESTS TO DETECT *HELCOBACTER PYLORI* INFECTION IN CHILDREN AND ADOLESCENTS: RESULTS OF A MULTICENTER EUROPEAN STUDY

FRANCIS MEGRAUD, ON BEHALF OF THE EUROPEAN PAEDIATRIC TASK FORCE ON *HELCOBACTER PYLORI*

**Objective**  To compare the current non-invasive tests for *Helicobacter pylori* infection in children and adolescents.

**Study design**  This multicenter, multinational study investigated the sensitivity, specificity, and positive and negative predictive values of four non-invasive tests: urea breath test (UBT), stool antigen test, and antibody detection in serum and urine, in comparison with biopsy-based tests.

**Results**  Of 503 patients included pre-treatment, 473 fulfilled the definition of *H pylori* status and among those 316 had results available for the four non-invasive tests (including 133 *H pylori*-positive patients). The specificity was excellent for all tests. The UBT had the best sensitivity in all age groups, followed by serology, stool test, and antibody detection in urine. A trend for better sensitivity with an increase in age was observed except for the stool test. The receiver operating characteristics (ROC) curves showed that sensitivity of serology, stool test, and urinelisa could be improved by changing the cutoff value. An inadequate storage of the specimens may explain the poor results of the stool test.

**Conclusion**  The UBT appears to be an excellent test for diagnosis of *H pylori* infection for children and adolescents. (J Pediatr 2005;146:198-203)

*Helicobacter pylori* is acquired early in life, and it persists for decades and maybe even lifelong. The chronic gastritis that it induces may not be symptomatic but is considered to be the background of severe diseases, ie, peptic ulcer disease and gastric malignancies that typically occur in adulthood. Although duodenal ulcer disease is rarely found in children, it does occur and, as in adults, can be the consequence of an *H pylori* infection. Moreover, *H pylori* infection has been incriminated in other syndromes, ie, recurrent abdominal pain and iron deficiency anemia, but remains to be confirmed, and for this purpose it is mandatory to compare the value of non-invasive tests.

The *H pylori* prevalence in childhood reflects the prevalence that will be found in adulthood in a given age cohort. There is a great contrast between developed countries, where only very few children are infected, and developing countries, where most children reach adulthood being *H pylori* positive.1

The need for an accurate non-invasive test in children to study the transmission of the disease and to monitor the success of eradication therapy by groups in Europe and in North America.2,3 There are now four types of tests available on the market, two based on detection of specific antibodies, in serum and urine, one based on the detection of *H pylori* antigen in stool, and the urea breath test (UBT), which detects the strong urease activity of this bacterium. Although several studies have already been performed on children, the four non-invasive tests have never been compared in the same study with a "gold standard" including all biopsy-based tests. In addition, the number of patients included in previous studies has not been sufficient enough to allow a meaningful analysis of age subgroups, especially for children <6 years of age in which the information is the most important to establish.4

**METHODS**

This is an open, prospective multicenter study investigating the diagnostic properties of non-invasive tests in comparison with a gold standard for the diagnosis of *H pylori* infection in children (2-5 and 6-11 years of age) and adolescents (12-17 years of age).

| UBT | Urea breath test |
|HpSA| *H pylori* stool antigen test |
|ROC| Receiver operating characteristics |

See editorial, p 164.
Patients between 2 and 17 years of age were included if an upper digestive tract endoscopy and *H. pylori* testing was required. An informed consent was obtained from the legal guardian and when possible from the patient. Patients were excluded in the case of previous *H. pylori* eradication therapy, consumption of antibiotics, antisecretory drugs, bismuth salts, or sucralfate in the previous 2 weeks, or if they manifested cough or another disorder leading to a contraindication for endoscopy and/or biopsies.

The size of the study population was calculated to be 600 children and 200 adolescents in order to have at least 30 *H. pylori* positive patients per age group (children 2-11 years of age, and adolescents 12-17 years of age) and to demonstrate with a probability of 90% that sensitivity and specificity were >85% using one-sided confidence intervals. It was decided that the study could be stopped when more than 30 *H. pylori* positive patients would be included in each age group.

The following diagnostic tests were performed within 1 week:

**Endoscopic examination with at least five biopsies sampled.** Four biopsies were sampled from the antrum (one each for local culture, central culture, histology, and rapid urease test) and one from the corpus for histology. Biopsies for histology were processed and interpreted blindly according to the Sydney System in the local laboratory of each participating center.

In addition to culture performed locally, one antral biopsy per patient was stored frozen at −80°C and transported in dry ice twice during the study to a central laboratory in Bordeaux where they were processed blindly according to a protocol previously described. The rapid urease test (RUT) used was PyloriTek (BARD, Billerica, Mass). Reading was performed within 1 hour.

**Definition of *H. pylori* status.** A positive *H. pylori* status was defined as a positive culture (either local or central, or both) or in case of negative culture, positive results for both histology and rapid urease test. A negative *H. pylori* status was confirmed when all invasive tests performed gave concordant negative results. Cases with discrepant results, ie, histology positive and rapid urease test negative or vice versa, were excluded.

**13C-urea breath test.** The Helicobacter Test INFAI (INFAI GmbH, Cologne, Germany) was used. The test was performed on fasting children (>4 hours after their last meal and at least 2 hours after the endoscopic examination). The test meal used was 200 mL of orange or apple juice: 150 mL of juice was given; thereafter, 13C-labeled urea dissolved in 20 mL of juice was administered and another 30 mL of juice was used to rinse the mouth of the tracer. A total of 100 mL of juice was given to children 2 to 4 years of age. The 13C-urea dosage was 75 mg for adolescents and 45 mg for children. The analysis was performed blindly on expired air samples collected before and 30 minutes after urea ingestion, by mass spectrometry centrally (INFAI Laboratory, Cologne, Germany). A δ

### Table I. Characteristics of the 316 children and adolescents studied with known *Helicobacter pylori* status and the four tests performed

<table>
<thead>
<tr>
<th></th>
<th>N</th>
<th>% H. pylori +</th>
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<tr>
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<tr>
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<td></td>
</tr>
<tr>
<td>Male</td>
<td>144</td>
<td>38.9</td>
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<tr>
<td>Female</td>
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<td>44.4</td>
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<td><strong>Age (y)</strong></td>
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<td>27.1</td>
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<tr>
<td>6-11</td>
<td>150</td>
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<td>12-17</td>
<td>118</td>
<td>44.9</td>
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<tr>
<td><strong>Mean age</strong></td>
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<tr>
<td>9.9 ± 3.7</td>
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<td><strong>Reason for endoscopy</strong></td>
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<td>Symptoms of malabsorption</td>
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<tr>
<td>Failure to thrive</td>
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<td>42.1</td>
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<tr>
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<td>32.8</td>
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<td><strong>Endoscopy finding</strong></td>
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<tr>
<td>Duodenal ulcer</td>
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<td>100</td>
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<tr>
<td>Gastric ulcer</td>
<td>2</td>
<td>100</td>
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<tr>
<td>Duodenal erosion</td>
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<td>64.2</td>
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<td>Gastritis erosion</td>
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<tr>
<td>Duodenal nodules</td>
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<td>83.2</td>
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<tr>
<td>Normal endoscopy</td>
<td>123</td>
<td>16.2</td>
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</table>

*Some children had several reasons to be endoscoped.

**Stool antigen test.** The kit Premier *H. pylori* Stool Antigen test (HpSA) (Meridian, Milan, Italy) was used. Stool samples were obtained from patients, frozen at −20°C, transported frozen under the manufacturer’s responsibility separately from the other specimens, and processed blindly in a central laboratory (Y. Glucpzynski, Yvoir, Belgium).

**Serology.** The kit Pyloritest EIA-G III (Orion Diagnostics, Espoo, Finland) was used. Serum samples were kept frozen at −20°C, transported in dry ice in a central laboratory in Bordeaux, and tested blindly.

**Antibody detection in urine.** The kit Urinelisa (Otsuka Diagnostic, Frankfurt, Germany) was used. Urine samples were collected in a conservation medium and sent by regular mail to a central laboratory in Bordeaux, where they were processed blindly.
The sensitivity, specificity, accuracy, and positive and negative predictive values of the different tests, on this population of 316 children and adolescents, are presented in Table II. The results were not different from those of the 473 regarding all of the characteristics studied. The ROC curves indicated that the cutoff values proposed by the manufacturers were not optimal for HpSA and Urinelisa. Indeed, the sensitivity of HpSA could be increased to 80.3%, and the sensitivity of Urinelisa to 72.2%, both with a minor loss in specificity by decreasing the cutoff values (Figure).

When the sensitivity of the four tests was compared according to the three age groups, a trend for a better sensitivity in adolescents compared with children was observed for all tests except HpSA. It was more marked for Urinelisa, but it did not reach statistical significance ($P = .2$).

In order to know the predictive values of the tests in centers with the lowest prevalence, we carried out a subgroup analysis in which 76 cases were included. The positive and negative predictive values were the following: UBT: 76.4% and 98.3%, Pyloriset-EIA-G III 76.4% and 98.3%, HpSA 83.3% and 93.7%, Urinelisa 83.3% and 93.7%, Rapirun 84.7% and 75%, respectively. The accuracy of the tests also was calculated using as reference the combination of the histology and RUT instead of culture. The accuracy was then 88.7% for UBT, 83.7% for Pyloriset-EIA-G III, 78% for HpSA, 73.7% for Urinelisa, and 59.5% for Rapirun.

### DISCUSSION

Because of the current rarity of symptomatic $H$ pylori infection in this period of life, previous studies did not include large enough numbers of young patients to break down the results by age. In this study we could differentiate adolescents from children. However, we could not include enough children between 2 and 5 years of age to obtain reliable results.

The rate of $H$ pylori infection observed in this study is high and cannot be interpreted as the prevalence rate in children in Europe. It can be explained mainly by a strong recruitment of immigrant children in centers of Western

### RESULTS

Of 503 patients recruited in the study, 473 fulfilled the definition of a positive or negative $H$ pylori status and 316 had further results available for the four non-invasive tests. There were 191 $H$ pylori positive cases among the 473 with defined $H$ pylori status; culture was positive for 167 (88%), both locally and centrally in 74% of them, and locally or centrally in 13%, and an additional 24 cases were both urease and histology positive. Chronic gastritis was present in all. The characteristics of the population of 316 children and adolescents for which results were available for the four non-invasive tests are presented in Table I.

### Table II. Performances of the diagnostic tests for the 316 patients with gold standard and four tests performed (UBT, HpSA, Urinelisa and Pyloriset EIA-G)

<table>
<thead>
<tr>
<th>Age group (y)</th>
<th>Helicobacter test INFAI</th>
<th>HpSA</th>
<th>Urinelisa</th>
<th>Pyloriset EIA-G</th>
<th><strong>Rapirun</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Sensitivity</td>
<td>Global</td>
<td>96.2 [91.9-98.6]</td>
<td>72.9 [64.9-80.0]</td>
<td>63.2 [54.7-71.0]</td>
<td>88.7 [82.5-93.3]</td>
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<tr>
<td>Specificity</td>
<td>Global</td>
<td>97.3 [94.0-99.0]</td>
<td>97.3 [94.0-99.0]</td>
<td>97.3 [94.0-99.0]</td>
<td>97.3 [94.0-99.0]</td>
</tr>
<tr>
<td>Accuracy</td>
<td>Global</td>
<td>96.8 [94.4-98.4]</td>
<td>87.0 [83.0-90.4]</td>
<td>82.9 [78.4-86.8]</td>
<td>89.5 [86.0-93.0]</td>
</tr>
<tr>
<td>PPV</td>
<td>Global</td>
<td>96.2 [91.9-98.6]</td>
<td>95.1 [89.5-98.2]</td>
<td>94.4 [88.0-97.9]</td>
<td>90.8 [84.8-94.9]</td>
</tr>
<tr>
<td>NPV</td>
<td>Global</td>
<td>97.3 [94.0-99.0]</td>
<td>83.2 [77.7-87.7]</td>
<td>78.4 [72.7-83.4]</td>
<td>91.9 [87.3-95.2]</td>
</tr>
</tbody>
</table>

*NPV, Negative Predictive Value; PPV, Positive Predictive Value.*

**Rapirun was performed only on 272 children including 119 $H$ pylori positive. Age groups were the following: 2-5 y (n = 41 including 11 $H$ pylori positive), 6-11 y (n = 130 including 59 $H$ pylori positive) and 12-17 y (n = 101 including 49 $H$ pylori positive).***

**Sensitivity and specificity values obtained after defining the optimal cutoff based on ROC curves.**
Europe. Therefore, it must be acknowledged that the predictive values of the test performances may vary according to local \textit{H pylori} prevalence. For this reason we calculated the positive and negative predictive values for the centers with a low prevalence of the infection, but the small number of cases limited the power of the results.

A relatively low rate of ulcers was noted (8.6%), but they were all \textit{H pylori} positive. The rate was however higher than in a series of 622 upper endoscopy reports in the United States in which only 11 case patients had an ulcer and only 3 of them an \textit{H pylori} positive ulcer.\textsuperscript{12}

A strong point of this study is the reliability of the \textit{H pylori} status used as a gold standard. Culture was performed meticulously and turned out to be highly successful despite the fact that the centers were spread all over Europe. Among the 191 gold standard positive cases, 167 were positive by culture. In case of a negative culture usually explainable by a transport problem or lack of experience for certain centers, the other criterion used was to have both urease test and histology positive. The urease test chosen has been recognized as the most sensitive and practical test,\textsuperscript{13} and in this study, it also showed an excellent performance when compared with culture.

The evaluation concerned four different non-invasive tests based on three different principles, given that Serology and Urinelisa used the same principal, ie antibody detection. The results confirm the value of UBT, which exhibits an excellent sensitivity in all age groups, as previously demonstrated in several studies.\textsuperscript{14-16} We did not notice a lower specificity in young children in our small sample in contrast with previous data,\textsuperscript{17,18,19} and all of the DOB values of \textit{H pylori} negative cases were far from the cutoff (<1.7 \(\delta\) per mil), but this result must be interpreted with caution given the low number of such cases. The use of 45 mg of \textsuperscript{13}C-urea in children is therefore justified, as well as is the protocol consisting of a test meal of orange or apple juice after fasting with rinsing the mouth after tracer ingestion. The cutoff of 4 per mil is also the best alternative. These results confirm previous data presented by Bazzoli et al.\textsuperscript{20} who obtained the same results with 50 mg as with 100 mg of urea when performing the UBT in children.

A surprising finding of this study was the low sensitivity of the HpSA. This test has confirmed its value in the past both...
in adults\textsuperscript{21} and in children,\textsuperscript{22-27} especially for pretreatment diagnosis. The stability of the antigens to be detected has been previously mentioned by the manufacturer and confirmed in a study in which bacteria were experimentally spiked in stools.\textsuperscript{28} Therefore, in this study the recommendation was made to freeze the specimens at $-20^\circ C$ only. They were then transported frozen to the central laboratory performing this test. It must be acknowledged that, for customs reasons, some samples arrived thawed. In addition, asking parents to bring in stool specimens introduces a lack of full control on the time between defecation and storage in the ward. Therefore, the possibility of inadequate storage may explain the poor results. The falsely negative results, however, were randomly distributed among the centers and occurred throughout the study period, which does not suggest problem limited to certain shipments. If \textit{H pylori} antigens present in stools are not stable, the requirement for maintenance before testing must be reinforced in everyday practice in order to ensure proper results. An alternative hypothesis would be that the quality of the reagent, a polyclonal antibody, was different in this study compared with previous ones. Inter-test variability has been previously described.\textsuperscript{29} However, by lowering the cutoff, it was possible to increase the sensitivity of HpSA to 80.3\%. Recently, a novel \textit{H pylori} antigen stool test using monoclonal antibodies has shown very promising results (sensitivity 98\%, specificity 99\%), when applied to a similar patient population without variation according to age, and the problem of lot variation.\textsuperscript{30}

Serology ranked second in sensitivity. The kit was initially chosen on the basis of a previous evaluation in which it had the best accuracy.\textsuperscript{31} It was confirmed to be excellent in our laboratory for adult patients,\textsuperscript{32} and it now proves to be true for the children in this study. It also may be that the third generation of this test has a higher accuracy compared with the previous ones.\textsuperscript{33} Serologic tests have a bad reputation, which could be linked to two reasons: the variability of accuracy between kits, and the fact that they suffer from a comparison to a “low quality” gold standard because culture is rarely performed and histology may not be accurate. The outcome is then an apparent lack of specificity, whereas most false positives are probably true positives. The other antibody test, used on urine samples, did not perform well on children. The amount of antibodies in urine reflects the amount present in serum, but at a lower level. It is therefore logical that this test would be less sensitive than serology. Indeed, we found 44 cases positive in serum and negative in urine, versus only 3 cases negative in serum and positive in urine. Interestingly, the sensitivity increased significantly with age, reflecting a higher antibody response in adolescents than in children. Decreasing the cutoff allowed a notable increase in the sensitivity. The current kit is based on antigens from Japanese strains, which are known to have a special genetic pattern. It may well be that their antigen spectrum is different from that of most European strains isolated in this study, causing the low sensitivity that was not noticed in Japan.\textsuperscript{34} The urine “near patient” test, which could be the ideal non-invasive test if its performance were satisfactory, exhibited a very low sensitivity as occurs with “near patient” blood tests compared with laboratory enzyme linked immunosorbent assays.\textsuperscript{35}

In conclusion, all of the tests showed a trend for improved sensitivity with age except for the stool test. Of the methods evaluated in this study, UBT is the non-invasive test of choice, but more data are needed for children <5 years of age. Serology using Pyloriset EIA-G gave satisfactory results.

\textit{We acknowledge the contribution of Cédric Scribans and Frédérique Richy from Bordeaux, France, for data analysis and Serim (USA) for providing PyloriTek kits.}

\textbf{APPENDIX}

European Paediatric Task Force on \textit{Helicobacter pylori}

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This Task Force was formed by the European Helicobacter Study Group and the European Paediatric Society for Hepatology Gastroenterology and Nutrition.

\textbf{REFERENCES}


THE CURRENT MANAGEMENT OF HEPATOBLASTOMA: A COMBINATION OF CHEMOTHERAPY, CONVENTIONAL RESECTION, AND LIVER TRANSPLANTATION

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Objective To review our experience in the management of children who present with hepatoblastoma.

Study design Thirty patients treated for hepatoblastoma at a single institution were reviewed.

Results Ten patients presented with stage I to stage II disease and underwent resection. Seventeen presented with stage III disease; two underwent initial resection of which one required rescue transplantation. The remaining 15 underwent biopsies, which were followed by chemotherapy. Nine patients had a reduction in tumor size and underwent conventional resection. One patient expired during chemotherapy. Three patients presented with stage IV disease and underwent biopsies, which were followed by chemotherapy. One patient responded but required “rescue” transplantation after conventional resection. Seven patients underwent primary transplantation for unresectable disease. One patient expired during chemotherapy. Three patients presented with stage IV disease and underwent biopsies, which were followed by chemotherapy. One patient responded but required “rescue” transplantation after conventional resection. Seven patients underwent primary transplantation for unresectable disease. One patient expired during chemotherapy. Three patients presented with stage IV disease and underwent biopsies, which were followed by chemotherapy. One patient responded but required “rescue” transplantation after conventional resection. Seven patients underwent primary transplantation for unresectable disease. Five-year survival was 82.5% ± 7.1%. There was no operative mortality during surgical therapy. All transplant recipients were tumor free, but one died from lymphoma 7 years post-transplant.

Conclusion Chemotherapy may reduce tumor size, allowing for conventional resection. If aggressive resection is necessary or bi-lobar disease persists, primary transplantation is recommended. (J Pediatr 2005;146:204-11)

Hepatoblastoma is the most common malignant liver tumor in children, with an incidence of 0.7 to 1 per million children <15 years of age. Currently, long-term survival rates are 75% to 80%; improved survival rates are largely a result of advances in chemotherapy and surgical decision making. Complete surgical resection remains the most critical intervention required to achieve long-term cure; however, only 60% of patients present with tumors that are resectable. For patients with initially unresectable tumors, the addition of adjuvant chemotherapy and liver transplantation has expanded the treatment alternatives to achieve the goal of curative therapy.

The role of chemotherapy in the management of children with hepatoblastoma was demonstrated by several cooperative groups, including the Children’s Cancer Group (CCG), who reported improved survival in children with hepatoblastoma who were treated using a combination of vincristine, adriamycin, cyclophosphamide, 5-fluorouracil, and surgical resection. Subsequent studies found that hepatoblastomas are more sensitive to cisplatinum-based chemotherapy, which is now included in virtually all chemotherapeutic treatment regimens. Chemotherapy is used to reduce tumor size in lesions that appear unresectable at the time of presentation and to control residual microscopic disease after definitive resection. More than 60% of lesions that initially appear unresectable will decrease sufficiently in size with adjuvant chemotherapy to allow for conventional resection. In those cases in which tumor remain unresectable, usually because of invasion...
of major vascular structures or involvement of all sectors of the liver, liver transplantation should be considered to render a patient tumor free. The initial studies reviewing the use of liver transplantation in children with unresectable hepatoblastoma report a 50% survival rate. Tumor recurrence was the most common cause of treatment failure; however, many of these children did not receive adjuvant chemotherapy. Recent studies using adjuvant chemotherapy and liver transplantation have reported an improved outcome with survival rates ranging from 63% to 93%.11-15,16

This report details our experience using the combination of chemotherapy, conventional resection, and/or liver transplantation in the treatment of children with hepatoblastoma, with a focus on outcomes in patients with difficult-to-resect or unresectable tumors.

METHODS

A retrospective review of the tumor and transplant registries at Cincinnati Children’s Hospital and Medical Center (CCHMC) from 1986 to 2002 identified 30 patients who underwent treatment for hepatoblastoma. Their medical records were reviewed, recording patient demographics, presenting symptoms, laboratory studies, radiographic evaluation, treatment course, and subsequent outcome. Approval for the study was obtained from the Institutional Review Board at CCHMC before initiation of the study. Health Insurance Portability and Accountability Act (HIPAA) guidelines were followed to ensure patient privacy. CCHMC is a member of the Children’s Oncology Group (COG) and previously the Children’s Cancer Group (CCG). Patients were generally treated according to CCG and COG protocols. Some of the patients reported here participated in either CCG or COG clinical trials.

Treatment Strategy

Patients underwent cross-sectional imaging at the time of presentation. If the tumor appeared resectable, the patient underwent conventional resection. If the tumor appeared unresectable, biopsy of the lesion was performed to confirm the diagnosis. The decision to resect was based on the ability to obtain clear margins. Invasion/close proximity to major vessels, and/or extensive bi-lobar disease was the radiographic characteristic that made a tumor unresectable. Patients were staged after the initial surgical procedure according to the CCG/Pediatric Oncology Group (POG) surgical pathologic staging system (Table).

Children with stage I disease and pure fetal histology were treated with surgery alone17,18; all other patients received chemotherapy after resection. All children who only underwent biopsy at the time of presentation were treated with chemotherapy in an effort to reduce tumor size. In these patients, serial cross-sectional imaging of the abdomen using computerized tomography was performed to assess the response to treatment. Serial measurements of serum α-fetoprotein (AFP) levels were monitored in most patients. If lesions decreased sufficiently to allow conventional resection

| Table. CCG/POG post-resection staging system |
|---|---|
| Stage | Surgical procedure |
| I | Completer resection, margins negative |
| II | Gross resection with microscopically positive margins |
| III | Gross residual disease after attempted resection or Biopsy only |
| IV | Metastatic disease |

in which clear margins could be obtained, surgical intervention was performed. If after four cycles of chemotherapy the tumor remained unresectable, the patients underwent primary transplantation. Several children underwent “rescue” liver transplantation after an attempt at conventional resection failed because gross residual tumor remained at the surgical margin.

Chemotherapy Regimens

Over the time span of the study, four cisplatinum-based CCG/POG chemotherapy regimens were used: CCG 823F regimen consisted of cisplatin 90 mg/m² day 1, adriamycin 20 mg/m²/day continuous infusion days 1 through 4, repeated every 21 days; CCG 881A regimen consisted of cisplatin 90 mg/m² day 1, vincristine 1.5 mg/m² day 3 and 5-fluorouracil 600 mg/m² day 3, repeated every 21 days; CCG 881B regimen consisted of cisplatin 90 mg/m² day 1, adriamycin 20 mg/m²/day continuous infusion days 1 through 4, repeated every 21 days; POG 9645 regimen consisted of cisplatin 100 mg/m² day 1, vincristine 1.5 mg/m² days 3, 10, and 17, and 5-fluorouracil 600 mg/m² day 3, ± amifostine 740 mg/m² day 1, repeated every 21 days. The goal was to administer a total of six cycles of chemotherapy. A minimum of two cycles of chemotherapy was given post-resection or post-transplantation.

Statistics

Survival was estimated by the Kaplan-Meier method, and standard deviations were estimated by the Greenwood formula. Kaplan-Meier curves were compared using the log rank test.

RESULTS

Patient Population

There were 24 males and 6 females in our patient population. The median age at diagnosis was 15 months with a range of 2 days to 13 years. The median follow-up was 53 months with a range of 3 days to 16 years. Twenty-six of the patients were diagnosed at CCHMC, where all treatment decisions were made. Four patients were initially diagnosed at outside institutions, underwent treatment at those institutions, and were subsequently transferred to CCHMC for transplant evaluation.

The majority of patients presented with an asymptomatic mass found by a primary caregiver or primary care
physician (n = 22). Other patients presented with failure to thrive (n = 2), abdominal tenderness (n = 3), acute abdomen secondary to tumor rupture (n = 1), or an elevated AFP level (n = 2). In addition to hepatoblastoma, several children had other medical conditions including trisomy 18 (n = 1), fetal alcohol syndrome (n = 1), biliary atresia (n = 1), autoimmune hepatitis (n = 1), and a history of prematurity (n = 2). Among the two patients who were diagnosed based on an elevated AFP, the high levels were noted in one patient who was undergoing routine AFP monitoring because of underlying cirrhosis from chronic liver disease (autoimmune hepatitis) and in the other patient while undergoing an endocrine workup for vanishing testis. No patient had a genetic condition that had a predisposition to hepatoblastoma such as Beckwith-Wiedemann syndrome, hemihypertrophy, or familial adenomatous polyposis.

### Staging

Twelve patients underwent conventional resection at the time of presentation. Seven had negative margins and were classified with stage I disease. Three patients had surgical margins positive for microscopic disease and were classified with stage II disease. Two patients underwent incomplete resection with gross residual disease in the remaining parenchyma and were classified with stage III disease.

Eighteen patients presented with bulky lesions that were judged nonresectable at the time of presentation. All underwent biopsy to confirm the diagnosis of hepatoblastoma. Fifteen patients had no evidence of metastatic disease and were classified with stage III disease. Three patients had metastatic disease to the lung at the time of presentation and were classified with stage IV disease.

Histologic analysis of the 30 tumors showed fetal histology in 20% (n = 6), embryonal in 40% (n = 12), mixed in 27% (n = 8), teratoid in 3% (n = 1), and unknown in 10% (n = 3).

### Overall Outcome

The 5-year event-free survival and 5-year overall survival was 82.5% ± 7.1% (Figure 1). A total of eight patients did not survive, but only five expired because of progressive, recurrent, or metastatic spread of hepatoblastoma. Three patients died during the treatment of their hepatoblastoma but not as a direct manifestation of tumor spread. The causes of death included a massive cerebrovascular accident (CVA) in the postoperative period in a patient with stage I disease, complications related to trisomy 18 one year after a liver resection for a patient with stage I disease, and post-transplant lymphoproliferative disease (PTLD) 7 years after liver transplantation in a patient with who was treated for stage III disease.

### Outcome According to the Stage of Disease

Patients with stage I or II disease had a significantly better prognosis overall than patients with either stage III or IV disease (Figure 2); 5-year survival was 88.9% ± 10.5% for stage I and II patients combined and 87.8% ± 8.1% for stage III; however, two stage III patients died 7 years after diagnosis, one of relapsed disease and one of PTLD (the patient with stage I disease who died from a CVA on post-operative day 3 was not included in the Kaplan-Meier survival analysis). Patients with stage IV disease had a poor prognosis with a 2-year overall survival of 33.3% ± 27.2% (P = .0144).

### Stage I and II

The hepatic resections performed on the 10 patients with stage I and II disease were lobectomy (n = 9) or trisegmentectomy (n = 1). Seven received adjuvant chemotherapy after resection: CCG 823F (n = 1), CCG 881A (n = 3), CCG 881B (n = 1), and unknown (n = 2). Three patients whose tumors were of the pure fetal histologic subtype received no chemotherapy. None of the patients with stage I or II developed recurrent or metastatic disease. The median length of follow-up was 7 years with a range of 3 days to 16 years. The causes of death were a CVA in the postoperative period (n = 1) and unrelated disease (trisomy 18, n = 1).

### Stage III

Seventeen patients had stage III disease. Two patients underwent attempted conventional resection at the time of presentation and had incomplete resections (lobectomy [n = 1], trisegmentectomy [n = 1]) with gross residual disease at the surgical margin. Both received postoperative chemotherapy according to the CCG 881A protocol. The patient who underwent lobectomy had complete resolution of residual disease during chemotherapy, did not require a second resection, and has not developed recurrent or metastatic disease during a 10-year follow-up. The other patient, who had undergone trisegmentectomy, had complete resolution of the tumor during chemotherapy but developed hepatic insufficiency after treatment and underwent cadaveric liver transplantation for manifestations of end-stage liver disease. The patient initially did well but developed PTLD 7 years after transplantation and died despite aggressive chemotherapy.
Fifteen patients with stage III disease had tumors that were considered unresectable by imaging criteria at presentation and had their diagnosis confirmed by biopsy. There were no biopsy-related complications. All 15 received chemotherapy according to CCG protocols: CCG 881A (n = 4), CCG 881B (n = 2), POG 9645 (n = 6), and unknown (n = 3).

Fourteen of the 15 patients responded to chemotherapy as demonstrated by a reduction in tumor size during serial radiographic analysis. Among these 14 patients, serial measurements of AFP were available in 8 patients, and all showed a decrease during treatment. The one patient with stage III disease who failed to respond to treatment had underlying cirrhosis from autoimmune hepatitis and expired after only one cycle of chemotherapy as a result of complications from progressive end-stage liver disease; the tumor response to chemotherapy could not be assessed.

Nine of the 14 patients (64%) who responded to treatment had tumors that decreased sufficiently in size to allow for conventional resection, and they underwent lobectomy (n = 4), trisegmentectomy (n = 4), or central liver resection (n = 1). One of these patients had gross residual tumor at the surgical margin after right trisegmentectomy and underwent rescue cadaveric liver transplantation followed by two cycles of chemotherapy.

Five patients with stage III disease underwent primary liver transplantation. Four patients received four cycles of chemotherapy and one patient received two cycles of chemotherapy before transplantation. Vascular invasion (n = 1), proximity to great vessels (n = 2), or bi-lobar disease (n = 2) were the reasons these tumors were considered unresectable. Primary transplantation took place in one patient after only two rounds of chemotherapy because, despite a decrease in size, the tumor remained so large the patient was ventilator dependent. Four patients underwent cadaveric liver transplantation; one patient underwent living-related left lateral segment transplantation. After transplantation, four patients received chemotherapy such that a total of six cycles were administered during the treatment course. One patient received a total of seven cycles of chemotherapy; five cycles pre-transplant then two cycles after transplantation.

Two patients, who underwent conventional resection, developed metastatic lung disease after definitive resection. One was treated with intensification of chemotherapy followed by three lung resections to clear metastatic disease. The patient remains disease-free 6 years after his last intervention. The other patient developed metastatic disease shortly after resection. He was initially treated on CCG 881B, then carboplatin, vincristine, and 5-fluorouracil when he developed progressive disease. Chemoembolization with cisplatin, Adriamycin, and mitomycin was also attempted, but the patient expired 11 months after presentation. One patient who underwent a central liver resection developed local recurrence of hepatoblastoma with extra-hepatic spread. He underwent several abdominal explorations and resections including a pancreatecooduodenectomy to control local disease but eventually expired of metastatic spread 7 years after initial presentation.

Overall survival in patients who presented with stage III disease was 77%. The median length of follow-up was 5 years with a range of 2 months to 10 years. Of the four patients who expired, two died because of complications related to hepatoblastoma, one of underlying chronic liver disease, and one of complications related to liver transplantation.

A comparison of patients with stage III disease who underwent conventional resection after chemotherapy versus those who underwent primary liver transplantation after chemotherapy had similar overall 5-year outcomes. Overall 5-year survival was 88.9% ± 10.5% for those who underwent conventional resection versus 100% for those who underwent primary liver transplantation (P = .46; Figure 3).

Stage IV Disease

One patient with stage IV disease presented with tumor rupture and hemodynamic instability from intra-abdominal bleeding. After stabilization in the intensive care unit, the patient underwent biopsy and was diagnosed with hepatoblastoma. The patient was started on chemotherapy (POG 9645) but developed multi-system organ dysfunction and expired after one cycle of chemotherapy.

Both remaining patients underwent biopsy followed by adjuvant chemotherapy: CCG 823F (n = 1) and unknown (n = 1). One patient responded well with complete resolution of his lung disease and radiographic evidence of tumor reduction. The patient underwent attempted conventional resection (right trisegmentectomy) after four cycles of chemotherapy, but because there was gross residual tumor at the surgical margins he required rescue living-related liver transplantation for salvage. The patient continues to do well without evidence of tumor recurrence 2 years post-transplant. The other patient failed to respond to chemotherapy and succumbed to progressive metastatic disease 15 months after presentation, never having undergone resection. Overall survival in patients with stage IV disease was 33%, with the single survivor alive and well 2 years after diagnosis. Deaths of the other two patients were a result of hepatoblastoma.
Outcome among Children Who Underwent Liver Transplantation

Eight children underwent liver transplantation; 5 underwent primary transplantation for stage III disease in which the tumor decreased in size during chemotherapy but not sufficiently to allow for conventional resection. Two underwent rescue liver transplantation for gross residual disease after attempted conventional resection. One underwent liver transplantation for hepatic insufficiency that developed after conventional resection and chemotherapy. Seven of eight are currently alive and well. The patient who developed hepatic insufficiency after conventional resection and who required liver transplantation died 7 years post-transplant of PTLD-related lymphoma.

Four of the eight patients had at least one episode of rejection; all episodes were managed by a short course of steroids. Two patients required bile duct revision 11 and 18 months post transplant. One patient developed pneumatoasis intestinalis during chemotherapy 2 months post-transplant that resolved with non-operative management. Three patients had fever and neutropenia during post-transplant chemotherapy that required hospitalization. Two patients developed PTLD; one progressed to lymphoma and expired. The other responded to reduction of immunosuppression. All seven survivors are currently receiving tacrolimus mono-therapy as the sole immunosuppressive agent.

DISCUSSION

The management of a child who presents with hepatoblastoma has changed markedly over the last 20 years. In this study, we reviewed our experience in which children with hepatoblastoma were treated with a combination of chemotherapy, conventional resection, and liver transplantation. In children who presented with lesions that were amenable to conventional resection, surgery followed by chemotherapy was performed. In lesions that were not resectable at the time of presentation, adjuvant chemotherapy was used to reduce tumor burden, followed by definitive resection using either conventional techniques or liver transplantation. Primary liver transplantation was employed if it did not appear by cross-sectional radiographic imaging that clear margins could be achieved by conventional resection. Rescue liver transplantation also was utilized for salvage in several patients after failed attempts at conventional resection. The result was effective tumor control in 25 of 30 patients (83%). Our 5-year event-free survival and 5-year overall survival of ~83% is consistent with results reported in a recent study in which a similar combination of treatment interventions was used.

The results of the current study support the efficacy of adjuvant cisplatinum-based chemotherapy in the treatment of children with hepatoblastoma. In patients with stage I or II disease, adjuvant chemotherapy was utilized in 7 of 10 patients, and none developed metastatic or recurrent disease. Fifteen of 18 patients with stage III or IV disease responded to chemotherapy, as demonstrated by radiographic reduction of tumor size. Although only 9 could undergo attempted conventional resection, a 55% rate of resection by conventional surgery after adjuvant chemotherapy is consistent with the findings of previously reported studies.

The treatment algorithm used in the current study was based on the ability to resect at the time of presentation. In contrast, the ongoing The International Society of Pediatric Oncology (SIOPEL) studies based in Europe stage patients radiographically at the time of presentation followed by chemotherapy to reduce tumor size. Histologic assessment of the tumor is not always required. If the lesion decreases in size so that it is amenable to complete removal, conventional resection is employed. If the lesion remains unresectable, total hepatectomy followed by liver transplantation is employed. Although our treatment strategy was to consider resection at the time of presentation, 60% of the children in the series were unresectable and underwent chemotherapy before definitive resection. Of the 12 patients who underwent resection at the time of presentation, 7 had negative margins, 3 had microscopic residual disease, and 2 had gross residual disease; all responded to post-operative chemotherapy with no evidence of tumor recurrence. Both patients who underwent resection at the time of presentation and who had gross residual disease (stage III disease) were treated in the early 1990s and had lesions that arose in the medial segment of the left lobe that encroached on the right hepatic vein. If these patients presented today and were evaluated using current imaging modalities such as magnetic resonance imaging, these lesions may have been considered unresectable.

The favorable outcome of the eight patients who underwent liver transplantation further supports its important role in the treatment of patients with unresectable hepatoblastoma. To date, no patient has developed tumor recurrence. Liver transplantation probably offers the child with an unresectable tumor the only potential hope of long-term cure. Identification of those patients who will not be able to be resected by conventional techniques is difficult. Trisegmentectomy or central liver resections are considered aggressive conventional resections, and the need to perform
Figure 4. Current algorithm for the management of a child who presents with a hepatoblastoma.

*Consider continuation of chemotherapy or living-related liver transplantation if cadaveric liver transplant not available in a timely fashion*
a trisegmentectomy has been shown to be a negative predictor of outcome.\textsuperscript{20} In the current study, eight patients underwent aggressive conventional resection. Four of these patients had poor outcomes, with three requiring liver transplantation for salvage, and a fourth patient developed locally invasive recurrent disease and eventually succumbed of metastatic spread. Although we were able to "rescue" the two patients in our series who failed conventional resection with gross residual disease, heroic conventional resections should be avoided given the efficacy of liver transplantation. We consider primary liver transplantation a better alternative than an aggressive conventional resection if radiographic analysis does not reveal a clear margin between the tumor and vasculature to the residual liver parenchyma. This finding is supported by Otte et al, who reviewed 147 patients with hepatoblastoma treated with liver transplantation\textsuperscript{16}; 106 patients received a primary liver transplant, whereas 41 patients received a rescue transplant following either an incomplete resection or recurrence of their liver tumor. Survival in patients who were treated with a primary transplant was significantly better, with a 6-year overall survival of 82\% (95\% CI, 72\%–92\%) compared with 30\% (95\% CI, 14\%–46\%) in the patients who were transplanted only after initial treatment had failed.

Liver transplantation, however, is not without significant adverse effects. Among the eight patients who were transplanted, all but one patient experienced at least one postoperative complication. Most of the complications were readily managed with standard post-transplant care, but one child died of PTLD-induced lymphoma. Liver transplantation for children with unresectable hepatoblastoma may be the only alternative, but its role needs further definition. A multi-institutional trial, designed to identify when liver transplantation should be employed in the treatment of a child with hepatoblastoma needs to be conducted.

Although there were no patients in this series who developed tumor recurrence following transplantation, several recent studies have reported recurrence rates as high as 25\%. The recurrence rate following transplantation is similar to that found following conventional resection. Pimpalwar et al found that tumor susceptibility to chemotherapy, as manifest by decreasing AFP levels or reduction in tumor size, indicated by cross-sectional imaging, was a better predictor of outcome than the manner by which the tumor was completely removed.\textsuperscript{12} In patients who had a poor response to adjuvant chemotherapy, the outcome was worse regardless of whether the patient underwent conventional resection or transplantation when compared with those who had a good response to chemotherapy before surgery.

Patients with bulky disease not amenable to conventional resection after chemotherapy who also have metastatic lung lesions at diagnosis may still be considered for transplantation. Although the overall prognosis is worse for this group of patients, there is a subset that responds well to adjuvant chemotherapy. Thus, if complete clearance of their lung disease can be achieved, transplantation may be offered.\textsuperscript{16,22} Thoracoscopic or open resection may be necessary to remove all metastatic lesions. The length of time a patient needs to be free of metastatic disease before transplantation remains uncertain.

Children who present with or develop metastatic disease continue to have the worst prognosis. In the current study, 2 of 3 patients who presented with metastatic spread expired before resection could be attempted and 2 of 3 patients who developed metastatic spread eventually succumbed of the disease. In these cases, the biologic characteristics of the tumor may be a more accurate predictor of outcome than any other risk factor.

Our current algorithm in the management of a child who presents with hepatoblastoma utilizes a combination of conventional resection, chemotherapy, and transplantation (Figure 4). The treatment algorithm is tailored to the individual child. In some of our patients we have utilized living-related donors in order to optimize the timing of liver transplantation with respect to chemotherapy course. Ideally, two cycles of chemotherapy are administered post-transplantation, and, in general, these have been tolerated well in our post-transplant patients.

REFERENCES


50 Years Ago in The Journal of Pediatrics

THE TIME OF PASSAGE OF THE FIRST STOOL AND FIRST URINE BY THE NEWBORN INFANT

Journal articles that report important findings and remain unchallenged for 50 years are uncommon, but this is one of them. In this elegant little study, Drs Sherry and Kramer timed the passage of first stool and first urine in 500 term newborn infants. They found that 94% of newborn infants had their first stool at 24 hours of age and in the same time period, 92% of infants had voided. Two years later, the same authors reported the results of a similar study in premature infants.1

The manuscript is as simple as its findings are important. The first passages of stool and urine are reassuring indicators that exclude, with high sensitivity and specificity, some important congenital abnormalities. Every provider of medical care to newborn infants is taught these data and applies them routinely. And only two journal pages were required, including tables, discussion, and relevant references. An editor’s dream.

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REFERENCE
SEX DIFFERENCES IN PATIENTS REFERRED FOR EVALUATION OF POOR GROWTH

ADDIA GRIMBERG, MD, JESSICA KATZ KUTIKOV, MD, AND ANDREW J. CUCCHIARA, PHD

Objectives  The objective of this study was to compare sex differences among referrals for evaluation of poor growth.

Study design  This study was based on chart reviews of all new-patient encounters at Children’s Hospital of Philadelphia Diagnostic and Research Growth Center for short stature or poor growth evaluations during 2001. Outcome measures were patient growth characteristics, frequency of underlying pathology, and frequency of laboratory and radiologic investigations before referral.

Results  One hundred eighty-two boys and 96 girls were referred (P < .0001). Girls were shorter, relative to the general population (median height z score, −2.4 vs −1.9 for boys, P = .02) and mid-parental target heights (median deficit, 1.9 vs 1.3 SD, P < .01). Differences were more pronounced starting at age 9 years. Median time to referral from initial fall-off on the growth curve was 35 months in girls and 24 months in boys (not significant). The percentage of girls (41%) with organic disease significantly exceeded that of boys (15%). Conversely, more boys (72%) than girls (48%) were of normal height or short but healthy (P < .0001). Sex was not associated with frequency of tests before referral; neither was severity of short stature.

Conclusions  Sex differences in short stature referrals may delay diagnosis of diseases in girls while promoting overzealous evaluations of healthy boys who do not appear to be tall enough. (J Pediatr 2005;146:212-6)

Growth is perhaps the most sensitive indicator of a child’s overall health; growth failure may be the first and only sign of underlying disease in a child. For example, growth failure may precede abdominal symptoms by months or years in children with inflammatory bowel disease. Other diseases that can present with growth failure alone include celiac disease, cystic fibrosis, renal tubular acidosis, and HIV infection. Hence, growth should be carefully monitored, and when an abnormality is identified, it should be investigated to differentiate normal variants in healthy children from underlying pathology that requires treatment. The American Academy of Pediatrics acknowledged the importance of growth in its March 2000 “Recommendations for Preventative Pediatric Health Care,” stating that a child’s height and weight should be measured at least at birth, age 2 to 4 days, 1, 2, 4, 6, 9, 12, 15, 18, and 24 months, and every year thereafter through age 21 years.

Because growth can serve as a marker for potential underlying disorders, growth failure should be given equal import when evaluating children of either sex. On the contrary, social pressures focus more on growth in boys than girls. Studies have linked height to elementary school teachers’ perceptions of competence in the male students, male success in mating selection, occupational success, achievement as an academic, perceptions of presidential candidates, and perceived performance and leadership in military service. Growth hormone registries indicate preferential treatment of boys with poor growth; boys receive growth hormone therapy by ratios of approximately 2:1, depending on the diagnosis, with the highest for idiopathic short stature. This study proposed to determine whether there are sex differences among children referred to a pediatric endocrine center for evaluation of short stature or poor growth.

METHODS

All encounters at The Children’s Hospital of Philadelphia Diagnostic and Research Growth Center for short stature or poor growth evaluations from January 1, 2001, through December 31, 2001, were reviewed. Only new patients with the chief complaint of short
stature were included in this study (n = 278). Patients with prior evaluation by an endocrinologist or previous treatment with growth hormone (n = 34), children referred for pituitary evaluation (secondary to brain tumor, meningitis, septo-optic dysplasia, and other brain malformations) in whom growth was not the primary concern (n = 15), and girls with known Turner syndrome (n = 6) were excluded.

Sex, age, patient height, parent height, final diagnosis, and laboratory and radiologic evaluations before referral were extracted from patient charts and entered into a database. The patient’s height at the first clinic visit was the arithmetic mean of three sequential measurements, using a wall-mounted Holtain stadiometer for older children and a recumbent stadiometer for infants and toddlers. Parents’ heights were self-reported. For 115 children, a copy of their growth curves was available for estimating the age at first deviation, that is, the age at which the plotted heights first fell across major percentiles.

The patient’s height measurement was converted into a height z score, standardized for age and sex, using the Growth Calculator electronic program (developed by Phillip Cheng MD, 2000; referenced to the National Center for Health Statistics percentiles). Mid-parental target height was calculated by the method of Tanner and then transformed into mid-parental target height z score using Growth Calculator. The patient’s height deficit was calculated by subtracting the patient’s height z score from the mid-parental height z score, and the time to referral was calculated by subtracting the age at first deviation across major percentiles from the age at the first visit. Normal height was defined as healthy children whose height z score was within 2 SD of both the population mean and their mid-parental target. Diagnoses were tabulated for children whose heights did not meet the definition of normal.

Descriptive statistics and tests of hypotheses were calculated through the use of JMP Software (SAS Institute; Cary, NC) and StatXact (Cytel Software Corp, Cambridge, MA). Differences between male and female means were evaluated by Wilcoxon rank sum test for continuous outcomes (due to skewed data sets) and by $\chi^2$ tests for categoric ones. Linear trend analysis of male proportions as a function of age or third-order interactions. Logistic regression analyses were applied to investigate if laboratory or radiologic testing, both the total number of tests and frequency of each individual test, were related to sex, height z score, or height deficit as explanatory variables in main effect, second-order or third-order interactions.

Approval of the Children’s Hospital of Philadelphia Institutional Review Board was obtained before commencing this study.

### RESULTS

Referrals to our Growth Center included 182 boys and 96 girls, a significant ($P < .0001$) departure from the null hypothesis of equal proportions. At the time of referral, the height deficit was greater in girls than in boys, both relative to the general population (Figure 1) and relative to their mid-parental target heights (Figure 2). Median height z score for girls was $-2.4$ versus $-1.9$ for boys ($P < .01$), and median deficit from mid-parental target height z score was $1.9$ for girls versus $1.3$ for boys ($P < .001$). Mid-parental height $z$ score could not be determined for 6 girls and 13 boys because of unknown height of one or both parents. Although the median time to referral was longer in girls (35 months) than boys (24 months), the difference was not statistically significant ($P = .30$) (Figure 3). The percentage of girls (44%) versus boys (40%) for whom prior growth curves were provided was not statistically different ($P = .55$).

The proportion of male referrals by age is shown in Figure 4; the linear trend was positively associated with age across the entire group ($P < .01$). From Figure 4, it appears that the male predominance was particularly evident starting at age 9 years. Indeed, boys comprised 57% of the 117 referrals under the age of 9 years and 71% of the 161 children age 9 years and older ($P < .05$). Under age 9 years, neither height $z$ scores (medians $-2.4$ for girls and $-2.1$ for boys; $P = .48$) nor height deficits from the mid-parental target heights (medians, 1.8 for girls and 1.5 for boys; $P = .34$) were significantly different between sexes. However, for children age 9 years and older, the median height $z$ score for girls was $-2.4$ versus $-1.9$ for boys ($P < .01$), and median deficit from mid-parental target height $z$ score was 2.1 for girls versus 1.2 for boys ($P < .001$).

Organic disease was more common ($P < .0001$) among girls (39/96) than boys (27/182). The risk ratio was 2.7, with 95% confidence interval of [1.8 to 4.2]. Even if the 9 girls found to have Turner syndrome were excluded, more girls (31%) than boys (15%) had organic disease. Conversely, the percentage of boys referred at normal height (38%) exceeded ($P < .01$) that of girls (20%). Of the boys, 25% had constitutional growth delay, 7% had familial short stature, and 2% had both; for girls, the proportions were 15%, 7%, and 6%, respectively ($P = .31$ for all three diagnoses combined). Nonorganic diseases (failure to thrive/nutritional dwarfing and psychosocial dwarfing) were similar (9% of boys and 8% of girls).

Sex was not significantly associated with the number of laboratory and/or radiologic studies obtained before referral ($P = .90$) (Table). Logistic regression analysis identified one statistically significant association of sex and laboratory testing: chromosomes ($P < .001$). A trend existed in prior measurement of celiac antibodies, more often obtained in boys than girls ($P = .053$), yet the one case of celiac disease found in our cohort was a girl. Prior evaluation by the primary physician was not found to be statistically associated with the severity of short stature, indicated by either height $z$ score ($P = .14$) or deficit from mid-parental target height $z$ score ($P = .85$).

### DISCUSSION

Our data demonstrate a sex bias in referrals for poor growth evaluation. It is unclear if this discrepancy results from dilution of the frequency of referrals for organic disease in boys.
by all the healthy boys of normal or short height. More worrisome is the possibility that underappreciation of growth problems in girls results in missed diagnoses in girls who are not referred. Additional studies with access to the total primary care population are needed to determine the characteristics of the children who are not referred. For example, in a Utah school-based screening study, 50% of children found to have growth hormone deficiency (2.7 male-to-female ratio) and 17% of the girls found to have Turner syndrome had been previously advised to see an endocrinologist; only 25% of the former and none of the latter had done so. It is also unclear what portion of the observed differences in referrals was due to bias by the primary physicians or to selection bias by the patients and their families. In response to the greater social pressures for tall stature in men, boys and their families may be more likely to request specialist referrals from their primary physicians or else to seek specialist care directly. Our study suggests that age plays a role, with the
Sex Differences In Patients Referred For Evaluation Of Poor Growth

Can predispose to other autoimmune conditions. Conversely, early diagnosis and treatment are associated with better final height outcomes in growth hormone deficiency and Turner syndrome and can obviate the need for delaying puberty.

Chart reviews of the University of North Carolina Turner Syndrome Clinic found an average 5.2-year delay in diagnosis from the time the height had fallen below the 5th percentile. There may also be significant consequences of overzealous evaluation and treatment of healthy boys. It reinforces to the boys and their families that their height is a bona fide problem that requires medical intervention, which may serve to exacerbate rather than alleviate the self-esteem issues with which the boys may be grappling. Despite the social stresses demonstrated in multiple studies, other studies have countered that short stature does not preclude normal psychosocial adjustment in children or adults. On a societal level, it raises questions about resource allocation and ethical debates about medical versus cosmetic treatment, a subject already extensively argued in the literature. Our study demonstrates a sex bias in referrals to a pediatric endocrine center for poor growth evaluations. In a survey of pediatric endocrinologists, the specialists recommended growth hormone treatment 1.3 times as often for identical hypothetical case scenarios that described male rather than female patients. The growth hormone registries already document a male predominance among treated children. With the new FDA-approved indication for growth hormone therapy to treat idiopathic short stature, these multilayered gender biases have the potential to translate into even more significant therapeutic discrepancies.

This study adds to a worrisome trend in the recent literature that indicates suboptimal use of growth monitoring. Despite the explicit American Academy of Pediatrics recommendations, 35% of well-child encounters at an academic pediatric clinic failed to plot growth measurements and/or document a growth abnormality. Often, recommendations for measuring and plotting length rather than height in younger children are not followed, or improper equipment is used. Our chart reviews found cases exemplifying these previously published errors, but most troublesome was the lack of prior growth curves for 163 of the 278 children referred. Failure to forward the growth curves does not necessarily mean that the primary physicians did not construct such curves for use in their practices. Rather, it highlights an underappreciation of the importance of growth curves as a tool for assessing growth. Growth velocity cannot be determined from a solitary height measurement, so 59% of the children referred specifically for the evaluation of poor growth did not have the data necessary to include growth velocity in that evaluation. Although incomplete, the percentage of growth curves provided for each sex did not differ statistically. The median time to referral for girls was nearly 1 year longer than for boys, but the difference was not statistically significant. This may be due to overlap of the two groups or to insufficient power, associated with the reduced sample size involving only 41% of the subjects in this study. A World Health Organization survey revealed suboptimal growth curve use to be a global
greatest pressures felt in the peripubertal years. It would be interesting to explore other potential factors in referral patterns, such as parental height, parental socioeconomic background, cultural background, and insurance status.

Comparison with other studies confirms the sex bias in referrals. In our center, 72% of boys and 48% of girls were of normal height or short with familial short stature, constitutional growth delay, or both. These same categories comprised 63% of short stature referrals to the Pediatric Endocrine Ambulatory Center at North Shore University Hospital during 1973 to 1991; the sex distribution was not reported. However, such marked differences were not seen in the Utah school-based screening program; of the 555 children identified as growing abnormally, 83% of boys and 77% of girls had familial short stature, constitutional growth delay, or both.

In a prospective study of 220 pediatric endocrine centers children referred for short stature whom the endocrinologists thought would be seen at least once more for follow-up, 69% of the 21,736 children enrolled during 1981 through 1999 were male (personal communication, Genentech). Although this population differs slightly from our study of all new short stature referrals, the 2:1 ratio was also found.

Either scenario portends a disservice to children. There may be significant medical consequences to delayed or missed diagnoses in girls whose growth problems are underappreciated. For example, long-standing unrecognized celiac disease
table. Frequency of prereferral investigations, distributed by sex

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*Significance is expressed as P < .05.
† Other laboratory values for boys: HIV (1), lead level (1), insulin level (0.5), ferritin/iron/total iron binding capacity (0.5), homocysteine (0.5), plasma amino acids (0.5); for girls: lead level (4), hepatitis serologies (1).
issue, including North America, one that we hope will be addressed for all children.

We are grateful to Serono, Inc, for providing the Growth Calculator and the University of Pennsylvania General Clinical Research Center for biostatistical guidance.

REFERENCES


CHRONIC IMMUNE THROMBOCYTOPENIC PURPURA IN CHILDREN:  ASSESSMENT OF RITUXIMAB TREATMENT

JULIE WANG, MD, JOSEPH M. WILEY, MD, RUTH LUDDY, MD, JAY GREENBERG, MD, MICHAEL A. FEUERSTEIN, BA, AND JAMES B. BUSSEL, MD

Objectives  This study examined the efficacy and safety of rituximab in children with chronic immune thrombocytopenic purpura.

Study design  Twenty-four patients, 2 to 19 years of age, with platelet counts <30,000/mcL (microliter), received 375 mg/m² rituximab in 4 weekly doses. Platelet response was characterized as complete (CR) if a count >150,000/mcL was achieved; partial (PR) if 50,000 to 150,000/mcL; minimal (MR) if the count increased by >20,000/mcL to a peak count >30,000/mcL but <50,000/mcL; or no response (NR).

Results  Fifteen of 24 patients (63%) achieved a CR lasting 4 to 30 months, 9 of which are ongoing. Two had PRs lasting 4 and 6 months; 2 had MRs lasting 5 and 8 months, and 5 did not respond. Pruritus, urticaria, and throat tightness (but no respiratory distress) occurred with the first infusion in a small number of children. Three patients had serum sickness after the first, second, and third infusions, respectively. No increased frequency or severity of infections was seen, although immunoglobulin levels decreased to below the normal range in 6 of 14 cases.

Conclusions  Rituximab may be a useful treatment for chronic immune thrombocytopenic purpura in children with a >50% CR rate lasting an average of 13 months, with 9 of 15 CRs ongoing (8 lasted 6 months or longer). There was no substantial toxicity other than transient serum sickness. (J Pediatr 2005;146:217-21)

Immune thrombocytopenic purpura (ITP) results from accelerated platelet destruction mediated by autoantibodies to platelet glycoproteins.1 Although children with ITP often have their thrombocytopenia resolve with or without intervention, ITP persists beyond 6 months in approximately 10% to 20% of children who are classified as having chronic disease.2 Repeated courses of steroids, intravenous immunoglobulin (IVIG), and anti-D can increase platelet counts in more than 60% to 80% of patients,3 but the disease may persist in those destined to be chronic despite repeated, transient responses to these interventions. Splenectomy is often effective and leads to a “cure” in 70% to 80% of cases.4 However, a fraction of patients do not respond, and patients increasingly are reluctant to undergo this procedure. More difficult cases of chronic ITP may require additional therapy, including danazol, azathioprine, cyclophosphamide, and vinca alkaloids5; these are often reserved until after splenectomy because of a combination of toxicity and inconsistent efficacy.

A promising therapy for refractory ITP in adults is rituximab, a chimeric murine/human anti-CD20 monoclonal antibody that was licensed for treatment of B-cell lymphomas.6 Its infusion leads to a substantial depletion of malignant and normal B cells. Rituximab has been infused in almost a half million adults with B-cell lymphoma; in general, safety has been well established in adults not receiving additional, intense immunosuppression.7 It was first investigated as a treatment for chronic ITP in adults on the basis of its ability to deplete B cells and the fact that ITP is an autoantibody-mediated disease.8,9 At least 4 studies have demonstrated efficacy rates for rituximab of 30% to 60%;
responses lasting more than 1 year were seen in 32% of adults with chronic ITP in the largest study.10

With the low toxicity profile of rituximab and studies showing durable responses after single courses of 4 infusions in a number of refractory adult ITP patients, this therapy was evaluated in 24 children with chronic ITP.

METHODS

Patients

Eligibility for this study included chronic ITP (duration >6 months) with platelet counts off other treatment of less than 30,000/mcL (Table I). Three patients had platelet counts greater than 30,000/mcL in the 4 weeks before the study as a result of steroid treatment for low platelet counts. The age, sex, duration of ITP, and previous therapies are listed in Table I. Patient ages ranged from 2 to 19 years; 4 patients had previously undergone splenectomy. Nine patients were treated at the New York Presbyterian Hospital–Weill Cornell center, 10 at The Children’s Hospital at Sinai in Baltimore, and 5 at Fairfax Hospital in Virginia. Permission for treatment was obtained from all patients’ parents and assent was obtained from the patients; all were aware that rituximab had not been approved for use in adults or children with ITP.

Treatment

Rituximab (anti-CD20, Genentech, South San Francisco, CA) was infused at the standard dose of 375 mg/m² weekly for 4 weeks. Patients were premedicated with acetaminophen, diphenhydramine, and/or prednisone or solumedrol. The 4 infusions were completed as scheduled, 375 mg/m² weekly for 4 weeks in 21 of the 24 patients. One patient (patient 2) received the 4 doses in only 2 weeks because of residence outside of New York. Patients 4 and 18 had serum sickness after the second and third infusions, respectively, and had their subsequent treatments discontinued. Although patient 20 had mild serum sickness after the first infusion, she was treated with 2 mg/kg per day prednisone with a slow taper of the dose and tolerated the remaining infusions.

Several patients who responded and relapsed were retreated with rituximab.

Laboratory Studies

All patients were monitored with weekly blood counts during the first 4 weeks; subsequent monitoring occurred at 1- to 4-week intervals, depending on response. Immunoglobulin levels were measured every 2 to 4 months for patients 1 through 9 and on occasion for patients 20 through 24. The patients treated at the New York Presbyterian Hospital–Weill Cornell center (patients 1 through 9) had their B-cell numbers measured.

Response Criteria

Responses were defined as: complete (CR) if the peak platelet count was >150,000/mcL; partial (PR) if a count of 50,000 to 150,000/mcL was achieved; minimal (MR) if there was an increase of >20,000/mcL to a peak count greater than 30,000/mcL but less than <50,000/mcL; and none (NR) if there was no platelet increase. Data analysis was primarily descriptive, using medians and ranges. A Kaplan-Meier analysis was performed to represent duration of responses in the CR group. Duration of response was considered from the day of the initial infusion until March 2004.

Adverse events were monitored clinically on the frequent and regular visits made during and after the treatment with rituximab. No systematic grading system was used to assess symptoms of bleeding.

RESULTS

Patients enrolled in this pilot study had chronic ITP lasting from 6 to 120 months. All patients had had only transient responses after 1 to 7 other therapies before the initiation of this study. Four patients had already failed splenectomy (Table I).

Treatment Response

Fifteen of the 24 patients (63%) achieved stable platelet counts >150,000/mcL for 4 to 30 months without additional therapy (Table I). Thirteen of the 15 complete responders (CRs) had platelet counts consistently >50,000/mcL within 4 weeks of starting rituximab and achieved their CRs within 10 weeks. Two of the CRs were not achieved until 9 and 10 months after starting rituximab treatment (patients 10 and 12), even though partial responses, platelet counts >50,000/mcL, were achieved at 1 and 2 months, respectively. Three of the 4 patients who had previously failed to respond to splenectomy achieved CRs. Two patients achieved PR by week 7 and consistently maintained their platelet counts above 50,000/mcL by 11 weeks after the start of rituximab. Two patients had MRs and 5 patients had NR to treatment.

Duration of Response

Six of the 15 complete responses continued for 3 to 18 months before relapsing and requiring additional treatments. Nine patients have ongoing complete responses (Table I). Of these 9 patients, 6 have lasted more than 1 year and 2 continue to maintain a complete response 24 and 30 months after rituximab was initiated. A Kaplan-Meier analysis suggests that approximately 50% of CRs will have a durable response for a mean of 15 months (data not shown).

Patients 4 and 18 were able to achieve complete responses despite receiving only 2 and 3 doses of rituximab, respectively, because of serum sickness. Patient 4 relapsed and received other treatments before going into a lasting remission; however, patient 18 has an ongoing CR. Patients 5 and 15 maintained PRs for 6 and 4 months, respectively; patient 5 subsequently underwent splenectomy but relapsed 9 months afterward. The two MRs had response durations of 5 and 8 months. Overall, the response rate among younger (<10 years) and older (≥10) patients was comparable in rate and duration. Two of the 3 patients achieving a CR after splenectomy relapsed (patients 1 and 2). Patient 13 achieved a CR after splenectomy and has had an ongoing response for 24 months.
Toxicity

Infusion reactions were generally mild and primarily occurred with the first infusion (Table II). All reactions resolved with slowing of the infusion rate and/or administration of additional diphenhydramine or steroids. Serum sickness developed in 3 cases. Two presented with maculopapular rash, arthralgia, low-grade fever, and malaise 7 to 14 days after the first infusion of rituximab. The third patient was diagnosed on the basis of an acute reaction followed by persistent arthralgia after the first infusion. With the exception of these 3 cases of serum sickness, there have been no significant clinical adverse events reported with a mean follow-up of 10 months in the 24 patients.

Immunologic Parameters (Patients 1 Through 9 and 20 Through 24)

Three patients (patients 2, 7, and 22) had preexisting neutropenia and leukopenia with normal (patients 2 and 22) and mildly hypocellular (patient 7) bone marrow examinations; neutropenia improved with rituximab in 2 patients, and 1 was unchanged (Table II). Patient 2 had an absolute neutrophil count (ANC) of 300 cells/mm³ at the start of rituximab; by week 12, the ANC was consistently above 500 cells/mm³, with the majority of counts greater than 1500 cells/mm³. Patient 22 had an ANC above 500 cell/mm³ by week 2. Patient 7’s ANC fluctuated between 500 cells/mm³ and 3700 cells/mm³ throughout the course of treatment. As expected, in 6 of 6 patients whose B cells were measured using CD19, there was a substantial reduction after infusion of rituximab. Six patients (1, 2, 5, 9, 20, and 22) had immunoglobulin (IgM and/or IgG fall to a level below the normal range after administration of rituximab (Table III). One patient (No. 2) had a baseline low IgM before rituximab treatment; he was entered because he was especially refractory, as seen in Table I, having failed 7 treatments, including splenectomy. His IgG levels did not fall out of the normal range and his IgM remained stable. Patients 20 and 22 did not have baseline

### Table I. Clinical and hematologic characteristics of patients with chronic immune thrombocytopenic purpura

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age/sex</th>
<th>Prior therapy</th>
<th>ITP duration (mo)</th>
<th>Lowest platelet count*</th>
<th>Response duration</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>3/F</td>
<td>Splenectomy, IVIG, St, Anti-D, Cy</td>
<td>15</td>
<td>4</td>
<td>CR (6 mo)</td>
</tr>
<tr>
<td>2</td>
<td>16/M</td>
<td>Splenectomy, IVIG, St, Anti-D, Danazol, Az, Vc</td>
<td>96</td>
<td>24</td>
<td>CR (15 mo)</td>
</tr>
<tr>
<td>3</td>
<td>16/F</td>
<td>IVIG, St, Anti-D, Az</td>
<td>24</td>
<td>39</td>
<td>CR (17 mo)-O</td>
</tr>
<tr>
<td>4</td>
<td>14/F</td>
<td>IVIG, St, Anti-D</td>
<td>9</td>
<td>11</td>
<td>CR (3 mo)</td>
</tr>
<tr>
<td>5</td>
<td>12/M</td>
<td>IVIG, St</td>
<td>6</td>
<td>3</td>
<td>PR (6 mo)</td>
</tr>
<tr>
<td>6</td>
<td>17/F</td>
<td>IVIG, St</td>
<td>108</td>
<td>14</td>
<td>MR (8 mo)</td>
</tr>
<tr>
<td>7</td>
<td>10/M</td>
<td>IVIG, St, Anti-D, Az, Vc</td>
<td>6</td>
<td>16</td>
<td>MR (5 mo)</td>
</tr>
<tr>
<td>8</td>
<td>11/M</td>
<td>St, Anti-D</td>
<td>12</td>
<td>18</td>
<td>NR</td>
</tr>
<tr>
<td>9</td>
<td>13/M</td>
<td>Splenectomy, IVIG, St, Anti-D</td>
<td>120</td>
<td>17</td>
<td>NR</td>
</tr>
<tr>
<td>10</td>
<td>17/F</td>
<td>St</td>
<td>12</td>
<td>5</td>
<td>CR (13 mo)</td>
</tr>
<tr>
<td>11</td>
<td>19/F</td>
<td>IVIG, St, Anti-D</td>
<td>47</td>
<td>23</td>
<td>CR (30 mo)-O</td>
</tr>
<tr>
<td>12</td>
<td>16/M</td>
<td>St</td>
<td>7</td>
<td>3</td>
<td>CR (18 mo)</td>
</tr>
<tr>
<td>13</td>
<td>12/F</td>
<td>Splenectomy, IVIG, St, Anti-D</td>
<td>13</td>
<td>1</td>
<td>CR (24 mo)-O</td>
</tr>
<tr>
<td>14</td>
<td>3/M</td>
<td>IVIG, St, Anti-D</td>
<td>22</td>
<td>4</td>
<td>NR</td>
</tr>
<tr>
<td>15</td>
<td>7/F</td>
<td>IVIG, St, Anti-D</td>
<td>19</td>
<td>82</td>
<td>PR (4 mo)</td>
</tr>
<tr>
<td>16</td>
<td>2/F</td>
<td>IVIG, St, Anti-D, Vc, Csp</td>
<td>12</td>
<td>3</td>
<td>NR</td>
</tr>
<tr>
<td>17</td>
<td>18/F</td>
<td>IVIG, St</td>
<td>33</td>
<td>21</td>
<td>CR (6 mo)-O</td>
</tr>
<tr>
<td>18</td>
<td>12/F</td>
<td>IVIG, St, Anti-D</td>
<td>30</td>
<td>26</td>
<td>CR (6 mo)-O</td>
</tr>
<tr>
<td>19</td>
<td>18/M</td>
<td>St</td>
<td>8</td>
<td>85</td>
<td>CR (4 mo)-O</td>
</tr>
<tr>
<td>20</td>
<td>12/F</td>
<td>IVIG, Anti-D</td>
<td>96</td>
<td>2</td>
<td>CR (19 mo)-O</td>
</tr>
<tr>
<td>21</td>
<td>8/F</td>
<td>St, Anti-D</td>
<td>48</td>
<td>15</td>
<td>NR</td>
</tr>
<tr>
<td>22</td>
<td>8/M</td>
<td>St</td>
<td>36</td>
<td>1</td>
<td>CR (9 mo)</td>
</tr>
<tr>
<td>23</td>
<td>18/M</td>
<td>St, Anti-D, IVIG</td>
<td>60</td>
<td>30</td>
<td>CR (21 mo)-O</td>
</tr>
<tr>
<td>24</td>
<td>15/F</td>
<td>St, Anti-D</td>
<td>30</td>
<td>3</td>
<td>CR (15 mo)-O</td>
</tr>
</tbody>
</table>

*ITP, thrombocytopenic purpura; IVIG, intravenous immunoglobulin; St, steroids (prednisone, solumedrol, dexamethasone); Cy, cyclophosphamide; Az, azathioprine; Vc, vincristine; Csp, cyclosporine; O, ongoing response.

*Lowest platelet count within 1 month before initiation of rituximab; patients 3, 15, and 19 were receiving long-term steroid treatment to maintain their platelet counts before start of rituximab. Steroids were tapered and discontinued after rituximab treatment.

Patient 4 had serum sickness after the second dose (3rd and 4th doses omitted). Patient 18 had serum sickness after the third dose (4th dose omitted). Patient 20 had serum sickness after the first dose but was able to complete all 4 infusions.
immunoglobulin levels measured before treatment. Three patients had IgM and/or IgG fall to less than 50% of the lower limit of normal; no one received replacement IVIG. There was no increase in frequency or severity of infections during this study, including no cases of pneumonia, sepsis, or abscess.

### DISCUSSION

This is the largest report of rituximab treatment in children with hematologic disease to date. Rituximab treatment was initiated for 24 patients, 18 of whom were 10 years of age or older. All had ITP that had failed to respond to between 1 and 7 different treatments, including 4 patients who had failed splenectomy. The CR rate was 15 of 24 (63%); there were also 2 PRs and 2 MRs, for an overall response rate of 78%. Fifteen of the 17 good responders (CRs and PRs) had platelet increases to above 50,000/mcL within 4 weeks; thus, children who respond to rituximab probably will do so early. These 17 good responses lasted a median of 17 months. Nine patients, all CRs, have ongoing responses, 6 of whom are more than 1 year from their initial infusions. Six patients with CRs relapsed 3 to 18 months from their initial infusion. The long-term response rate, based on Kaplan–Meier analysis, suggested that the lasting CR rate is approximately 32%. There was no statistical difference between those who received multiple prior treatments for ITP compared with those who had only received 1 or 2 treatments in terms of response or duration of response. No major hemorrhage was seen in any patient reported in this series.

At the dose of rituximab licensed for use in adults, therapy was well tolerated, except for 3 cases of serum sickness, possibly relating to the immunocompetence of these children; we are aware of at least 2 additional cases of serum sickness occurring in children with ITP. Minor infusion-related effects were associated with the first dose and included pruritus, throat tightness, headache, and urticaria. Initial reports of more serious reactions have been found to be rare with appropriate premedication and rate of infusion increase. In addition, they are especially low in healthy outpatients without comorbid medical conditions.11

At one center, immunology data were available. As expected, B-cell levels decreased dramatically after rituximab treatment in all tested patients and returned to near baseline within 6 months. Three patients with preexisting neutropenia

---

**Table II. Adverse events during treatment with rituximab**

<table>
<thead>
<tr>
<th>Patient</th>
<th>Pruritus§</th>
<th>Throat tightness§</th>
<th>Urticaria§</th>
<th>Headache§</th>
<th>Chest pain‡</th>
<th>Serum sickness‡</th>
<th>LowANC¶</th>
</tr>
</thead>
<tbody>
<tr>
<td>2</td>
<td>Yes</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>N</td>
</tr>
<tr>
<td>4</td>
<td>N</td>
<td>N</td>
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<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>N</td>
</tr>
<tr>
<td>6</td>
<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>7</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>N</td>
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<td>N</td>
</tr>
<tr>
<td>10</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>Yes</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>18</td>
<td>N§</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>19</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>20</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>N</td>
<td>N</td>
</tr>
<tr>
<td>22</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>N</td>
<td>Yes</td>
<td>N</td>
</tr>
</tbody>
</table>

Patients 1, 3, 5, 8, 9, 11, 12, 13, 14, 15, 16, 17, 21, and 23 had no clinical adverse events.

§No respiratory distress or difficulty swallowing was seen.

‡Immediately after completion of infusion.

¶After second infusion for patient 4, after the third infusion for patient 18, after first infusion for patient 20.

§Only with first infusion.

Preexisting neutropenia; patient 2 improved on rituximab.

Nonspecific, nonpruritic rash during the first infusion.

**Table III. IgM/IgG levels (mg/dL) in six patients whose levels dropped below normal ranges**

<table>
<thead>
<tr>
<th>Patient</th>
<th>IgM start (normal for age)</th>
<th>IgM nadir† (wk)</th>
<th>IgG start (normal for age)</th>
<th>IgG nadir† (wk)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>84 (22–159)</td>
<td>11 (11)</td>
<td>1690 (441–1135)</td>
<td>370 (19)</td>
</tr>
<tr>
<td>2</td>
<td>39 (56–352)</td>
<td>32 (52)</td>
<td>1370 (639–1347)</td>
<td>889 (35)</td>
</tr>
<tr>
<td>5</td>
<td>87 (56–352)</td>
<td>26 (42)</td>
<td>1890 (639–1347)</td>
<td>488 (32)</td>
</tr>
<tr>
<td>9</td>
<td>60 (56–352)</td>
<td>35 (13)</td>
<td>1310 (639–1347)</td>
<td>1070 (5)</td>
</tr>
<tr>
<td>20</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>250 (12)</td>
</tr>
<tr>
<td>22</td>
<td>(48–207)</td>
<td>&lt;20 (4)</td>
<td>(633–1280)</td>
<td>&lt;300 (4)</td>
</tr>
</tbody>
</table>

*Baseline immunoglobulin levels were not obtained for patients 20 and 22.

†Nadir was less than 50% of the lower limit of normal for 3 patients.
had low ANCs recorded during this study, two of whom showed improvement in the ANC, suggesting that they may have had autoimmune neutropenia. Decreases in IgM and/or IgG levels below the normal ranges were noted in 6 of the cases, but there was no increase in frequency or severity of infections.

In the largest adult study that included 57 patients, of which 31 failed splenectomy, 18 had complete responses. Of these 18, 15 have maintained their complete responses for more than 1 year after rituximab treatment,10 whereas the response durations of our 15 complete responders tended to be shorter (mean, 13 months; lasting CR rate, 50%). On the other hand, there was a higher overall complete response rate in the children (63% vs 32%, P = .01 by χ² test). The two children with PRs relapsed within 6 months, as did 11 of the 13 adult patients. Other differences in our experience between adult and pediatric patients is that the children generally responded faster than the adults (within 4 weeks versus within 8 weeks) and were more likely to have their IgG and/or IgM levels decrease to less than the lower limit of normal for age (6 of 13 compared with 1 of 44).

The efficacy and safety of rituximab treatment in pediatric cases (median age of 2 years in the larger study) has been reported for autoimmune hemolytic anemia.12,13 Both studies report high response rates (6 of 6 responses ranging from 15 to 22 months12 and 13 of 15 responses, with 10 having sustained responses lasting on average 13 months).13 Treatment was well tolerated, with 3 patients having infections, 2 with fevers, and 1 with upper airway edema in both studies combined (21 patients). However, children in these studies were maintained on IVIG after treatment to prevent rituximab-induced hypogammaglobulinemia.

Rituximab may be a good option for children who fail splenectomy, since 3 of our 4 splenectomized patients achieved complete responses with a minimum duration of 6 months. In the future, rituximab may also be used to replace or delay this surgical option. Toxicity was generally not substantial, although there were 3 cases of serum sickness. It appeared that immunoglobulin levels sometimes fell to less than normal, although 4 of the 6 patients in whom this was reported were older than 10 years of age and no significant infections were noted. The 8 patients younger than 10 years of age had response types, durations, and toxicity that were no different from the older patients.

Trials with larger numbers of children will be needed to determine the true rates of efficacy before and after splenectomy, what the duration of complete responses are, and whether there are prognostic factors for response such as primary compared with secondary ITP. A multicenter, single-arm study has begun to answer these questions.

REFERENCES

ANTIBIOTIC PRESCRIBING FOR UPPER RESPIRATORY TRACT INFECTION: THE IMPORTANCE OF DIAGNOSTIC UNCERTAINTY

SANDRA R. ARNOLD, MD, MSc, TERESA TO, MSc, PhD, WARREN J. MCISAAC, MD, MSc, CCFP, and ELAINE E. L. WANG, MD, MCM, MSc

Objectives Antibiotic misuse for viral upper respiratory tract infections (URI) in children is a significant problem. We determined the influence on antibiotic prescribing of clinical features that may increase concern about possible bacterial infection (age, appearance, fever) in children with URI.

Study design We created 16 scenarios of children with URI and distributed them by mail survey to 540 pediatricians and family practitioners in Ontario, Canada. The association of patient clinical features, parental pressure, and physician characteristics with antibiotic prescribing was determined through the use of logistic regression analysis.

Results A total of 257 physicians responded (48%). Poor appearance (OR, 6.50; 95% CI, 5.06 to 3.84), fever above 38.5°C (OR, 1.48; 95% CI, 1.21 to 1.82), and age older than 2 years (OR, 2.27; 95% CI, 1.85 to 2.78) were associated with prescribing, whereas parental pressure was not. Physician characteristics associated with antibiotic use were family practitioner (OR, 1.54; 95% CI, 1.22 to 1.96), increasing number of patients seen per week (OR, 1.05; 95% CI, 1.01 to 1.08 for every 20-patient increase), and increasing physician age (OR, 1.17; 95% CI, 1.11 to 1.24, 5-year increments).

Conclusions Clinical factors, which may lead physicians to be concerned about possible bacterial infection in children, are associated with antibiotic use for pediatric URI. (J Pediatr 2005;146:222-6)

Antibiotic exposure has been linked to an increased risk of harboring or becoming infected with an antibiotic-resistant bacteria.1-4 Misuse of antibiotics for viral upper respiratory tract infections (URI) has been well documented, with prescribing rates as high as 40% to 75% for such illness visits5,6 in both the United States and Canada. Research has identified 4 factors as determinants of antibiotic misuse: (1) physician-patient interaction, (2) physician characteristics, (3) physician time constraints, and (4) diagnostic uncertainty.7-13

Although the first three have received extensive review,7,9-12 diagnostic uncertainty, as a factor in antibiotic overuse, has been inadequately studied. We define diagnostic uncertainty as the difficulty in distinguishing a self-limited viral infection from a bacterial infection requiring antibiotic therapy. It has been shown that physicians frequently overestimate the probability that an adult patient with a URI has a bacterial infection, such as pharyngitis,13 sinusitis,14 and bronchitis.14,15 Diagnostic uncertainty regarding the presence of one of these bacterial syndromes may be an important determinant of antibiotic misuse in children as well. In addition, the difficulty in predicting invasive bacterial infections in young, febrile children16,17 may also contribute to the use of antibiotics in children with fever with or without symptoms of URI.

The objective of this study was to determine the relative effect of patient and illness characteristics, which enhance diagnostic uncertainty, on a physician’s decision to prescribe an antibiotic for pediatric URI contrasted with the effect of pressure from a parent for a prescription.

METHODS

Participants

We distributed a self-administered mail survey to a quasi-randomized sample (obtained from a medical list provider, Southam Medical Lists) of English-speaking family

NAMCS National Ambulatory Medical Care Surveys URI Upper respiratory infection

From the Department of Pediatrics, The Hospital for Sick Children, University of Toronto, Toronto, Ontario, Canada; the Department of Pediatrics, Le Bonheur Children’s Medical Center, University of Tennessee Health Science Center, Memphis, Tennessee; Population Health Sciences, Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada; the Departments of Public Health Sciences and Health Policy, Management, and Evaluation and Pediatrics, Faculty of Medicine, University of Toronto, Toronto, Ontario, Canada; and Ray D. Wolfe Department of Family Medicine, Mount Sinai Hospital, Department of Family and Community Medicine, University of Toronto, Toronto, Ontario, Canada.

Supported by a research fellowship funded through the Research Institute, The Hospital for Sick Children, Toronto, Ontario, Canada (Dr Arnold) and by the Canadian Institutes of Health Research and the Ontario Ministry of Health and Long-Term Care through an Investigator Award (Dr To).

The results and conclusions are those of the authors; no official endorsement by the Ministry is intended or should be inferred.

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physicians (n = 270) and pediatricians (n = 270) in Ontario, Canada. Pediatricians in Ontario consist of a mix of primary care physicians, consultant general pediatricians, and pediatric specialists. Most family practitioners provide primary care. The cover letter indicated that the survey was directed at physicians providing primary care to children. The survey was administered according to the Total Design Method for mail surveys by Dillman. There were 3 mailings approximately 2 weeks apart. The first and third mailings consisted of a cover letter, survey booklet, and reply card; the second consisted of a reminder card only. The survey booklet contained a page of demographic questions (specialty of physician, duration of time in practice, sex, age, location of medical school, number of hours spent on continuing medical education yearly, number of patients seen in a busy week) followed by written case simulations derived from principles of clinical judgment analysis (see Appendix on-line at www.elsevierhealth.com/jpeds). Four patient variables with two levels were used to create 16 simulations, consisting of all possible combinations of these binary variables: patient age (younger or older than 2 years), temperature (less than or more than 38.5°C), overall appearance (appears well or ill), and parental pressure for an antibiotic (yes or no). Each child was described as having signs and symptoms of a viral URI without any indication of a complicating bacterial infection. Physicians were asked how likely they were to prescribe an antibiotic for the child in each simulation (highly unlikely, unlikely, likely, or highly likely).

Sample Size

A sample size calculator for multivariable logistic regression was used (Size: A Sample Size Program for Clinical and Epidemiologic Studies, Murray Hill, NJ, 1991). Calculations assumed a 2-tailed test of significance, an α level of 0.05, a power of 0.8, and an estimated rate of antibiotic prescribing for pediatric URI of 60%, based on previous studies. This led to a sample size of 300, which was increased to 540 to allow for a response rate as low as 55%.

Variable Selection

The three patient variables (age of patient, height of fever, and general appearance) were chosen, as they have been studied extensively in relation to the risk of invasive bacterial infection in children presenting with acute febrile illness. Parental pressure for an antibiotic was selected because it is one of the most frequently cited factors by physicians in antibiotic overuse in children. The independent contribution of each patient and/or physician variable to the decision to prescribe an antibiotic was modeled by using multivariable logistic regression with antibiotic prescribing as the dependent variable. The four response categories of highly likely, likely, unlikely, and highly unlikely were collapsed (highly likely and likely = yes; highly unlikely and unlikely = no) to create a binary variable. The case simulation was the unit of analysis. Variables for inclusion in the model were selected by a stepwise procedure with the significance level for inclusion set at P < .05. To account for the clustering of patients within physicians, a variable for the physician was forced into the logistic regression model. The independent association between each explanatory variable and the prescribing of an antibiotic was expressed as the odds ratio (and 95% CI). All statistical analyses were performed with the use of SAS 8.2 software (SAS, Cary, NC, 1999 to 2001).

The possibility of response bias was investigated by using the confidence interval approach, proposed by Tennant and Badley. This study was approved by the Research Ethics Board at the Hospital for Sick Children, Toronto, Ontario, Canada.

RESULTS

There were 257 completed surveys returned of 540 mailed, 47.6% from family practitioners and 52.4% from pediatricians. There were 419 simulations for which there was no missing value for either the response variable or a demographic variable, leaving 3693 cases of a potential 4112 (89.8%) for analysis.

Table I displays the categoric demographic characteristics of the survey respondents. In addition, pediatricians reported spending more hours on continuing medical education each year (80.0 vs 50.0, P < .0001) and seeing fewer patients per week (111 vs 125.0, P < .0001), compared with family practitioners.

Demographic characteristics of respondents were similar to those published for primary care physicians in Ontario and Canada except for a greater proportion of women among respondents. Physician age, proportion graduating from a Canadian medical school, and site of practice were similar.

One hundred sixty-three physicians indicated that they would prescribe an antibiotic in at least one case simulation, giving an overall antibiotic prescribing rate of 13.5%: 14.5% for family practitioners and 12.0% for pediatricians (P = .02). Table II displays the univariate associations between patient and physician variables and antibiotic prescribing, with patient general appearance, age, and temperature having the strongest associations. Parental pressure was not associated with prescribing. The continuous variables of physician age and the number of patients seen per week were significantly associated with antibiotic prescribing, but the number of continuing medical education hours per year was not.

The results of the multivariable logistic regression model are presented in Table III. There were strong associations between sick overall appearance, age older than 2

Antibiotic Prescribing For Upper Respiratory Tract Infection: The Importance Of Diagnostic Uncertainty
years, and temperature more than 38.5°C and antibiotic prescribing. Parental pressure was not associated with antibiotic prescribing in this model.

The analysis was repeated, assuming that either all missing values for the response variable were either positive or negative. The results were similar to those using the original data set.

In the assessment for response bias, the proportion of prescribers (physicians prescribing an antibiotic in at least one simulation) in each wave of responses was examined.23 The 95% CIs for the proportion of prescribers in each wave of respondents and nonrespondents overlapped, indicating no response bias. However, the point estimate and confidence interval for the proportion of antibiotic prescribers in the third wave was higher than those for the initial two waves, indicating that there may be a trend toward an increasing proportion of prescribers in the last wave and, by extension, nonrespondents.

DISCUSSION

The results of this study suggest that patient clinical characteristics, which may lead to diagnostic uncertainty, are a more important determinant of antibiotic overuse than parental pressure.7-10 Physicians were more likely to prescribe antibiotics to a child with URI if the child had a temperature greater than 38.5°C or appeared unwell, regardless of age. This may be due to diagnostic uncertainty and resultant concern about missing an evolving or occult invasive bacterial infection.16,17 There is often no way to definitively differentiate a bacterial from a viral infection. Diagnostics aids such as rapid streptococcal antigen testing for pharyngitis26 or clinical criteria for the diagnosis of occult bacteremia25 are underused, and physicians rely on clinical judgment, which may be inaccurate.13,28,29 Ultimately, many children may receive unnecessary treatment out of the physician’s desire to not miss a treatable condition.

Children older than 2 years of age were more likely to be prescribed an antibiotic, even though younger children are at higher risk of serious bacterial infection.30,31 In the 1992 National Ambulatory Medical Care Surveys (NAMCS) analysis of antibiotic prescribing for children with colds,

<table>
<thead>
<tr>
<th>Table I. Demographic characteristics of respondents</th>
</tr>
</thead>
<tbody>
<tr>
<td>Family practitioners n = 119 (%)</td>
</tr>
<tr>
<td>Sex</td>
</tr>
<tr>
<td>Male</td>
</tr>
<tr>
<td>Female</td>
</tr>
<tr>
<td>Duration of time in practice</td>
</tr>
<tr>
<td>0–5 y</td>
</tr>
<tr>
<td>6–10 y</td>
</tr>
<tr>
<td>11–15 y</td>
</tr>
<tr>
<td>16–20 y</td>
</tr>
<tr>
<td>&gt;20 y</td>
</tr>
<tr>
<td>Location of practice</td>
</tr>
<tr>
<td>Urban</td>
</tr>
<tr>
<td>Suburban</td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>Location of medical school</td>
</tr>
<tr>
<td>Canada/United States</td>
</tr>
<tr>
<td>Europe</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Table II. Proportion of physicians who prescribed an antibiotic according to patient and physician characteristics (univariate analysis)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Categoric variables</td>
</tr>
<tr>
<td>-------------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Patient appearance</td>
</tr>
<tr>
<td>Ill</td>
</tr>
<tr>
<td>Not ill</td>
</tr>
<tr>
<td>Patient age</td>
</tr>
<tr>
<td>&lt;2 y</td>
</tr>
<tr>
<td>≥2 y</td>
</tr>
<tr>
<td>Patient temperature</td>
</tr>
<tr>
<td>&gt;38.5°C</td>
</tr>
<tr>
<td>≤38.5°C</td>
</tr>
<tr>
<td>Parental pressure</td>
</tr>
<tr>
<td>Present</td>
</tr>
<tr>
<td>Absent</td>
</tr>
<tr>
<td>Type of MD</td>
</tr>
<tr>
<td>Family Pract</td>
</tr>
<tr>
<td>Pediatrician</td>
</tr>
<tr>
<td>Gender</td>
</tr>
<tr>
<td>Male</td>
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<tr>
<td>Female</td>
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<td>Duration of time in practice</td>
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<tr>
<td>11–15 y</td>
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<tr>
<td>16–20 y</td>
</tr>
<tr>
<td>&gt;20 y</td>
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<tr>
<td>Location of practice</td>
</tr>
<tr>
<td>Urban</td>
</tr>
<tr>
<td>Suburban</td>
</tr>
<tr>
<td>Rural</td>
</tr>
<tr>
<td>Location of medical school</td>
</tr>
<tr>
<td>North America</td>
</tr>
<tr>
<td>Europe</td>
</tr>
<tr>
<td>Other</td>
</tr>
</tbody>
</table>

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URI, or bronchitis, office visits for school-aged children were more likely to result in an antibiotic prescription than for preschool-aged children.\(^6\) The authors hypothesized that physicians may feel pressure to prescribe an antibiotic in an effort to hasten the older child’s return to school because the parents may not have alternate child care arrangements for an older child.\(^6\) Regardless, it appears that physicians are at least as likely to prescribe antibiotics for older children with URI symptoms and temperature more than 38.5°C, despite their inherently low risk for invasive bacterial infections.

Contrary to previously published literature,\(^7,8\) parental pressure for an antibiotic was not associated with antibiotic prescribing in this study. There are several potential explanations for this observation. Physicians may have become less responsive to pressure from parents due to medical and lay press stories about the antibiotic resistance problem. It may also be that reading a case simulation in which a parent is demanding an antibiotic does not lead to the same pressure to prescribe as a real patient encounter. Finally, perceived parental pressure may be a projection of the physician’s own concerns, and, in controlling for factors that increase physician concern, the effect of parental pressure diminishes. We cannot conclude an explanation from this study.

Pediatricians prescribed fewer antibiotics than did family physicians. This association has been demonstrated previously. In the 1992 NAMCS analysis, nonpediatrician specialty was an independent predictor of antibiotic use in children.\(^34,35\) In the 1999 to 2000 NAMCS, pediatricians remained less likely to prescribe antibiotics for URIs and bronchitis, although the gap between pediatricians and family/general practitioners was smaller.\(^35\) In this study, family physicians saw more patients (mean, 135 vs 111, \(P = .0004\)) and may be prescribing more frequently out of a perceived need to use antibiotics to move patients in and out quickly. Pediatricians have undertaken focused training in pediatrics and spend 100% of their clinical time with children, whereas family/general practitioners see children less frequently and have greater variability in their experience with children. Thus, pediatricians may have a greater comfort level in treating children with URIs and may be less likely to use antibiotics as a security measure.

The use of clinical judgment analysis as a study design may be considered a limitation. However, two studies have examined the validity of this method in respiratory tract infections\(^34,35\) and disease activity in rheumatoid arthritis.\(^36\) For otitis media,\(^35\) there was generally good agreement between the physician’s diagnosis or plan of action in the scenario and their true management of the same patient weeks or months previously, demonstrating the validity of this method.

With a response rate of 48%, response bias is a potential problem in this study. The confidence interval approach\(^11,23\) demonstrated no significant response bias. The overall rate of prescribing among respondents was similar to that reported in other recent studies.\(^11,33,37\) The trend toward an increasing proportion of prescribers in the third wave may indicate a group of nonrespondents who prescribe more antibiotics for viral URIs. Inclusion of responses from a group of high prescribers may have led to a higher overall prescribing rate but possibly negated the association of prescribing with patient characteristics, as these physicians may have prescribed antibiotics regardless of the clinical features.

When performing multiple comparisons, one must be wary of making a type 1 error (rejecting the null hypothesis when it is true). To minimize this possibility, a sample size of 540 was calculated; however, there were only 257 respondents. We used the Bonferroni adjustment for multiple comparisons (divides the cutoff level of significance, usually .05, by the number of variables [12 in this study]) to create a lower cutoff value, \(P = .004\), to assess statistical significance. The only variable with a \(P\) value for its association with prescribing above .004 was location of medical school training. Excluding this variable does not affect the main conclusions of the study.

Despite these limitations, physicians appear to be more likely to prescribe antibiotics to children who appear ill or have a high fever. To reduce “just in case” prescribing, physician education programs regarding appropriate use of antibiotics should encourage alternatives to prescribing. It should be emphasized that the risk of invasive bacterial infection has been reduced due to widespread use of pneumococcal conjugate vaccine.\(^39-40\) Physicians should be encouraged to use appropriate tests, such as valid diagnostic criteria, when available,\(^27,41\) rapid streptococcal screening test, and chest radiography to reduce diagnostic uncertainty, especially for older children in whom the risk of invasive bacterial infection is low.

The appendix is available for viewing online at www.elsevierhealth.com/jpeds

Table III. Logistic regression results modeling outcome of antibiotic prescribing

<table>
<thead>
<tr>
<th>Variable</th>
<th>Odds ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient appearance (ill appearance vs well appearance)</td>
<td>6.50 (5.06–8.34)</td>
</tr>
<tr>
<td>Patient temperature (&gt;38.5°C vs &lt;38.5°C)</td>
<td>1.48 (1.21–1.82)</td>
</tr>
<tr>
<td>Patient age (&lt;2 y vs &gt;2 y)</td>
<td>0.44 (0.36–0.54)</td>
</tr>
<tr>
<td>Parental pressure (yes vs no)</td>
<td>1.09 (0.89–1.33)</td>
</tr>
<tr>
<td>Type of physician (family physician vs pediatrician)</td>
<td>0.65 (0.51–0.82)</td>
</tr>
<tr>
<td>Other location of medical school (excluding Europe) vs North America</td>
<td>1.21 (0.91–1.61)</td>
</tr>
<tr>
<td>Europe location of medical school vs North America</td>
<td>0.61 (0.40–0.94)</td>
</tr>
<tr>
<td>Age of physician (5-y units)</td>
<td>1.17 (1.11–1.24)</td>
</tr>
</tbody>
</table>

REFERENCES


Antibiotic Prescribing For Upper Respiratory Tract Infection: The Importance Of Diagnostic Uncertainty
9. Hamm RM, Hicks RJ, Bemben DA. Antibiotics and respiratory infections: are patients more satisfied when expectations are met? J Fam Pract 1996;43:56-62.
INVASIVE *HAEMOPHILUS INFLUENZAE* TYPE B DISEASES IN BANGLADESH, WITH INCREASED RESISTANCE TO ANTIBIOTICS

**Objective**

To determine the prevalence, age-group distribution, serotype, and antibiotic susceptibility patterns of invasive *Haemophilus influenzae* type b (Hib) isolates in Bangladeshi children because data regarding Hib diseases in developing countries are scarce, which has led to delay of the introduction of Hib vaccine in these countries.

**Methods**

Children diagnosed with meningitis (n = 1412) and pneumonia (n = 2434) were enrolled in this surveillance study for Hib invasive diseases. Cerebrospinal fluid (CSF) and blood specimens, and the subsequent isolates, were processed using standard procedures.

**Results**

During 1993 to 2003, 455 *H influenzae* strains were isolated from patients with meningitis (n = 425) and pneumonia (n = 30), and an additional 68 Hib meningitis cases were detected by latex agglutination (LA) testing. Overall, 35% of pyogenic meningitis cases were a result of *H influenzae*, 97.1% of which were Hib. Most (91.4%) cases occurred during the first year of life. Resistance to ampicillin, chloramphenicol, and cotrimoxazole was 32.5%, 21.5%, and 49.2%, respectively. There was a trend toward increasing resistance for all three drugs. Resistance to ampicillin and chloramphenicol was almost universally coexistent and was associated with increased sequelae compared with the patients infected with susceptible strains (31% [23/75] vs 11% [21/183]; *P* < .001).

**Conclusion**

Hib is the most predominant cause of meningitis in young Bangladeshi children. Resistance to ampicillin and chloramphenicol and the high cost of third-generation cephalosporin highlight the importance of disease prevention through vaccination against Hib. (J Pediatr 2005;146:227-33)

*Haemophilus influenzae* type b (Hib) has emerged as the predominant cause of meningitis in developing countries wherever it has been studied using good microbiologic procedures.1-3 Hib is an important etiology of pneumonia as well. However, it goes largely undetected because blood culture is not a sensitive enough test. Microbiologic evaluation of meningitis cases can be used to estimate the overall burden of Hib diseases as well the antibiotic susceptibility of invasive Hib strains in any population.4 Available evidence suggests that Hib is the predominant cause of bacterial meningitis and the second most common cause of bacterial pneumonia, in early childhood, and is responsible globally for 300,000 to 400,000 childhood deaths, mostly in developing countries.5-6 Preliminary data from Bangladesh indicate that *H influenzae* is the most predominant cause of meningitis in this country, and most strains are type b1, which could be prevented by currently available vaccine. Conjugate vaccine, which has approximately 98% efficacy against invasive Hib diseases, is considered to be one of the major public health accomplishments of the last few decades.3 However, the benefit of Hib vaccine is still limited primarily to children of the developed world. This is mainly because of the high cost of the vaccine and marked underestimation of the
burden of Hib disease. The underestimation of Hib is a result, in part, of the failure of most laboratories in developing countries, especially in Asia, to detect this fastidious organism.7-9

In addition to attempting to prevent the disease through widespread availability of vaccine, protocols for effective antibiotic management of acute lower respiratory tract infection and meningitis are critical. Because most laboratories in developing countries are not isolating Hib routinely and because treatment for very severe disease cannot be delayed while waiting for culture results, empiric therapy remains the rule in both primary and tertiary facilities. Therefore, it is of paramount importance to have comprehensive data on the burden and antibiotic susceptibility of Hib in order to set proper guidelines for empiric therapy. Recent reports on antibiotic susceptibility of H influenzae, most of which have reported on non-type-b isolates, indicate the emergence of resistance of this organism to various antibiotics, especially β-lactamase–mediated resistance to ampicillin. However, prevalence of resistance of H influenzae to antibiotics varies widely in different regions of the world.10,11 There are a few reports from South Asia,12,13 and none from Bangladesh. We sought to determine the prevalence, age-group distribution, serotype, biotype, and antibiotic susceptibility patterns of invasive H influenzae isolates and the clinical outcome of Hib cases presenting to Dhaka Shishu (Children) Hospital (DSH) in Bangladesh.

MATERIALS AND METHODS

Setting

The study was carried out at DSH, the largest pediatric hospital in Bangladesh that provides both primary and tertiary care. DSH has 349 beds of which 212 (61%) provide free care, including investigations, food, and essential first-line medicines/antibiotics. Nonpaying beds are reserved for those who cannot afford to pay for their care. Patients’ families are divided into low-, medium-, and high-income groups if their incomes are <$25/person/month, $25-50/person/month, or >$50/person/month, respectively.

Cases

All patients (0-144 months of age) with features of pyogenic meningitis (cerebrospinal fluid [CSF] containing ≥100 leukocytes/mm³ with >50% polymorphonuclear cells and/or growth of the organism in culture and/or detection of antigen by latex agglutination [LA] test) were enrolled during the period of September 1993 to August 2003 in a surveillance study. Pneumonia cases, diagnosed as per World Health Organization criteria,14 were enrolled only if blood cultures were obtained. As blood culture from pneumonia patients is not a routine practice at DSH, cultures were only obtained from pneumonia case patients enrolled in other clinical studies or in vaccine trials during the period of August 2000 to September 2003.

Results of pneumococcal cases were published elsewhere,15-16 and only data on H influenzae isolates are presented here.

Laboratory Procedures

Blood and chocolate agar were made from blood agar base (Oxoid, Basingstok, UK) with 5% sheep blood. Each batch of media was tested for adequate growth of reference strains on the respective media before culturing clinical specimens. CSF specimens were inoculated directly onto the media. Blood specimens were obtained aseptically, were inoculated in trypticase soy broth (Oxoid, Basingstok, UK) with 0.25% sodium polyethanol sulphonate (Sigma, St. Louis, Mo), and were incubated at 37°C for 7 days. Inoculated broths were sub-cultured on blood and chocolate agar plates on days 2, 3, and 7.

Colonies suspected to be H influenzae were further confirmed on the basis of their growth requirement for hemin and Nicotinamide dinucleotide (NAD), using the “X” and “V” factor disks (Difco, Detroit, Mich). H influenzae strains were serotyped by the slide agglutination method using type-specific antisera (Murex, Kent, UK). Randomly selected strains (n = 174) were biotyped using the Analytical Profile Index system (BioMerieux, Marcy l’Etoile, France) and were interpreted on the basis of growth–independent rapid tests of indole, urease, and ornithine decarboxylase.17 Serotyping, biotyping, and antibiotic susceptibility of 50 randomly selected strains were confirmed at Nagasaki University of Japan by blinded comparison with our results.

Reference strains employed in this study included H influenzae ATCC 49247, and β-lactamase positive (99-1, 99-5, and 99-42) and negative (99-2 and 99-10) strains of H influenzae with known Minimum Inhibitory Concentration (MIC) values (kindly provided by Dr. Gary Doern, Medical Research Center, University of Iowa) for quality control.

CSF specimens were tested for antigens of five common organisms (Hib, Streptococcus pneumoniae, Neisseria meningitidis, Group B Streptococcus, and Escherichia coli) using LA reagents (Murex, Kent, UK) according to the instruction of the manufacturer. To reduce costs, the LA test was done on the first day, only if the Gram’s stain result was not obviously positive. An aliquot of CSF was preserved, and the LA test was done on the second day if there was no growth from the CSF.

Susceptibility Tests

Susceptibility testing was done with antimicrobial agents commonly used in the treatment of meningitis and pneumonia (ie, ampicillin, amoxicillin-clavulanic acid, chloramphenicol, cotrimoxazole, ceftriaxone, gentamicin, ciprofloxacin, and azithromycin). To determine the antibiogram, the strains were tested by E-test and micro-broth dilution using standard techniques for H influenzae following National Committee for Clinical Laboratory Standards guidelines.18-20

Beta-lactamase production of the strains was detected by using paper disks impregnated with chromogenic cephalosporin (Becton Dickinson, Sparks, Md). “Multi-drug resistance”
Invasive Haemophilus Influenzae Type B Diseases In Bangladesh, With Increased Resistance To Antibiotics

RESULTS

A total of 425 (30%) and 30 (1.2%) *H influenzae* strains were isolated from 1412 CSF and 2434 blood specimens, respectively. Of these 455 strains, 440 (96.7%) were type b (96.9% and 93.3% of CSF and blood isolates, respectively). The rest of the strains were either nontypable (n = 12) or type a (n = 3). An additional 68 Hib meningitis cases were identified as positive by LA testing of CSF. Overall, a bacteriologic etiology of meningitis could be identified in 67% (930/1412) of the cases, and *H influenzae* was the predominant cause (53%; 493/930). Altogether, 523 (67%) (930/1412) of the cases, and *H influenzae* cases were detected by culture and/or LA test, of which 97.1% (508/523) were Hib. Among the enrolled case patients, 41 cases with a CSF white blood cell count <100/mm³ (range 0-95/mm³) were positive for etiology either by culture or LA test and 15 (36.6%) of these cases were Hib. Biotyping of 174 randomly selected strains showed a predominance of types I (52%), V (22%), and VII (13%), followed by II (6%) and VIII (6%). This pattern was consistent throughout the study period. Biotypes did not show any relation with β-lactamase production and drug resistance (data not shown).

Four hundred and forty-seven (85.5%), 68 (13%), and 8 (1.5%) cases were from low-, medium-, and high-income groups, respectively. In contrast, 52% of all admitted cases in DSH were from medium- or high-income groups. There was a marked predominance of males (66.3%; 347/523) over females (33.6%; 176/523).

Nearly all cases (91.4%) of invasive Hib diseases occurred in infants <12 months of age. There were only 7 (1.3%) and 10 (1.9%) cases during the first month and after the 24th month of life, respectively. Number of Hib cases rose sharply in the fourth month of life, and 50% of the cases occurred in infants 6 to 8 months of age (Figure 1). The mean and median age for Hib cases was 9 and 7 months, respectively.

The age distribution was similar in males and females, except in the neonatal age group, where 6 of 7 cases were in males. Separate analysis with non-b strains showed a similar distribution in different ages, as 67% of cases occurred during first year of life and 47% occurred in those 6 to 8 months of age.

Overall, *H influenzae* cases were found throughout the year. Analysis of resistance profiles showed that the only β-lactamase production and drug resistance (data not shown).

Antibiotic susceptibility patterns of isolates from the blood of pneumonia and CSF of meningitis cases were found to be similar. Overall resistance to ampicillin, chloramphenicol, and cotrimoxazole was 32.5% (147/452), 21.5% (97/452), and 49.2% (227/452), respectively. There was a trend toward increasing resistance for all three drugs over the study period, although the trend reversed in 2003 (Figure 2). All the ampicillin-resistant strains were β-lactamase producing (Minimum Inhibitory Concentration ranged from 8 to 256 μg/mL), and the enzyme was inhibitable by clavulanic acid.

Chloramphenicol resistance appeared in 1996, and its prevalence ultimately reached 55% (18/31) in 2002, with a drop to 35% (5/17) in 2003 (Figure 2). Cotrimoxazole resistance was high throughout the study period and ranged from 21% to 65%.

Analysis of resistance profiles showed that the only multi-drug resistance combination (>2 unrelated drugs) was ampicillin, chloramphenicol, and cotrimoxazole, which emerged in 1996 (1.5%; 1/66) and increased in prevalence until it reached a peak in 2002 (35.5%; 11/35). During the period of 2000 to 2002, none of the strains were resistant to ampicillin or chloramphenicol alone, yet overall resistance to each of these drugs increased from 31.4% to 51.6% and 23.7% to 54.8%, respectively, as the resistance markers increasingly appeared in combination. It was noted that all the non-b (n = 15) *H influenzae* strains were negative for β-lactamase production and were susceptible to both ampicillin and chloramphenicol.

When susceptibility to antibiotics was stratified by age, it was observed that younger children were at higher risk for infection with a resistant strain; resistance to ampicillin and chloramphenicol was two and three times higher, respectively, among children <2 years of age than among those ≥2 years of age. However, there were relatively few patients in the later age group (n = 10), and the difference was not statistically significant (P = .25).

Overall, 22% of Hib meningitis case patients died, and another 24% were discharged with sequelae. When restricted to culture positive cases (n = 425), those with an isolate resistant to at least one antibiotic had a case fatality rate of 30% (73/242) compared with 24% (44/183) (P = .16) in the group infected with a susceptible isolate. Meningitis with Hib strains resistant to both ampicillin and chloramphenicol had a significantly higher rate of sequelae compared with those with disease because of susceptible strains (31% [23/75] vs 11% [21/183]; P < .001; Table).

DISCUSSION

In this study, a bacteriologic etiology of meningitis could be identified in 67% (930/1412) of the cases, and *H influenzae* was the predominant cause (53.0%; 493/930). *H influenzae* strains were isolated from 1.2% of blood cultures from patients with pneumonia cases enrolled either as part of an Hib vaccine trial or of other clinical trials. Similar to data from the United States, India, and other countries, but in contrast with Pakistan, 97% (480/493) and 93% (28/30) of our *H influenzae* strains isolated from CSF and blood, respectively, were Hib and were, thus, preventable by Hib conjugate vaccine. This result is in concordance with an earlier report by Saha et al on meningitis in Bangladesh, but it is in contrast with another report in which *H influenzae* was not identified from CSF. Our personal communication with the author (Dr Setarunnahar, Institute of Public Health, Dhaka, Bangladesh) of the contrasting report revealed that the likely reason for
their failure to isolate *H influenzae* was that the specimens had been kept in the refrigerator overnight, before inoculation, which is lethal for *H influenzae*.24 The proportion of cultures positive for *H influenzae* in our case series was much higher in comparison with the recent report from India by the Invasive Bacterial Infection Surveillance (IBIS) Group.12 Culture positivity for *H influenzae* among meningitis cases in the IBIS Group study was only 8.5% (80/939), in contrast with 35% (493/1412) in our series. The most striking observation of the IBIS Group was that, overall, Hib (2.2%) and *S pneumoniae* (5.4%) were not the most important causes of meningitis and pneumonia, rather “other organisms” surfaced as the predominant (16.1%) etiology. Moreover, the authors of the IBIS Group study mentioned that the “other organisms” were mostly contaminants, which may have overgrown and prevented the isolation of fastidious and slow-growing etiologic agents such as *H influenzae* and *S pneumoniae*. The 8.5% isolation rate of *H influenzae* from meningitis cases of the IBIS Group study also is low in contrast with other reports (ie, isolation rates 30%-45%).25-29 The low isolation rate of *H influenzae* by the IBIS Group has undermined the importance of Hib, and it has

Figure 1. Age distribution of *H influenzae* cases.

Figure 2. Resistance of invasive *H influenzae* isolates: 1993-2003.
Table. Impact of resistance markers on clinical outcome of Hib meningitis cases (n = 425)

<table>
<thead>
<tr>
<th>Cured (%)</th>
<th>P value*</th>
<th>Sequel (%)</th>
<th>P value*</th>
<th>Died (%)</th>
<th>P value*</th>
<th>Lost (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>With any R-Marker (n = 242)</td>
<td>104 (43.0)</td>
<td>0.014</td>
<td>51 (21.1)</td>
<td>0.013</td>
<td>73 (30.2)</td>
<td>0.16</td>
</tr>
<tr>
<td>Ampicillin only (n = 10)</td>
<td>6 (60.0)</td>
<td>0.891</td>
<td>0</td>
<td>0.548</td>
<td>4 (40.0)</td>
<td>0.44</td>
</tr>
<tr>
<td>Chloramphenical only (n = 6)</td>
<td>4 (66.7)</td>
<td>0.955</td>
<td>2 (33.3)</td>
<td>NA</td>
<td>0</td>
<td>0.37</td>
</tr>
<tr>
<td>Ampicillin+chloramphenical (n = 75)</td>
<td>27 (36.0)</td>
<td>0.002</td>
<td>23 (30.7)</td>
<td>&lt;0.001</td>
<td>23 (30.7)</td>
<td>0.27</td>
</tr>
<tr>
<td>Susceptible to all (n = 183)</td>
<td>104 (56.8)</td>
<td>0.002</td>
<td>21 (11.5)</td>
<td>0.37</td>
<td>44 (24.0)</td>
<td>0.16</td>
</tr>
</tbody>
</table>

*Compared with cases with susceptible strains.

contributed to the debate, erroneously we believe, about the need for Hib vaccine in this part of the world. Our data highlight the principle that all CSF specimens should be cultured and Gram stained, irrespective of the cell count, given that of 41 cases of bacterial meningitis with a cell count of <100 leukocytes/mm³, 15 Hib cases were identified by culture and/or LA, and all these cases showed several thousand leukocytes when a second lumbar puncture was done between days 3 and 5.

In our series, none of the CSF specimens were contaminated, and the contamination rate for blood cultures was 6.9%. If we observed contamination on the first subculture from the blood bottle, it was documented and discussed; and another subculture was done for *H influenzae* and *S pneumoniae* on the selective media, bacitracin chocolate and gentamicin blood agar plates, respectively.

We observed a higher prevalence of meningitis in male than in female children (2:1), as has been reported previously from India (1.33:1) (IBIS) and from North America (1.46:1). Differential care-seeking for illness likely cannot explain this result because we are dealing with cases of very severe diseases for which care-seeking should be similar regardless of sex; in a recent randomized clinical trial of cotrimoxazole treatment of nonsevere pneumonia cases at the Outpatient Department of DSH, there were similar proportions of both sexes among 1010 randomized cases (our unpublished data).

The age distribution of Hib disease, which is very important for vaccination strategies, varies widely worldwide, especially between industrialized and non-industrialized countries. This hospital-based study conducted over a decade revealed that Hib diseases in Bangladesh occurred predominantly in infants. This age distribution is similar to the Australian Aborigines (median age 6 months), Taiwanese (median age 7 months), Indian (>90% cases during the first year), and Navajo and Apache populations (80% of cases during the first year). However, this distribution is in contrast with that found in Finland, where peak prevalence occurred between 12 and 24 months of age and where only 3% of cases occurred within the first 6 months of life, and in the United States, where <15% of cases occurred before 6 months of life. Early onset of Hib diseases in the Bangladesh population indicates that immunization is appropriate at 6 weeks of age and that existing combination vaccines of Diphtheria, Pertussis and Tetanus (DPT) and Hib (DPTHib or DPTHepBHib) are appropriate for the children of Bangladesh.

Beta-lactamase-mediated ampicillin resistance varies considerably with geographical region, ranging from 1.8% in Italy to 65% in South Korea. There have been several reports of beta-lactamase negative, ampicillin-resistant *H influenzae* strains. These are relatively uncommon, however, as judged by the recent national and multinational surveillance. In our study, overall, one-third of *H influenzae* strains were resistant to ampicillin, and all of them were beta-lactamase producing. As reported by other studies, the enzyme was completely inhibited by clavulanic acid, and the ampicillin resistant strains were highly sensitive to a combination of ampicillin and clavulanic acid.

There was a marked increasing trend of resistance to ampicillin and chloramphenicol, which were almost universally coexistent. These data on coexistence of markers for ampicillin and chloramphenicol resistance suggest that all of them may be from the same clonal origin and that the markers may be carried on a single plasmid, as seen with the European strains.

High case fatality (22%) and sequelae (24%) of Hib cases indicate the severity of Hib infection in Bangladeshi children, which also has been reported from India and other developing countries. The significantly higher rate of mortality and sequelae among cases that are a result of isolates resistant to ampicillin+chloramphenicol, in comparison with cases that are a result of susceptible isolates (31% vs 11%; P <0.001), was possibly because of a delay in the start of treatment with a third generation cephalosporin, as it cannot be provided free by the hospital and most of the patients cannot afford to buy the expensive drugs.

Several possible limitations of our study and their implications for future research need consideration. Because this was a hospital-based surveillance study, our data preclude estimation of total disease burden in the population. Although DSH is the largest pediatric hospital at a national level for primary and tertiary care, there are several general hospitals and private clinics serving the same population, and, therefore, some children with meningitis and bacteremic pneumonia were admitted to those hospitals as well. Moreover, because...
Bangladesh is a developing country with high poverty, low literacy levels, and poor access to healthcare, many children may not be brought to the hospital and may die at home. Nevertheless, this study revealed that Hib is a primary cause of invasive disease among infants of Bangladesh and that antibiotic resistance to the commonly used therapeutics, ampicillin and chloramphenicol, has markedly increased over the past decade.

Combined resistance to first-line drugs (ampicillin and chloramphenicol) and its impact on clinical outcome indicate that a third-generation cephalosporin would be the most rational empirical therapy, but this class of agents may not be affordable by the majority of Bangladeshi people. This highlights the importance of disease prevention through vaccination. However, despite formation of the Global Alliance for Vaccine and Immunization, Bangladesh, which is eligible for Alliance funding, has failed to include Hib vaccine in the Extended Program for Immunization (EPI) program.

It is now the responsibility of public health practitioners and scientists to establish the true burden of Hib disease in Bangladesh and elsewhere in the developing world, perhaps through use of “rapid tool” or vaccine probe studies and, thus, to provide convincing evidence to persuade policy makers to include Hib vaccine in EPI programs. Until the vaccine is available, a third-generation cephalosporin should be within the reach of the people of Bangladesh for effective treatment of Hib meningitis cases.

We gratefully acknowledge the technical assistance rendered by Dr Monir Hossain and Ms Maksuda Islam.

REFERENCES


50 Years Ago in The Journal of Pediatrics

INTERSTITIAL PLASMA CELL PNEUMONIA


In a fatal neonatal case of “interstitial plasma cell pneumonia,” the first in the United States, these authors described the clinical features of failure to thrive, a dry hacking cough, severe cyanosis during feeding, dyspnea upon exertion, and a “ground-glass cloudiness” on the chest roentgenogram. Pathologic findings were confined to the lungs, which were heavy, firm, densely infiltrated by plasma cells, and contained periodic acid Schiff–positive material within air spaces. Several similar cases had previously been reported in Europe, and although the cause was unknown, the authors astutely suggested that this was an infectious disease because of the ability to produce similar pathologic changes in guinea pigs by injecting them with patients’ blood or secretions. They noted that the disease only affected “premature and weak full-term infants” but spared “full-term infants in good condition.”

Fifty years later, we know that the disease is caused by a micro-organism, Pneumocystis carinii (jiroveci).1 In addition to children with congenital immune defects, other susceptible individuals include children or adults immunosuppressed because of treatment for malignancy or organ transplantation or consequent to infection by the human immunodeficiency virus. Initially thought to be a protozoan,2,3 P carinii is now classified as a fungus-based on ribosomal RNA that is homologous to that in fungi.4 The organism has 4 morphologic forms: trophozoites, cysts, precysts, and sporozoites. The diagnosis can be established by identification of the diagnostic form, the cyst, a 4 to 8 µm spheric or crescent–shaped object in bronchoalveolar lavage, or in an open lung biopsy. For definitive diagnosis, routine cytologic stains such as Giemsa, Papanicolaou, and Grocott methenamine silver are used for detecting the cysts; sometimes immunohistochemistry or polymerase chain reaction is necessary. Staining of bronchoalveolar lavage fluid with Calcofluor white, a nonspecific fluorochrome that binds cellulose, can provide rapid screening (within minutes) and permit prompt institution of therapy.5

Treatment has significantly reduced mortality rates, and effective drug regimens include co-trimoxazole, pentamidine or clindamycin with primaquine, dapson with trimethoprim, or atovaquone and trimetrexate with folic acid. Glucocorticosteroids may be added, and high-risk (immunosuppressed) patients are treated prophylactically with co-trimoxazole.6-8 Immunization of these high-risk patients will be possible in the future.9

References are available online at www.us.elsevierhealth.com/jpeds

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10.1016/j.jpeds.2004.10.051
Objective To compare parental feeding practices and evaluate their relationship to weight status among children with Down syndrome (DS) and their unaffected siblings.

Study design Cross-sectional study of sibling pairs, one child with DS (n = 36) and one child without DS (n = 36), between 3 and 10 years of age. Parents completed the Child Feeding Questionnaire (CFQ), which assesses six aspects of control in feeding, separately for each child. Children’s height and weight were measured using standard research procedures for calculation of body mass index (BMI) and BMI Z scores (BMIZ).

Results Mean BMIZ was higher among children with DS than their siblings (1.1 ± 0.9 vs 0.1 ± 1.1; P < .001), but there were no between-group differences in parents’ perception of children’s weight status. Parents reported greater use of restriction, greater feelings of responsibility for feeding and concern about child weight status, and lower pressure to eat for children with DS than for their siblings. After adjustment for BMIZ, differences remained significant only for concern (10.6 ± 3.5 vs 6.4 ± 3.4; P < .002). Perceived child overweight and concern were positively associated with BMIZ, whereas pressure was inversely associated with BMIZ.

Conclusions Differences in child-feeding practices may play a role in the development of obesity in DS. (J Pediatr 2005;146:234-8)

Down syndrome (DS) is the most common cause of developmental disability in the United States, and it is present in 1 of every 800 live births.1 In the United States, approximately 45% of men and 56% of women with DS are overweight, defined as body mass index (BMI) ≥27.8 for men and ≥27.3 for women2 compared with 33% of non-DS men and 36% of non-DS women using the same definition.3 The high prevalence of obesity together with increases in life expectancy in recent decades for individuals with DS4 raise concerns about risk for chronic diseases that are exacerbated by obesity. Indeed, standardized mortality ratios from a large cohort study of persons with DS were 3.9 [95% CI 2.7-5.4] for ischemic heart disease and 6.0 [95% CI 3.5-9.6] for cerebrovascular disease.5 Therefore, prevention of obesity during childhood is an important health issue for this population.

Available data suggest that children exposed to controlling feeding practices have less accurate short-term energy intake regulation,6 are more likely to eat in the absence of hunger,7,8 and have a higher BMI9 and greater fat mass.10 However, studies of child-feeding practices have been limited to nondisabled children. Guidance based on such studies advises caregivers to provide a variety of nutritious, developmentally appropriate foods in a neutral fashion, allowing the child to determine if and how much to eat.11 It is unclear whether this approach is appropriate for children with an intellectual disability who are predisposed to overweight.

In this cross-sectional study, we compare caregiver child-feeding practices among children with DS (n = 36) and their non-DS siblings (n = 36) and assess the relationship of child-feeding practices with weight status. We hypothesize that parents use more
control in feeding for their child with DS than for their nondisabled child and that this control is associated with higher relative BMI.

**METHODS**

**Subjects**

All data were collected during baseline visits of an ongoing cohort study. Thirty-six families were enrolled between November 2001 and June 2003 through referring physicians and DS parent groups in the greater Philadelphia area. A special effort was made to reach minority families for an inclusive sample. Families were contacted by phone and screened for eligibility to participate in the study if they had one child with DS and one child with no disabilities. Several conditions were identified as potential confounders of the relationship between DS and nutrition and were excluded: (1) congenital cardiac defects requiring open-heart surgery; (2) gastroenterologic anomalies requiring bowel resection and/or ongoing medical intervention; (3) leukemia or other cancer; (4) hypothyroidism requiring thyroid hormone replacement or other chronic condition affecting energy balance or growth; and (6) BMI (kg/m²) below the 5th and above 95th percentile of age- and sex-specific reference growth curves.12 The final sample included some children whose BMI-for-age was above the 95th percentile because of discrepancies between reported and measured height and weight. Children <3 years of age were excluded because of concerns about compliance with the protocol. Children >10 years of age were excluded in an effort to enroll only prepubertal children. If more than one eligible sibling was identified (two families), the caregiver selected one to participate based on the child’s interest. Written informed consent from a parent and child assent were obtained before study enrollment. All procedures were reviewed and approved by the Institutional Review Board at The Children’s Hospital of Philadelphia.

**Procedures**

Anthropometric and questionnaire data were collected during inpatient visits in the General Clinical Research Center at The Children’s Hospital of Philadelphia. One parent (the mother for 34 families and the father for 2 families) completed the Child Feeding Questionnaire (CFQ), which assesses parental feeding practices and attitudes toward children’s weight and eating.13 Parents were instructed to complete two separate questionnaires (one for their child with DS and one for their child without DS) while thinking specifically about each child as they completed the questionnaire for that child. The order in which the questionnaires were to be completed was not specified or recorded.

The CFQ has been shown to be internally consistent and related to independent measures of child weight status by confirmatory factor analysis in three large, multiethnic samples of children without disabilities.13 Table I presents brief descriptions of the CFQ subscales and Cronbach’s α scores for children with DS in this sample, indicating acceptable internal consistency. Weight and height were measured in triplicate by a trained anthropometrist using standard methods.14 Because median values for BMI follow a curvilinear pattern during childhood, BMI Z scores (BMIZ) were used to account for differences related to age and sex while capturing differences in relative weight.12

Physical activity data were collected for the 7 days following the inpatient visit using the Actitrac activity monitor (IM Systems, Baltimore, Md). The Actitrac is a bi-axial motion sensor that is worn on the waist to measure accelerations of the body. Parents were instructed to have both siblings wear the Actitrac activity monitor from the time they woke up in the morning until the time they went to bed at night, except when sleeping or immersed in water, over the 7-day period. The sum of 24-hour activity counts were averaged over 7 days for each child. A comprehensive analysis of this data will be the subject of a future manuscript.

---

**Table I. Description of CFQ subscales and internal consistencies for children with DS (n = 36)**

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of items</th>
<th>Scale</th>
<th>Measured attitude or behavior</th>
<th>Cronbach’s α</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perceived child</td>
<td>≤6</td>
<td>1-5</td>
<td>Degree to which a parent considers a child to be overweight at various ages on a scale from 1 (underweight) to 5 (markedly overweight). The number of items in the scale depends upon the child’s age.</td>
<td>0.76</td>
</tr>
<tr>
<td>Responsibility</td>
<td>3</td>
<td>3-15</td>
<td>How responsible the parent feels for feeding the child, determining portion sizes, and providing a healthy diet.</td>
<td>0.77</td>
</tr>
<tr>
<td>Concern</td>
<td>3</td>
<td>3-15</td>
<td>The degree to which the parent is concerned about the child’s current weight and possibility of the child becoming overweight.</td>
<td>0.77</td>
</tr>
<tr>
<td>Restriction</td>
<td>8</td>
<td>8-40</td>
<td>The extent to which parent restricts the types or quantities of palatable foods consumed by the child.</td>
<td>0.71</td>
</tr>
<tr>
<td>Pressure</td>
<td>4</td>
<td>4-20</td>
<td>The extent to which the parent encourages the child to eat.</td>
<td>0.73</td>
</tr>
<tr>
<td>Monitoring</td>
<td>3</td>
<td>3-15</td>
<td>The extent to which the parent keeps track of the child’s intake of sweets, snack foods, and high-fat foods.</td>
<td>0.92</td>
</tr>
</tbody>
</table>
**Table II. Sample characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Total Sample</th>
<th>DS</th>
<th>Non-DS</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N or mean ± SD</td>
<td>N or mean ± SD</td>
<td>N or mean ± SD</td>
</tr>
<tr>
<td>N</td>
<td>72</td>
<td>36</td>
<td>36</td>
</tr>
<tr>
<td>Gender, males*</td>
<td>36</td>
<td>18</td>
<td>18</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic (N)</td>
<td>8</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Non-Hispanic (N)</td>
<td>64</td>
<td>32</td>
<td>32</td>
</tr>
<tr>
<td>Race</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Asian (N)</td>
<td>2</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>African American (N)</td>
<td>7</td>
<td>3†</td>
<td>4†</td>
</tr>
<tr>
<td>Caucasian (N)</td>
<td>63</td>
<td>32†</td>
<td>31†</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>17.5 ± 3.1</td>
<td>18.5 ± 3.3</td>
<td>16.6 ± 2.5†</td>
</tr>
<tr>
<td>BMIZ (SD)</td>
<td>0.6 ± 1.1</td>
<td>1.1 ± 0.9</td>
<td>0.1 ± 1.1‡</td>
</tr>
<tr>
<td>Age (y)</td>
<td>6.9 ± 2.1</td>
<td>6.6 ± 2.0</td>
<td>7.2 ± 2.2</td>
</tr>
<tr>
<td>Total 24-hour physical activity counts</td>
<td>43,254 ± 19,624</td>
<td>38,883 ± 17,081</td>
<td>47,333 ± 21,205†</td>
</tr>
</tbody>
</table>

N, number.
*The sibling pairs were 20% both female, 20% both male, and 60% mixed gender.
†One pair of half-siblings was included in the sample; their fathers were different races.
‡DS vs non-DS, P < .0001.
§DS vs non-DS, P = .04.

**Statistical Analyses**

Generalized estimating equations and paired t tests were used for group comparisons and to account for sibling intercorrelation. Main effects of DS status on CFQ variables were evaluated first unadjusted and then with adjustment for possible confounding effects of age, sex, physical activity, family income, maternal education, race, and Hispanic ethnicity. An additional model included these variables as well as BMIZ. Confounders were chosen a priori based on existing literature on known or supposed associations with obesity. Main effects of CFQ variables on BMIZ also were determined using generalized estimating equations unadjusted, then with adjustment, for all potentially confounding variables and DS status. For these models, possible interactions of DS status and sex were examined. All statistical analyses were performed using Stata software (version 7.0; Stata Corp, College Station, Tex).

**RESULTS**

Sample characteristics are presented in Table II. Children with DS had higher BMIZ and lower physical activity levels, but age did not differ.

**Child-Feeding Practices and DS**

Table III presents comparisons of CFQ variables between children with DS and their siblings. Parents reported greater feelings of responsibility for feeding and concern about weight status, greater use of food restriction, and lower pressure to eat for their children with DS than for siblings. These results were confirmed by paired t tests. Interestingly, there was no between-group difference in parents’ perceiving children to be overweight, even though children with DS were significantly heavier. Differences remained significant for responsibility and concern but not for pressure and restriction after adjustment for demographic variables. With adjustment for demographic variables and BMIZ, between-group differences were significant for responsibility only.

**Weight Status and Child-Feeding Practices**

Results of models evaluating the relationship of BMIZ to CFQ variables are presented in Table IV. As expected, perceived child overweight and concern about child weight status were positively associated with BMIZ, whereas pressure to eat was inversely associated with BMIZ. No significant interactions of perceived child overweight, concern, or pressure with either sex or DS status were detected in these models. Therefore, further analyses were conducted in the entire sample without stratification. Adjusted models were significant for concern only.

**DISCUSSION**

The two main findings of this study are that parents employ certain controlling feeding practices more frequently for children with DS than for children without DS and that these practices are, to some extent, associated with differences in BMI. These findings are in keeping with those observed among children without disabilities. In laboratory studies, higher caregiver food restriction was related to impaired caloric compensation among girls.15,16 And, in observational studies, higher restriction was associated with higher child BMI, whereas higher pressure to eat is linked to lower child
In a cross-sectional study including both black and white children, higher maternal concern and lower pressure to eat were associated with higher fat mass even after adjusting for lean body mass, sex, race, socioeconomic status, and energy intake.\textsuperscript{10} The theoretical relationship between child-feeding practices and weight status is based on the premise that parents are more likely to impose control in feeding when they perceive a child to be at risk for becoming overweight.\textsuperscript{17} Further, it is suggested that excessive control in feeding trains children to ignore hunger and satiety cues, exacerbating obesity-proneness.\textsuperscript{17} Based on our results, this model, although it is derived from research among nondisabled children,\textsuperscript{9,10} may be appropriate for children with DS. Caregivers are probably aware that DS places their child at higher risk for obesity and may believe that the cognitive disabilities associated with DS extend into the domain of eating and warrant more control in feeding.

In our sample, the relationship of pressure and concern to BMIZ among children with DS was similar in direction and magnitude to the relationship observed among unaffected siblings in this study and among non-DS children in other studies.\textsuperscript{9,10} However, small sample size may have masked possible sibling and gender interactions. Further, in our cross-sectional study as well as in those of others,\textsuperscript{6,9} it remains unclear whether controlling feeding practices are the cause, the result, or simply the correlate of the child’s weight status, although recent longitudinal studies of feeding practices and child eating behaviors are suggestive of a possible causal relationship.\textsuperscript{8}

It is notable that BMIZ was significantly higher among children with DS than among their siblings even though, by design, the sample excluded children who were identified during telephone screening as overweight or having any condition other than DS affecting energy balance. Therefore, overweight in DS may be a greater problem than previously recognized,\textsuperscript{2} even among healthy prepubertal children. Our results suggest that differences in parental feeding practices may partially explain this phenomenon. It is not known why a predisposition toward obesity is associated with the syndrome but it has been suggested that persons with DS have lower than expected resting energy expenditure for their age, body mass, and sex.\textsuperscript{18-20} Lower physical activity levels, which were observed in our study, may also contribute to obesity in DS.

Limitations of the present study include the reliance on an indirect measure of adiposity, BMI\textsubscript{Z}, although its use is widely accepted. Additionally, the generalizability of these findings is limited by the fact that, by design, the study sample does not reflect the frequent gastroenterologic and cardiovascular problems present in the DS population. Further, intellectual quotient was not measured and may confound the relationship of feeding practices and weight status among children with DS.

### Table III. Comparison of CFQ variables among children with DS vs their unaffected siblings

<table>
<thead>
<tr>
<th>Variable</th>
<th>DS (n = 36)</th>
<th>Control (n = 36)</th>
<th>Unadjusted</th>
<th>Adjusted*</th>
<th>Adjusted†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean</td>
<td>SD</td>
<td>Mean</td>
<td>SD</td>
<td>P</td>
</tr>
<tr>
<td>Perceived child overweight</td>
<td>3.0</td>
<td>0.5</td>
<td>2.9</td>
<td>0.3</td>
<td>NS</td>
</tr>
<tr>
<td>Responsibility</td>
<td>13.0</td>
<td>2.1</td>
<td>12.4</td>
<td>2.0</td>
<td>0.003</td>
</tr>
<tr>
<td>Concern</td>
<td>10.6</td>
<td>3.5</td>
<td>6.4</td>
<td>3.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Restriction</td>
<td>23.9</td>
<td>6.0</td>
<td>21.6</td>
<td>6.4</td>
<td>0.026</td>
</tr>
<tr>
<td>Pressure</td>
<td>6.1</td>
<td>3.3</td>
<td>7.8</td>
<td>4.1</td>
<td>0.007</td>
</tr>
<tr>
<td>Monitoring</td>
<td>11.5</td>
<td>3.3</td>
<td>10.9</td>
<td>3.2</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, physical activity, race, ethnicity, income, and maternal education.
†Adjusted for age, sex, physical activity, race, ethnicity, income, maternal education, and BMI\textsubscript{Z}.

### Table IV. Relationships of CFQ variables to BMIZ as a dependent variable among children with and without DS (n = 72)

<table>
<thead>
<tr>
<th>Independent variable</th>
<th>Unadjusted parameter estimate (95% CI)</th>
<th>P value</th>
<th>Adjusted* parameter estimate (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Perceived child overweight</td>
<td>0.985 (0.40 – 1.57)</td>
<td>0.001</td>
<td>0.626 (-0.01 – 1.25)</td>
<td>NS</td>
</tr>
<tr>
<td>Responsibility</td>
<td>0.043 (-0.09 – 0.17)</td>
<td>NS</td>
<td>0.012 (-0.13 – 0.15)</td>
<td>NS</td>
</tr>
<tr>
<td>Concern</td>
<td>0.160 (0.11 – 0.21)</td>
<td>&lt;0.001</td>
<td>0.093 (0.02 – 0.17)</td>
<td>0.016</td>
</tr>
<tr>
<td>Restriction</td>
<td>0.031 (-0.01 – 0.07)</td>
<td>NS</td>
<td>0.004 (-0.04 – 0.05)</td>
<td>NS</td>
</tr>
<tr>
<td>Pressure</td>
<td>-0.093 (-0.16 – 0.03)</td>
<td>0.006</td>
<td>-0.096 (-0.17 – 0.02)</td>
<td>NS</td>
</tr>
<tr>
<td>Monitoring</td>
<td>0.031 (-0.05 – 0.11)</td>
<td>NS</td>
<td>-0.005 (-0.10 – 0.11)</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Adjusted for age, sex, physical activity, DS status, race, ethnicity, income, and maternal education.
its ethnically diverse sample and paired-sibling design, which permit observation of the relationships of obesity risk factors to weight status in DS while minimizing the confounding effects of familial predisposition to obesity and shared environmental effects. This design also accounted for potential selection bias related to parental interest in nutrition, as this effect would be the same for both members of the sibling pair.

As life expectancy in DS increases, obesity prevention in this population has become a necessity for lowering the risk for the development of diabetes, cardiovascular disease, and other obesity-related comorbidities. Our study suggests that guidance for caregivers on avoidance of controlling feeding practices may be one avenue for obesity prevention among children with DS.

The authors would like to thank the children and their parents who participated in the study as well as the following parent support groups: 21 Down, BUDS, and the Down Syndrome Interest Group of Montgomery County.

REFERENCES

ADOLESCENT GROWTH SPURTS IN FEMALE GYMNASTS

Martine Thoms, PhD, Albrecht L. Claessens, PhD, Johan Lefevre, PhD, Renaat Philippaerts, PhD, Gaston P. Beunen, PhD, FACSM, and Robert M. Malina, PhD, FACSM

Objectives
Three questions were addressed: (1) Do female gymnasts have adolescent growth spurts in height, sitting height, and leg length? (2) Are the sequence and magnitude of spurts comparable with female adolescent non-athletes? (3) How do the data compare with other female gymnasts and with short girls?

Study design
Height and sitting height were measured annually on 15 Belgian gymnasts from 8.7 ± 1.5 to 15.5 ± 1.5 years. The gymnasts trained, on average, approximately 15 h/wk. Leg length was estimated as height minus sitting height. The Preece-Baines Model I was fitted to individual growth records to estimate ages at peak velocity and peak velocities for the three dimensions. Age at menarche and skeletal age were also assessed.

Results
Gymnasts have clearly defined adolescent spurts in height, estimated leg length, and sitting height that occur approximately 1 year later and are slightly less intense than in nonathletic adolescent girls. Age at menarche and skeletal age are consistent with later somatic maturation. The pattern of adolescent growth and maturation is similar to that of other gymnasts, short normal late-maturing girls, and late-maturing girls with short parents.

Conclusions
The results emphasize a primary role for constitutional factors in the selection process of female gymnasts at relatively young ages. (J Pediatr 2005;146:239-44)

Female gymnasts are, on average, the shortest among elite young athletes, with mean statures that approximate the 10th percentiles of international reference data. Gymnasts are also later maturing, as indicated in differences between skeletal and chronologic ages and the age at menarche. The short stature and later maturation are often attributed to intensive gymnastics training beginning at an early age. Presently available data, however, do not permit cause-effect statements about the relation between training and the growth and maturation of young female gymnasts. Gymnasts are shorter than average before the start of systematic training and have shorter parents.

Longitudinal data for female gymnasts that span late childhood and adolescence are limited. Results of a short-term longitudinal study of 22 Swiss female gymnasts have led to the caution that “…gymnasts advance through puberty without a normal pubertal growth spurt.” A more recent short-term study over a period of 2 years also suggests a less intense spurt.

It is suggested that gymnastics training reduces the growth potential of the legs, leading to disproportionately short legs and short stature. Allowing for variation in methods of measuring or estimating trunk (upper segment) and leg (lower segment) lengths, cross-sectional data for several samples of gymnasts, including three from international competitions, indicate sitting height/stature ratios (relative leg length) that are similar to reference data for nonathletic girls. Although gymnasts are absolutely shorter and have absolutely shorter legs, the evidence indicates similar proportional relations of the legs and trunk as in nonathletes.

The current study addresses the adolescent growth spurt of female gymnasts in the context of 3 questions: (1) Do female gymnasts have clearly defined adolescent growth spurts in height, sitting height, and leg length? (2) How do the sequence and magnitude of the respective growth spurts compare with corresponding data for the general population of

<table>
<thead>
<tr>
<th>PHV</th>
<th>Peak height velocity</th>
</tr>
</thead>
<tbody>
<tr>
<td>PSHV</td>
<td>Peak sitting height velocity</td>
</tr>
<tr>
<td>PLLV</td>
<td>Peak leg length velocity</td>
</tr>
</tbody>
</table>
female adolescents? (3) How do the data compare with other female gymnasts and other samples of girls characterized by short stature?

METHODS

The study is based on the individual records of 15 Belgian female gymnasts from two prominent clubs in the Antwerp region. The gymnasts were followed longitudinally at yearly intervals for 6 (n = 4) to 7 (n = 11) years (6.7 ± 0.5 years) between 1990 and 1997. The study was approved by the Medical Ethics Committee of the Faculty of Kinesiology and Rehabilitation Sciences of the Katholieke Universiteit Leuven. Informed consent was provided by the parents at all time points. In addition, each gymnast provided written assent after she reached 10 years of age. More detailed descriptions of the gymnastics program and the overall project have been previously reported.16,17

The gymnasts entered the program as beginning level participants and during the course of the study moved to regional and national competitive levels. Ages at entry into the program ranged from 6.0 to 11.6 years (8.7 ± 1.5 years), whereas ages at the conclusion of the study ranged from 12.8 to 17.6 years (15.5 ± 1.5 years). The gymnasts were identified as regional/national caliber at 11.6 ± 1.2 years, with a range from 8.8 to 13.6 years. The gymnasts trained, on average, 15 h/wk during the course of the study, with a range from 8 to 19 h/wk.

Height and sitting height were measured at annual intervals. Reliability data between anthropometrists were as follows: mean differences of replicate measurements, 0.23 cm for height, and 0.43 cm for sitting height; coefficients of variation, 0.3% for height, and 1.0% for sitting height; and correlation coefficients, 0.99 for height and 0.95 for sitting height. Corresponding estimates within technicians were smaller.18 Sitting height was subtracted from height to estimate leg (subischial) length.

The Preece-Baines Model I19 was fitted to longitudinal data for individual gymnasts to derive estimates of ages at peak velocity (years) and peak velocities (cm/y) for height (peak height velocity, PHV), sitting height (peak sitting height velocity, PSHV) and leg length (peak leg length velocity, PLLV). The curve-fitting protocol was successfully fit to the height records of 13 of the 15 gymnasts. The curve fits had standard errors of estimate between 0.02 and 0.28 cm, which is comparable to corresponding errors for boys and girls in the general population.20 Curve fitting was unsuccessful in two gymnasts. One was followed from 8.1 to 15.1 years, and peak velocity of growth in height apparently occurred between the last two observations, but the associated error of estimate was large. The other gymnast was followed from 10.8 to 17.8 years; PHV apparently occurred between the first two observations, and the model did not provide a good fit. Of the 13 gymnasts with a successful fit for height, the Preece Baines protocol was unsuccessful for sitting height in one and for estimated leg length in three. Standard errors of estimate for sitting height and leg length were similar to those for height. The unsuccessful fits were due to an unrealistic estimate of age at peak velocity (before the first observation) or an apparent peak between the last two observations.

The velocity curve for adolescent growth in height in gymnasts was compared with a Belgian reference sample.21 The reference data are based on 50 girls from the Belgian Growth Study of the Normal Child (1955 to 1975) who were followed longitudinally from early childhood through adolescence. The data were also fitted with the Preece-Baines Model I. The estimated age at PHV in the reference sample does not differ from the estimated ages at maximum increment in more recent samples of Belgian girls.21 Moreover, mean ages at PHV and median ages at menarche in samples of northwestern European girls have been rather stable through the 1980s.22,23

Menarcheal status and age at menarche were obtained by questionnaire and interview at each annual visit. A radiograph of the left hand and wrist was also taken annually to assess skeletal maturity. An Elimax 60 (62 kV, 15 mA, De Vree, Antwerp, Belgium) assembled in a portable apparatus was used. The radiographs were rated twice by an experienced assessor, using the radius-ulna-short bone protocol of the Tanner-Whitehouse 2 (TW2) method.24 Skeletal ages (SA) at the time of PHV and menarche were estimated. This is relevant because variation in SA is reduced at the time of PHV and menarche.25-27 Skeletal age at PHV and menarche was estimated by linear interpolation of maturity scores for radiographs taken on the visits immediately preceding and after the two maturity milestones, respectively. The interpolated maturity scores were converted to the respective SAs by using the tables provided for the TW2 method.24

RESULTS

Measures of the adolescent growth spurt for the three dimensions are summarized in Table 1. PHV occurs at 12.9 ± 1.5 years, with a range of approximately 4 years (10.5 to 14.5 years). In contrast, PHV occurs at an SA of 12.4 ± 0.6 years, with a range of variation of approximately 2 years (10.9 to 13.2 years).

The velocity curve for adolescent growth in height in gymnasts and the Belgian reference is shown in the Figure. The mean velocities for the gymnasts are based on the curve-fitting protocol, which provides velocity estimates for each year before and after PHV. These in turn were converted to chronologic ages, with 12.9 years as the reference (mean age at PHV for the sample) to permit comparison with the Belgian reference sample. Minimum and maximum velocities for the gymnasts were also derived in this manner and plotted at each chronologic age from 8 to 15 years for the gymnasts. The data for gymnasts are shown relative to the 3rd and 97th percentiles of velocity reference values for Belgian girls. Growth velocities of gymnasts are well within the range of those for the general population, but the velocity curve is shifted to the right. PHV is later in gymnasts compared with the reference (12.9 ± 1.5 years versus 11.6 ± 0.9 years, respectively), but the magnitude of the spurt is only slightly less in gymnasts compared with the reference (6.8 ± 1.1 cm/y versus 7.5±1.1 cm/y, respectively).21
PLLV occurs, on average, before PSHV (Table I). This sequence is consistent with limited data for adolescent girls, but the respective ages at peak velocity occur later in gymnasts than in nonathletes (Table II). Estimated peak velocities of growth in leg length (3.7 ± 0.8 cm/y) and sitting height (3.8 ± 1.0 cm/y) of gymnasts are lower, on average, but overlap estimates for two samples of adolescent girls. This should be viewed in the context of the different measures of sitting trunk (upper segment) and leg (lower segment) lengths. Data for the English sample are derived as in the current study, for example, standing height minus sitting height, whereas data for the Polish sample are derived as height minus symphyseal height.

Nine of the 15 gymnasts attained menarche during the study. Mean chronological age (CA) at menarche is 14.5 ± 1.6 years, with a range 11.3 to 16.5 years, whereas mean SA at menarche is 13.6 ± 0.5 years, with a range of 13.0 to 14.8 years. Mean CA at last observation of the 6 premenarcheal gymnasts is 14.5 ± 0.8 years, with a range of 13.0 to 15.1 years, whereas mean SA is 12.9 ± 1.0 years, with a range of 11.4 to 14.4 years. Variation in CA and SA is reasonably similar in the 6 premenarcheal gymnasts, which contrasts the trend in the 9 postmenarcheal gymnasts. Longitudinal height data were successfully modeled in 8 of the 9 postmenarcheal gymnasts. Menarche occurs after PHV, on average, by 1.5 ± 0.8 years, with a range of 0.7 to 3.2 years.

The adolescent growth spurt in height for the present and two other samples of gymnasts is summarized in Table III. The Polish sample was followed from 1971 to 1977 and trained approximately 22 to 26 h/wk, whereas the Swiss sample was followed for a shorter interval during the 1980s and trained 18 to 26 h/wk. Allowing for different methods of estimating parameters of the growth curve and for variation in time of study and training volume, estimated mean ages at PHV (12.9 ± 1.5 years) and estimated peak velocities (5.5 to 6.8 cm/y) are similar in the three samples of gymnasts. Menarche occurs after PHV, on average, by 1.5 ± 0.8 years, with a range of 0.7 to 3.2 years.

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Female gymnasts present, on average, short stature and late maturation. It is thus of interest to compare gymnasts with other samples of short, late-maturing girls. Data for two samples of short girls who are not gymnasts are included in Table III. One sample includes 18 late-maturing girls with short parents (menarche >13.7 years, midparent stature <162.5 cm) followed longitudinally in the Wroclaw Growth Study of youth in southwestern Poland, 1961 to 1972. The other sample includes 27 American girls classified as short, normal, slow-maturing children. The girls are from 6 longitudinal studies that date to the 1930s and have a stature at or below the 10th percentile of US reference data and an SA at least 1 SD less than CA. The Polish data from approximately the same time period indicate that gymnasts and late-maturing girls with short parents compare closely in age at PHV. Age at PHV in short, normal, slow-maturing American girls is earlier, but American girls tend to mature, on average, earlier than European girls. Estimated peak velocities are slightly less in gymnasts compared with other short girls but are within the range of normal variation.

**DISCUSSION**

Female gymnasts in the current study show a clearly defined adolescent growth spurt in height. PHV occurs, on average, more than 1 year later (12.9 ± 1.5 years) compared with Belgian nonathletes (11.6 ± 0.9 years), but there is considerable overlap. Comparative longitudinal data for gymnasts are limited to 9 Polish gymnasts in the 1970s followed annually for 6 years from 10 to 12 years and 22 Swiss gymnasts in the 1980s followed at 5- to 7-month intervals for 2.0 to 3.7 years from 12.3 ± 0.2 years. Allowing for different methods of estimating parameters of the growth curve and for variation in time of study and training volume, estimated mean ages at PHV (12.9 ± 1.5 years) and estimated peak velocities (5.5 to 6.8 cm/y) are similar in the three samples of gymnasts.

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**Figures**

Velocity curve for adolescent growth in height in gymnasts and a Belgian reference sample. Means (middle) and minimum (lower) and maximum (upper) values are shown as solid lines for the gymnasts. Medians and 3rd and 97th percentiles are shown as broken lines for the reference. See text for details.

**Table I. Variables of the adolescent growth spurt in female gymnasts.**

<table>
<thead>
<tr>
<th>Adolescent variables</th>
<th>n</th>
<th>Mean</th>
<th>SD</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age at peak height velocity (y)</td>
<td>13</td>
<td>12.9</td>
<td>1.5</td>
<td>10.55–14.52</td>
</tr>
<tr>
<td>Peak height velocity (cm/y)</td>
<td>6.8</td>
<td>1.1</td>
<td>4.81–9.23</td>
<td></td>
</tr>
<tr>
<td>Age at peak leg length velocity (y)</td>
<td>10</td>
<td>12.1</td>
<td>1.5</td>
<td>10.10–14.16</td>
</tr>
<tr>
<td>Peak leg length velocity (cm/y)</td>
<td>3.7</td>
<td>0.8</td>
<td>2.88–5.21</td>
<td></td>
</tr>
<tr>
<td>Age at peak sitting height velocity (y)</td>
<td>12</td>
<td>13.3</td>
<td>1.4</td>
<td>11.01–14.82</td>
</tr>
<tr>
<td>Peak sitting height velocity (cm/y)</td>
<td>3.8</td>
<td>1.0</td>
<td>2.40–5.49</td>
<td></td>
</tr>
</tbody>
</table>
years, and there is no clear secular gradient in the estimated ages. It should be noted, however, that longitudinal samples are somewhat unique. They are usually limited in numbers of subjects and may not be representative of the population.

Peak velocity of growth in Belgian and Polish gymnasts (6.8 ± 1.1 cm/y and 5.8 ± 0.5 cm/y, respectively) are slightly less than corresponding mean velocities in European girls, 7.1 to 9.0 cm/y (standard deviations a little more than 1 cm/y). The standard deviations indicate overlap between gymnasts and nonathletic adolescent girls.

Mean age at menarche in 9 of the 15 gymnasts is later than average (14.5 ± 1.6 years). The remaining 6 were premenarchal at the conclusion of the study so that the mean age for the total sample would be later. Mean ages at menarche for other samples of adolescent gymnasts range from 14.3 to 15.6 years (SD, 0.6 to 2.1 years).

A question of interest is whether the slightly less intense adolescent spurt of gymnasts contributes to their shorter height as adults. This may be so, but gymnasts are later maturing and probably continue to grow in height into the late teen ages. The estimated growth rates of the Belgian gymnasts at 15 years are approximately 1.5 to 5.0 cm/y (Figure), which suggests that growth in height probably will continue into the late teen ages in many of the girls.

PLLV occurs earlier than PSHV in the gymnasts (Table I), which is consistent with data for other samples of adolescent girls. Estimated peak velocities of growth in leg length and sitting height are slightly lower in gymnasts, but the standard deviations indicate overlap with the general population of adolescent girls (Table II). Note, however, different methods are used to measure/estimate the upper (sitting height) and lower (leg length) segments.

It has been postulated that intensive training during puberty may delay the timing of the growth spurt and sexual maturation and alter pubertal progression in athletes or extremely active girls. The interval between ages at PHV and menarche in gymnasts may provide some insight into this issue. Menarche is a late event in the sequence of adolescent changes that occurs, on average, about 1 year or more after PHV. The interval between PHV and menarche in 8 gymnasts of the current sample is 1.5 ± 0.8 years (height data of one postmenarchal gymnast was not successfully modeled). The interval for gymnasts overlaps those for adolescent girls active in sport, though not in gymnastics (1.2 ± 0.6 and 1.1 ± 0.5 years) and 5 samples of girls not active in sport (1.1 ± 0.5, 1.2 ± 0.8, 1.3 ± 0.7, 1.3 ± 0.6, 1.5 years). The correlation between ages at PHV and menarche in the small sample of gymnasts of the current study (n = 8, r = 0.87) is also similar to corresponding coefficients for girls active in sport (r = 0.74, 0.85) and for adolescent girls in 13 longitudinal studies (r = 0.69 to 0.93).

Variation in skeletal age at the time of PHV and menarche is reduced in gymnasts (12.4 ± 0.6 years and 13.6 ± 0.5 years, respectively). These estimates compare closely with corresponding estimates of SA (TW2) for British girls in the

### Table II. Means and standard deviations for ages at peak velocity (PV, y) and peak velocities (PV, cm/y) for estimated leg length and sitting height in gymnasts and adolescent girls from several European longitudinal samples

<table>
<thead>
<tr>
<th>Sample/method*</th>
<th>N</th>
<th>Mean</th>
<th>SD</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gymnasts</td>
<td>10–12</td>
<td>12.1</td>
<td>1.5</td>
<td>3.7</td>
<td>0.8</td>
</tr>
<tr>
<td>England</td>
<td>35</td>
<td>11.6</td>
<td>0.9</td>
<td>4.3</td>
<td>0.6</td>
</tr>
<tr>
<td>Poland</td>
<td>232–236</td>
<td>11.2</td>
<td>1.0</td>
<td>5.2</td>
<td>1.9</td>
</tr>
<tr>
<td>Poland</td>
<td>49</td>
<td>11.8</td>
<td>0.9</td>
<td>13.3</td>
<td>1.4</td>
</tr>
<tr>
<td>Sweden</td>
<td>80</td>
<td>11.6</td>
<td>0.9</td>
<td>13.2</td>
<td>1.0</td>
</tr>
</tbody>
</table>

*Gymnasts, current study, Preece-Baines Model I; England, Harpenden, single logistic; Poland, Wroclaw, graphic; Poland, Warsaw, kernel regression; Sweden, graphic.

### Table III. Estimated ages at peak height velocity (PHV, y) and PHV (cm/y) in samples of gymnasts and other short female subjects

<table>
<thead>
<tr>
<th>Sample</th>
<th>Age at PHV (y)</th>
<th>PHV (cm/y)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>M</td>
</tr>
<tr>
<td>Gymnasts</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current study</td>
<td>13</td>
<td>12.9</td>
</tr>
<tr>
<td>Polish</td>
<td>9</td>
<td>13.1</td>
</tr>
<tr>
<td>Swiss</td>
<td>22</td>
<td>13.0</td>
</tr>
<tr>
<td>Short female subjects</td>
<td>Pol</td>
<td>18</td>
</tr>
<tr>
<td>United States, SNSM</td>
<td>27</td>
<td>12.4</td>
</tr>
</tbody>
</table>

*Ziemilska, polynomials (n = 9), kernel regression (n = 6), calculated by Malina.*
**Theintz et al, chronologic age with maximum velocity.*
#Kozliski, late-maturing/short parents, Preece-Baines Model I.
†Khamis and Roche, short normal/slow-maturing, kernel regression.
parents and short normal slow-maturing girls. The stature of adolescent growth spurt in female gymnasts appears to be similar to that of other samples of short late-maturing girls (Table III), that is, late-maturing girls with short parents and short normal slow-maturing girls. The stature deficit evident in short normal slow-maturing girls was apparent by 3 years of age, which is consistent with data for gymnasts. Select Dutch gymnasts, for example, had statures that were approximately 1 SD score below average by 2 years of age.

The pattern of adolescent growth and sexual maturation of this sample of Belgian gymnasts is similar to corresponding data for other samples of gymnasts and appears to emphasize a primary role for constitutional factors in the selection process for the sport (parent, self, and/or coach) at relatively young ages. In turn, regular training for gymnastics during adolescence, on average 15 h/wk, does not appear to influence the timing of the growth spurt and sexual maturation in this sample of gymnasts. If intensive training is a factor that influences the growth and maturation of gymnasts, its effects must be partitioned from constitutional and familial factors and perhaps specific components of the overall gymnastics environment before causality can be established.

Special thanks are extended to Mrs I. Wuyts, coach of the gymnasts, and to all of the girls who participated in this longitudinal study.

REFERENCES

PUBERTAL UPREGULATION OF ERYTHROPOIESIS IN BOYS IS DETERMINED PRIMARILY BY ANDROGEN

MATTI HERO, MD, SANNI WIKMAN, MD, PhD, RAJA HANNIHERVI, MD, PhD, MARTTI A. SIIMES, MD, PhD, AND LEO DUNKEL, MD, PhD

Objectives  To study the relative roles of androgens and the growth hormone–insulin-like growth factor I (GH-IGF-I) system in the regulation of erythropoiesis in boys during puberty.

Study design  We treated 23 boys with constitutional delay of puberty with low-dose testosterone (T), in combination with either a potent aromatase inhibitor, letrozole (Lz; 2.5 mg/d), or placebo (P). The study design was randomized, double-blinded, and placebo-controlled between the treated groups. Treatment with T + Lz was associated with high T and low IGF-I concentrations, whereas treatment with T + P resulted in moderately increased T and high IGF-I concentrations.

Results  The blood hemoglobin concentration increased by 1.6 g/dL in T + Lz–treated boys, despite their low IGF-I concentrations. The estimated red blood cell volume increased more in T + Lz–treated than in T + P–treated boys (349 vs 174 mL, respectively, \( P = .01 \)). Serum T concentrations during the treatment period correlated with the 12-month increments in hemoglobin and red blood cell volume. The changes in blood hemoglobin concentration and RBC in T + Lz–treated boys were similar to those we observed in a population of normal adolescent boys in the late stages of puberty.

Conclusions  The pubertal increase in hemoglobin concentration in boys is related to direct androgen effects. (J Pediatr 2005;146:245-52)

Male puberty is followed by a 2-g/dL increase in blood hemoglobin concentration. The regulation of this acceleration in erythropoiesis has remained poorly understood, although it has been connected with the pubertal increase in serum testosterone (T) concentration. \(^1\)\(^,\)\(^2\) The rise in blood hemoglobin concentration, however, is slowly progressive and takes about 6 years, whereas serum T increases more rapidly and in a more linear fashion from the early puberty onward. \(^3\) This lack of synchronism between the changes in T and hemoglobin makes it difficult to explain the puberty-associated changes in erythropoiesis exclusively, as the effects of testosterone.

The growth hormone–insulin-like growth factor I (GH-IGF-I) system is also activated during puberty. Previous reports have shown that GH and especially IGF-I stimulate erythropoiesis both in vitro \(^4\)\(^-\)\(^9\) and in vivo. \(^10\)\(^,\)\(^11\) The significance of the GH-IGF-I system in the regulation of erythropoiesis is demonstrated by the impaired erythropoiesis in children \(^12\)\(^,\)\(^13\) and adults \(^14\) with GH deficiency and by the normalization of erythropoiesis with GH treatment. \(^13\)\(^,\)\(^14\) Moreover, a blood hemoglobin level within normal limits is associated with the IGF-I and IGF binding protein-3 (IGFBP-3) concentrations in healthy prepubertal and early pubertal boys. \(^15\) The level is not associated with individual testosterone concentrations. \(^15\) These findings have led to the suggestion that testosterone effects are mediated by the GH-IGF-I system, which eventually induces the pubertal increase in hemoglobin. \(^15\) However, the distinct roles of androgens and the GH-IGF-I system have been difficult to separate in an in vivo situation because androgens, after aromatization to estrogens, stimulate GH secretion in adolescent boys, and thus the two hormones are interrelated. \(^16\)\(^,\)\(^17\)

In this study, we aimed to clarify the relative roles of androgens and the GH-IGF-I system in the regulation of erythropoiesis in adolescent boys.

FAI  Free androgen index
GH  Growth hormone
IGF-I  Insulin-like growth factor I
IGFBP-3  Insulin-like growth factor binding protein-3
Lz  Letrozole
MCV  Mean cell volume
P  Placebo
RBC  Red blood cell volume
STfR  Serum transferrin receptor
T  Testosterone

From Hospital for Children and Adolescents, University of Helsinki, Helsinki, Finland; Hyvinkää Hospital, Hyvinkää, Finland; and the Department of Pediatrics, Kuopio University Hospital, Kuopio, Finland.

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Drs Simes and Dunkel contributed equally to this article.

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METHODS

Subjects

The data reported were gathered from two populations: Boys with constitutional delay of puberty received T treatment combined either with placebo or with letrozole (Lz) or served as control subjects, and a population of normal adolescent boys, partly published earlier, were followed longitudinally throughout their puberty without any intervention.

Figure 1. Changes (mean ± SEM) in serum testosterone and IGF-I concentrations during the 18 months of follow-up in patients treated either with T + Lz or with T + P and in nontreated boys. Marked P values refer to differences between the treated groups. *P < .05, †P < .01 refer to within-group changes from baseline values.

Figure 2. Changes (mean ± SEM) in blood hemoglobin concentration and estimated RBC during the 18 months of follow-up in patients treated with either T + Lz or T + P and in the control subjects. *P < .05, †P < .01 refer to within-group difference from baseline values. Marked P values refer to difference between the treated groups.
Boys with Delayed Puberty. A group of 33 boys who were referred to the Hospital for Children and Adolescents, University of Helsinki, for assessment of their delayed puberty, short stature, or both, composed the study population of boys with constitutional delay of puberty. The criteria for delayed puberty were defined as a Tanner genital or pubic hair stage observed at an older age than the mean $+ 2 \text{SD}$ for healthy Finnish boys or a testicular volume of less than 4 mL after 13.5 years of age. None of the boys presented with signs of chronic illness in their medical history, physical examination, or laboratory evaluation.  

### Table I. Baseline characteristics of boys with delayed puberty

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Testosterone and letrozole (n = 10)</th>
<th>Testosterone and placebo (n = 12)</th>
<th>No treatment (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, y</td>
<td>15.2 (0.8)</td>
<td>15.0 (0.8)</td>
<td>15.0 (0.7)</td>
</tr>
<tr>
<td>Weight, kg</td>
<td>44.6 (7.5)</td>
<td>46.2 (7.4)</td>
<td>41.0 (6.9)</td>
</tr>
<tr>
<td>Height, cm</td>
<td>155.3 (6.7)</td>
<td>151.9 (8.3)</td>
<td>154.3 (4.4)</td>
</tr>
<tr>
<td>Pubertal stage, G; P*</td>
<td>2 (2–3);1 (1-2)</td>
<td>2 (2–3);1 (1-2)</td>
<td>2 (2–3);2 (1-2)</td>
</tr>
<tr>
<td>Testicular volume, mL</td>
<td>5.5 (1.9)</td>
<td>6.9 (4.3)</td>
<td>5.9 (2.7)</td>
</tr>
<tr>
<td>Serum testosterone, ng/mL</td>
<td>2.7 (3.2)</td>
<td>3.4 (2.9)</td>
<td>3.0 (3.1)</td>
</tr>
<tr>
<td>Serum IGF-I, ng/mL</td>
<td>231 (82)</td>
<td>216 (72)</td>
<td>209 (92)</td>
</tr>
<tr>
<td>Serum IGFBP-3, mg/L</td>
<td>3.7 (0.5)</td>
<td>3.8 (0.5)</td>
<td>3.7 (0.8)</td>
</tr>
<tr>
<td>Blood hemoglobin, g/dL</td>
<td>13.7 (0.9)</td>
<td>13.6 (0.8)</td>
<td>13.8 (0.7)</td>
</tr>
<tr>
<td>MCV, µm³</td>
<td>83 (2)</td>
<td>83 (3)</td>
<td>84 (3)</td>
</tr>
</tbody>
</table>

There were no statistically significant differences between groups. Kruskal-Wallis nonparametric analysis of variance was used for comparisons. Values are mean (SD), except for pubertal stage, which is median (range).

*Pubertal stage according to Tanner; G = genital stage; P = pubic hair stage.

### Table II. Iron parameters of boys with delayed puberty

<table>
<thead>
<tr>
<th></th>
<th>Testosterone and letrozole</th>
<th>Testosterone and placebo</th>
<th>No treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean cell volume, µm³</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mo</td>
<td>83 (2)</td>
<td>83 (3)</td>
<td>84 (3)</td>
</tr>
<tr>
<td>5 mo</td>
<td>80 (3)</td>
<td>82 (4)</td>
<td>84 (3)</td>
</tr>
<tr>
<td>12 mo</td>
<td>81 (3)</td>
<td>82 (5)</td>
<td>83 (4)</td>
</tr>
<tr>
<td>18 mo</td>
<td>83 (3)</td>
<td>85 (3)</td>
<td>85 (4)</td>
</tr>
<tr>
<td>Ferritin, ng/mL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mo</td>
<td>26.9</td>
<td>25.7</td>
<td>26.9</td>
</tr>
<tr>
<td>5 mo</td>
<td>13.8†</td>
<td>15.8</td>
<td>28.8</td>
</tr>
<tr>
<td>12 mo</td>
<td>20.0</td>
<td>15.1</td>
<td>25.7</td>
</tr>
<tr>
<td>18 mo</td>
<td>22.9</td>
<td>23.4</td>
<td>24.6</td>
</tr>
<tr>
<td>Transferrin, mg/dL</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mo</td>
<td>257 (20)</td>
<td>263 (30)</td>
<td>249 (50)</td>
</tr>
<tr>
<td>5 mo</td>
<td>282 (20)†</td>
<td>287 (30)†</td>
<td>242 (50)</td>
</tr>
<tr>
<td>12 mo</td>
<td>284 (30)†</td>
<td>288 (50)</td>
<td>244 (60)</td>
</tr>
<tr>
<td>18 mo</td>
<td>273 (40)</td>
<td>27 (30)</td>
<td>253 (50)</td>
</tr>
<tr>
<td>Transferrin saturation, %</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mo</td>
<td>21 (5)</td>
<td>27.7 (12)</td>
<td>22.8 (11)</td>
</tr>
<tr>
<td>5 mo</td>
<td>19.1 (14)</td>
<td>24.3 (13)</td>
<td>26 (14)</td>
</tr>
<tr>
<td>12 mo</td>
<td>20.6 (11)</td>
<td>23.7 (12)</td>
<td>25.5 (11)</td>
</tr>
<tr>
<td>18 mo</td>
<td>21.8 (8)</td>
<td>27.6 (9)</td>
<td>30.1 (14)</td>
</tr>
<tr>
<td>Transferrin receptor, mg/L</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0 mo</td>
<td>3.05 (0.4)</td>
<td>3.37 (0.9)</td>
<td>3.00 (0.7)</td>
</tr>
<tr>
<td>5 mo</td>
<td>4.26 (1.4)†</td>
<td>4.40 (1.3)†</td>
<td>2.85 (0.5)</td>
</tr>
<tr>
<td>12 mo</td>
<td>3.60 (0.6)†</td>
<td>4.14 (1.9)</td>
<td>2.70 (0.6)</td>
</tr>
<tr>
<td>18 mo</td>
<td>3.21 (0.6)</td>
<td>3.35 (1.0)</td>
<td>2.88 (0.5)</td>
</tr>
</tbody>
</table>

Values are mean (SD). Change within group from the start to indicated time point: *P < .05; †P < .01. Nonparametric tests were applied for every parameter. Ferritin values are geometric means because of skewed distributions.
routine laboratory measurements that could account for the delayed puberty. Two boys received inhaled corticosteroid treatment for asthma. After the 12-month follow-up visit, one T1P–treated boy received ferroaspartate (250 mg orally twice a day) for 3 months.

**POPULATION OF NORMAL ADOLESCENT BOYS.** A total of 60 boys were studied. The characteristics of the study population at the beginning of the study have been presented in detail previously. At the beginning of the study, 49 of 60 boys (82%) had a testicular volume of less than 4 mL (total range, 0.9 to 6.8 mL), and their mean serum testosterone concentration was 0.3 ng/mL (0.1 to 3.2 ng/mL). Two boys had serum ferritin levels below 12 ng/mL. The blood hemoglobin concentrations of the boys ranged from 11.7 to 14.5 g/dL (mean, 13.0 g/dL).

**Study Protocol**

**BOYS WITH DELAYED PUBERTY.** The study of boys with delayed puberty was based on a randomized, double-blinded, placebo-controlled design between the two groups receiving treatment. Of the 33 boys recruited, 10 boys (or their parents) wanted to wait for spontaneous progression of puberty and did not wish for any medical intervention. However, they were willing to participate in the study and composed the nontreated control group. The remaining 23 boys, who wished for medical intervention, were randomly assigned to receive either testosterone enanthate (Testoviron-Depot-250, Schering, Berlin, Germany) 6 times at a dose of 1 mg/kg IM every 4 weeks, combined with oral placebo once daily for 12 months; or testosterone enanthate (as above) and an aromatase inhibitor, letrozole (Femar, Novartis AG, Stein, Switzerland), 2.5 mg orally once daily for 12 months. The rationale for

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**Figure 3.** Correlations between average testosterone and changes in hemoglobin (Hb) and estimated RBC at 12 months, compared with correlations between average IGF-I and Hb and RBC. Average testosterone and average IGF-I were calculated from the 2-, 5-, and 12-month values. ● = T + Lz–treated boys, ○ = T + P–treated boys, ● = nontreated boys.
treat these boys with letrozole, an inhibitor of estrogen synthesis, was our hypothesis that blocking the estrogen effects would delay bone maturation and thereby increase adult height.20

We examined the boys at the start of the study and at 2, 5, 12, and 18 months, subsequently. Every visit included a physical examination, measurement of body weight and height (Harpenden stadiometer with 0.1-cm precision), assessment of pubertal stage according to Tanner,21 measurement of mean testicular volume, and laboratory tests. Venous blood samples were drawn between 7:30 and 10:15 AM. Mean testicular volume was calculated by a formula: length in cm $^3$ (width in cm)$^2$ 0.52.22 Of the 11 patients treated with T1Lz, of the 12 treated with T1P, and of the 10 control subjects, 10, 11, and 8 patients completed the 12-month follow-up and 10, 10, and 7 patients completed the 18-month follow-up, respectively. Elevations in the concentrations of serum gonadotropins and testosterone were absent in one boy treated with T1Lz during the treatment period. He was therefore considered noncompliant, and his results were excluded from the analyses. The patient selection and the trial profile have been published in detail previously.20

At the start of the study, the three groups did not differ in the stage of pubertal development, age, body weight or height, or mean concentrations of hormonal and hematologic indexes (Table I). None of the boys had a blood hemoglobin concentration below 12.0 g/dL or a serum ferritin concentration below 12 ng/mL. Six boys in the T1P group and two boys in the control group had elevated concentrations of the serum transferrin receptor (sTfR > 3.4 mg/L).

**Table III. Changes in testicular volume, serum testosterone, blood hemoglobin concentration, and serum ferritin in the population of normal adolescent boys**

<table>
<thead>
<tr>
<th>Age</th>
<th>Testicular volume, mL</th>
<th>S-testosterone, ng/mL</th>
<th>B-hemoglobin, g/dL</th>
<th>S-ferritin, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7 y (n = 60)</td>
<td>2.4 (2)</td>
<td>0.3 (0.4)</td>
<td>13.0 (0.7)</td>
<td>31.4</td>
</tr>
<tr>
<td>12.6 y (n = 60)</td>
<td>6.1 (4)$^\ddagger$</td>
<td>0.9 (1.0)$^\ddagger$</td>
<td>13.2 (0.6)</td>
<td>27.6$^\ddagger$</td>
</tr>
<tr>
<td>13.6 y (n = 58)</td>
<td>10.0 (6)$^\ddagger$</td>
<td>2.1 (1.9)$^\ddagger$</td>
<td>13.3 (0.8)$^\ddagger$</td>
<td>20.0$^\ddagger$</td>
</tr>
<tr>
<td>14.6 y (n = 56)</td>
<td>14.2 (6)$^\ddagger$</td>
<td>2.7 (1.5)$^\ddagger$</td>
<td>13.6 (0.8)$^\ddagger$</td>
<td>25.9$^\ddagger$</td>
</tr>
<tr>
<td>16.6 y (n = 19)</td>
<td>19.8 (5)$^\ddagger$</td>
<td>5.8 (2.2)$^\ddagger$</td>
<td>15.1 (0.7)$^\ddagger$</td>
<td>33.1$^\ddagger$</td>
</tr>
</tbody>
</table>

Values are mean (SD), except the values of ferritin, which are presented as geometric means, because of skewed distributions. $^*P < .05; ^\ddagger P < .01; ^\ddagger\ddagger P < .001; P$ values refer to change from 11.7 years value to indicated time point.

Figure 4. Changes in blood hemoglobin and estimated RBC (mean $\pm$ SEM) at different ages in the population of normal adolescent boys followed longitudinally throughout puberty. $\ddagger P < .01.$

**Table III. Changes in testicular volume, serum testosterone, blood hemoglobin concentration, and serum ferritin in the population of normal adolescent boys**

<table>
<thead>
<tr>
<th>Age</th>
<th>Testicular volume, mL</th>
<th>S-testosterone, ng/mL</th>
<th>B-hemoglobin, g/dL</th>
<th>S-ferritin, ng/mL</th>
</tr>
</thead>
<tbody>
<tr>
<td>11.7 y (n = 60)</td>
<td>2.4 (2)</td>
<td>0.3 (0.4)</td>
<td>13.0 (0.7)</td>
<td>31.4</td>
</tr>
<tr>
<td>12.6 y (n = 60)</td>
<td>6.1 (4)$^\ddagger$</td>
<td>0.9 (1.0)$^\ddagger$</td>
<td>13.2 (0.6)</td>
<td>27.6$^\ddagger$</td>
</tr>
<tr>
<td>13.6 y (n = 58)</td>
<td>10.0 (6)$^\ddagger$</td>
<td>2.1 (1.9)$^\ddagger$</td>
<td>13.3 (0.8)$^\ddagger$</td>
<td>20.0$^\ddagger$</td>
</tr>
<tr>
<td>14.6 y (n = 56)</td>
<td>14.2 (6)$^\ddagger$</td>
<td>2.7 (1.5)$^\ddagger$</td>
<td>13.6 (0.8)$^\ddagger$</td>
<td>25.9$^\ddagger$</td>
</tr>
<tr>
<td>16.6 y (n = 19)</td>
<td>19.8 (5)$^\ddagger$</td>
<td>5.8 (2.2)$^\ddagger$</td>
<td>15.1 (0.7)$^\ddagger$</td>
<td>33.1$^\ddagger$</td>
</tr>
</tbody>
</table>

Values are mean (SD), except the values of ferritin, which are presented as geometric means, because of skewed distributions. $^*P < .05; ^\ddagger P < .01; ^\ddagger\ddagger P < .001; P$ values refer to change from 11.7 years value to indicated time point.
of 11.7 years. Venous blood samples were drawn between 8:30 AM and 2 PM.

In both study populations, informed written consent was obtained from the patients and their guardians. The protocols concerning the boys with delayed puberty and the population of normal boys were accepted by the Ethics Committee of the Hospital for Children and Adolescents. The protocol of the boys with delayed puberty was also accepted by the National Agency for Medicines.

Biochemical Analyses

**BOYS WITH DELAYED PUBERTY**. The methods used for determining serum testosterone, IGF-I, and IGFBP-3 concentrations have been described elsewhere.20 Blood hemoglobin concentrations were measured with either Advia 120 or with a Coulter Counter T890, using the photometric, cyanmethemoglobin method. The analytical variation of these methods was <1.7%. Serum soluble transferrin receptor concentrations were measured by a noncompetitive “sandwich type” immunoenzymometric assay, using monoclonal anti-sTfR antibody (Idea, Orion Diagnostica, Espoo, Finland). Serum ferritin concentrations were determined by a 2-site chemiluminometric immunoassay (Architect 2* 2000i, Abbott Laboratories, Abbott Park, Ill). Erythropoietin was quantified with the use of an immunochromiluminometric assay on the IMMULITE analyzer (DPC, Los Angeles, Calif). The detection limit of the assay is 0.2 U/L. Within-run coefficient of variation (CV) was <9% and the total CV <9% in the concentration ranged from 7 to 148 U/L.

The estimated red blood cell volume (RBC) was calculated from the formula hematocrit value × 64 mL/kg (estimated blood volume) × body weight.23 To have a single measure that would reflect the influence of testosterone and IGF-I during the 12 months of treatment, we calculated the average values for both variables, using the 2-, 5-, and 12-month values.

**POPULATION OF NORMAL ADOLESCENT BOYS.** Methods used for determining blood hemoglobin, serum testosterone, and serum IGFBP-3 concentrations have been described previously.15 To provide a more sensitive measure of androgen influence, we calculated the free androgen index (FAI) from the formula total testosterone/sex hormone binding globulin × 100.24

**STATISTICAL ANALYSES.** The values in the tables are means (SD), except for the values for Tanner stages, which are presented as medians (range), and for ferritin, which are presented as geometric means because of skewed distributions. Values in the figures are presented as means (SEM). The differences in the baseline values between the groups were evaluated by use of the Kruskal-Wallis nonparametric analysis of variance. The differences in hormonal and hematologic indexes between the treatment groups were evaluated by means of the Mann-Whitney U test. The Wilcoxon matched-pairs, signed-rank test was used to evaluate the changes within the groups during the follow-up. The Pearson correlation coefficient was used to evaluate the relations between the changes in hemoglobin and average testosterone or IGF-I and between RBC and average testosterone or IGF-I. For correlations concerning groups, we used the Spearman correlation coefficient. We used the Student paired t test for analyzing the longitudinal differences in hormonal and hematologic indexes in the population of normal adolescent boys. All the statistical tests were 2-sided. We used SPSS statistical software for Windows, release 10.0.7 (SPSS, Chicago, Ill), for analyzing the data.

**RESULTS**

**Boys with Delayed Puberty**

Treatment with the T + P increased both T and IGF-I concentrations, whereas in the T + Lz-treated, T and IGF-I changed to opposite directions, resulting in high T and lowered IGF-I concentrations (Figure 1). The blood hemoglobin level increased by 1.61 g/dL in the T + Lz-treated boys during the 12 months of treatment (Figure 2). The respective rise was 0.96 g/dL in the boys receiving T + P and 0.88 in the nontreated boys. The difference in hemoglobin increment between the T + Lz–treated and T + P–treated boys failed to reach significance (P = .17). Simultaneously, during the 12 months of treatment, RBC rose in the boys of all three groups (Figure 2). The respective elevation in RBC was greater in the boys treated with T + Lz than in those who received T + P (349 vs 174 mL, respectively). During the 6 months of the follow-up period after discontinuation of the treatments, the hemoglobin concentration decreased from 15.2 to 14.2 g/dL (P < .01) in the T + Lz–treated boys but not in the T + P–treated boys (14.6 g/dL at 12 and 18 months) or in the control subjects (from 14.5 to 14.8 g/dL).

Treatment with T + Lz was associated with a decline in serum ferritin and mean cell volume (MCV), whereas serum transferrin and sTfR concentrations increased at 5 months after the start of treatment (Table II). The changes in iron consumption markers were less pronounced in the T + P–treated boys; serum transferrin and sTfR concentrations increased after 5 months of treatment, but no significant changes occurred in serum ferritin or MCV. In contrast, the above-mentioned iron parameters of the control subjects did not change during the study period.

The individual elevations in hemoglobin and RBC at 12 months were positively associated with average T concentrations in boys with delayed puberty (Figure 3). When analyzed according to received treatment, association with T remained only in those who received T + Lz (r = 0.71, P < .05 and r = 0.65, P = .06, respectively) and in control subjects (r = 0.74, P < .05 and r = 0.91, P < .01, respectively). We observed no associations between the average IGF-I or IGFBP-3 and the changes in hemoglobin or RBC in the treated boys. However, the IGF-I and IGFBP-3 were associated with the 12-month increments in hemoglobin (r = 0.95, P < .001 and r = 0.86, P < .01, respectively) and RBC (r = 0.91, P < .01 and r = 0.71, P < .05) in the
control subjects. Serum erythropoietin concentrations did not change in the T + Lz–treated, in the T + P–treated, or in the control subjects during the study period, nor did they correlate with the changes in hemoglobin and RBC.

Body weight gains at 5 months were negatively associated with serum ferritin concentrations at 5 months \((r = -0.43; \ P < .05)\) and positively associated with sTfR concentrations at 5 months \((r = 0.39; \ P < .05)\). In addition, at 5 months, the T concentration was negatively associated with ferritin \((r = -0.45, \ P < .05)\) and positively associated with sTfR \((r = 0.48, \ P < .01)\). Such associations were not observed thereafter during the study period.

**Population of Normal Adolescent Boys**

During the 5 years of follow-up, from the average age of 11.7 years up to 16.6 years, there was a 2.1-g/dL increase in blood hemoglobin concentration (Table III). The blood hemoglobin concentration did not increase significantly until the average age of 13.6 years (Table III). At that age, the mean testicular volume was 10.0 mL (range, 2.2 to 25.6 mL) and the serum T concentration was 2.1 ng/mL (range, 0.1 to 9.4 ng/mL). Using a cutoff point of 4 mL for testicular volume, 10% of the boys were prepubertal at the average age of 13.6 years. The first significant annual increment in hemoglobin concentration occurred at the average age of 14.6 years, when the mean hemoglobin was 13.6 g/dL, the testicular volume was 14.2 mL (range, 3.4 to 29.4 mL), and the serum T concentration was 2.7 ng/mL (range, 0.1 to 6.2 ng/mL). Most of the increase in hemoglobin took place at late puberty, between the ages 14.6 and 16.6 years, when the hemoglobin concentration increased by 1.6 g/dL in 2 years (Figure 4). During those 2 years, the serum T concentration increased to 5.7 ng/mL (range, 3.6 to 11.7 ng/mL) and the testicular volume increased to 19.8 mL (range, 12.4 to 26.7 mL). The change in RBC was more linear and increased steadily throughout puberty (Figure 4). The serum ferritin decreased during the first year of follow-up, reaching the nadir at the average age of 13.6 years (Table III). Thereafter, the serum ferritin concentration increased.

At the average age of 11.7 years, the blood hemoglobin concentration correlated with the serum IGF-I concentration \((r = 0.36, \ P = .008)\) but not with the serum T concentration. Thereafter, the serum T concentration correlated with the blood hemoglobin concentration at the average ages of 13.6 years \((r = 0.35, \ P < .01)\) and 14.6 years \((r = 0.27, \ P < .05)\). At these two ages, there was a close correlation between the blood hemoglobin concentration and the FAI \((r = 0.50, \ P < .001)\) and \(r = 0.50, \ P < .001\) respectively. The correlations between T and hemoglobin and between FAI and hemoglobin failed to reach significance at the average ages of 12.6 and 16.6 years.

**DISCUSSION**

This study demonstrates for the first time that in vivo androgens are able to stimulate erythropoiesis independent of the GH-IGF-I system during male puberty. Treatment with T + Lz was followed by a substantial increase in blood hemoglobin and an increase in RBC that exceeded the respective increment in the T + P–treated boys. This acceleration in erythropoiesis in the T + Lz–treated boys took place despite their low IGF-I concentration and was associated with serum T. Furthermore, discontinuation of T + Lz treatment resulted in a substantial decrease in hemoglobin level, despite the simultaneous increase in IGF-I concentration.

T + Lz treatment significantly raised RBC and significantly decreased the markers of iron consumption. This is in keeping with androgen-induced upregulation of erythrocyte production rate, followed by an increase in iron demand. In fact, 5 of 10 boys treated with T + Lz had serum ferritin below 12 mg/L 5 months after the onset of treatment, indicating depleted iron stores. This decrease in iron availability may have been a rate-limiting factor of erythropoiesis in the T + Lz–treated boys. With iron supplementation, the acceleration in erythropoiesis could have been even more pronounced.

The effects of androgens on erythropoiesis are well documented. Androgens were the main pharmacologic agents used to stimulate erythropoiesis before the availability of recombinant hematopoietic growth factors, and androgens have been shown to stimulate erythropoiesis in bone marrow cultures. Furthermore, in adult men, temporary withdrawal of sex hormone effects by using GnRH-analogue treatment is associated with a reversible decrease in hemoglobin level. Cross-sectional studies of pubertal boys have shown that hematocrit levels increase as they mature and that the pubertal increase in hemoglobin correlates closely with serum T. The increase in hemoglobin level appears to follow the induction of testosterone secretion with a 5-month delay. These findings suggest that sex hormones play a role in the maintenance of hemoglobin level typical for adult males and that androgens may trigger the pubertal upregulation of erythropoiesis in boys. However, previous studies have not taken into account that aromatizable androgens stimulate the GH-IGF-I system. To the best of our knowledge, our study is the first in vivo study designed to specify the individual effects of androgen and IGF-I on erythropoiesis.

Our current findings are not in conflict with earlier studies demonstrating that the GH-IGF-I system has a significant role in the regulation of erythropoiesis in human beings. Even though anemia is not a key feature in GH deficiency, it is associated with impaired erythropoiesis in both adults and children. Treatment of GH-deficient adults with GH increased plasma volume and red cell mass but did not have an effect on hemoglobin level, whereas the treatment of GH-deficient children elevated hemoglobin level. Furthermore, in healthy prepubertal and early pubertal boys, blood hemoglobin level within normal limits correlated with serum IGF-I and IGFBP-3 levels. These findings suggest that the GH-IGF-I system influences erythropoiesis throughout life and may play a key role in the gradual increase of RBC in growing children and adolescents. However, the male-limited increase in erythrocyte production rate and concomitant rise in blood hemoglobin level during puberty appears to be regulated by the direct action of androgens.
In pubertal boys, there is a smooth, progressive increase in hemoglobin level occurring mostly at late stages of puberty. Most of the increase in blood hemoglobin level is gained years after the onset of testosterone secretion, soon after the growth spurt in body height. Despite the close temporal connection between the acceleration in longitudinal growth and the increase in hemoglobin level, these two phenomena are differentially regulated. The pubertal acceleration in chondrocyte proliferation in the bone growth plates is inducted by estrogen,\textsuperscript{31} whereas the upregulation of hemoglobin level is dependent on androgen. The fact that pubertal girls have an estrogen-mediated spurt in longitudinal growth but no changes in hemoglobin level supports this view. The reason for the delay between the increase in serum T concentration and the increase in hemoglobin level is unclear. However, in the normal population of adolescent boys, serum ferritin concentration declined in earlier stages of pubertal development, suggesting an increase in the rate of erythropoiesis. This did not lead to an increase in blood hemoglobin level, possibly because of the diluting effect of simultaneous increase in growth rate and, presumably, in plasma volume.

REFERENCES

Objectives  Medical dictionaries and anthropologic sources define brachycephaly as a cranial index (CI = width divided by length × 100%) greater than 81%. We examine the impact of supine sleeping on CI and compare orthotic treatment with repositioning.

Study design  We compared the effect of repositioning versus helmet therapy on CI in 193 infants referred for abnormal head shape.

Results  Eighty percent of the infants had a pretreatment CI > 81%. Their initial mean CI at mean age 5.3 months was 89%, and after treatment, their mean CI was 87% (±2 SE = 0.9%) at mean age 9.0 months. For 92 infants with an initial CI at or above 90%, their initial mean CI of 96.1% was reduced to a mean of 91.9%.

Conclusions  Post-treatment CI was 86% to 88%. CI in neonates delivered by cesarean section was 80%, and CI in supine-sleeping Asian children was 85% to 91%, versus 78% to 83% for prone-sleeping American children. Repositioning was less effective than cranial orthotic therapy in correcting severe brachycephaly. We recommend varying the head position when putting infants to sleep. (J Pediatr 2005;146:253-7)

Brachycephaly refers to a head, which is shortened in the anteroposterior dimension and wide between the biparietal eminences, with a cranial index (CI = width divided by length × 100%) > 81% (Figure 1). Dolichocephaly is present when the CI is less than 76%, so the normal CI ranges from 76% to 81%. The most frequent cause of brachycephaly is constant supine positioning during infancy, which must be distinguished from bilateral coronal synostosis, which is comparatively rare. The increasing prevalence of both plagiocephaly and brachycephaly in recent years is a consequence of the success of efforts to prevent sudden infant death syndrome (SIDS). The prone sleeping position has a protective effect for both positional preference and deformational brachycephaly. In less than a decade, the normal head shape of the American infant has changed from normocephalic (CI = 76% to 81%) to brachycephalic, causing many families to consult their primary care providers for guidance in correcting unusual head shapes. In Asia, where infants have traditionally been positioned for sleep on their backs, brachycephaly is a normal head shape. We provide new normative data for current infant head shapes, present results from treating infants with excessive brachycephaly, and compare these head shapes with those in other populations.

METHODS

Longitudinal data were collected on 193 normal infants with no malformations or neurologic problems who were referred and treated for abnormal head shapes at Cedars-Sinai Medical Center between 1997 and 2001. Institutional review board review was obtained for use of anonymized patient care data. Cranial measurements were made with anthropometric precision metal cranial calipers (B. Braun Medical Products, Aesculap Division, Tuttingen, Germany). Each measurement was repeated 3 times by one of two pediatric nurse practitioners and averaged. Comparisons between both practitioners measuring the same patient demonstrated no significant differences in measurement techniques. The CI was compared before and after treatment, and we examined the
impact of treatment on positional brachycephaly. Practitioners instructed parents in how to perform neck physical therapy, if there was any positional preference, and repositioning was initiated at the onset of treatment. Statistical tests, using the SAS software package, were used to compare the effect of repositioning versus helmet therapy. For those infants treated with cranial orthotic therapy, custom-designed plastic helmets were individually fitted by using methods previously described by Clarren in 1979 and 1981.4,5

RESULTS

We determined the mean and median post-treatment CI for the entire group of 193 infants who were treated with either repositioning or orthotic therapy during infancy (Table I). The initial mean CI at a mean age of 5.3 months for this entire group was 89%, and the mean final CI was reduced to 87% at a mean age of 9.0 months (P < .0001). Among a subgroup of 96 infants treated by repositioning from an average age of 4.6 months to 7.7 months, the mean final CI was 0.86. Among a subgroup of 97 infants treated with helmets from an average age of 6.0 months to 10.3 months, the mean final CI was 0.88. The change in CI for the subgroup of 96 infants treated with repositioning was not significant, whereas the change in CI with helmet therapy was significant (Table I). For infants treated with helmet therapy, treatment at a younger age resulted in more improvement in the CI. Changes in CI with helmet therapy were significant at each age group, but results decreased in magnitude with advancing age (Table II). Within a subgroup of 92 infants with an initial CI at or above 90% (Table III), their initial mean CI of 96.1% at a mean age of 5.2 months was reduced to a mean of 91.9% at a mean age of 8.8 months.

By using “cephalic index” as a search term, we examined the medical and anthropologic literature to determine the CI for children from different cultures to examine how CI varied with different sleeping practices (Table IV).6-18 Generalized linear model analysis comparing the mean CI among cultures with prone sleepers (80%), supine sleepers (84%), and newborn infants (80%) yielded a P value of .0033. Pairwise t tests showed that supine sleepers have a significantly higher mean CI than both prone sleepers and newborn infants, which were not significantly different from each other.

DISCUSSION

In 1912, Franz Boas17 suggested that exposure to the American environment resulted in significant differences in CI between same-age American and European children, though a recent re-analysis of Boas’ data found these differences to not be significant.18 The average CI for this population of 4677 children who slept prone was 79.8%, which is not different from the neonatal CI of 80.0%.6 Brachycephaly refers to a head, which is short in the anteroposterior dimension and wide between the biparietal eminences, with a CI above 81%.1 Comparison of CI between prone-sleeping cultures and supine-sleeping cultures (Table IV) indicates a significantly higher CI in supine-sleeping cultures.

The human infant cranium is highly malleable, and mechanical forces have been used by many cultures to deform

Table I. Initial and final means for age and CI in 193 infants by treatment group

<table>
<thead>
<tr>
<th>No. of infants</th>
<th>Reposition</th>
<th>Helmet</th>
<th>All</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial mean age (mo)</td>
<td>96</td>
<td>6.0</td>
<td>5.3</td>
</tr>
<tr>
<td>Initial mean CI (confidence interval)</td>
<td>86.3% (84.8%–87.9%)</td>
<td>91.5% (89.8%–93.2%)</td>
<td>89.0% (87.8%–90.1%)</td>
</tr>
<tr>
<td>Final mean age (mo)</td>
<td>7.7</td>
<td>10.3</td>
<td>9.0</td>
</tr>
<tr>
<td>Final mean CI (confidence interval)</td>
<td>85.7% (84.3%–87.0%)</td>
<td>88.4% (87.1%–89.6%)</td>
<td>87.0% (86.1%–87.9%)</td>
</tr>
<tr>
<td>Reduction of brachycephaly (confidence interval)</td>
<td>0.7% (0.08%–1.4%)</td>
<td>3.2% (2.3%–4.0%)</td>
<td>1.9% (1.3%–2.5%)</td>
</tr>
<tr>
<td>P value for change in CI</td>
<td>.08</td>
<td>.0001</td>
<td>.0001</td>
</tr>
</tbody>
</table>

Table II. Reductions in CI in helmet-treated infants by age group

<table>
<thead>
<tr>
<th>Age group</th>
<th>N</th>
<th>Reduction in CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>3–4.5 mo</td>
<td>16</td>
<td>5.1%</td>
</tr>
<tr>
<td>4.5–6.0 mo</td>
<td>41</td>
<td>3.2%</td>
</tr>
<tr>
<td>&gt;6 mo</td>
<td>38</td>
<td>2.9%</td>
</tr>
</tbody>
</table>
the shape of an infant’s head, resulting in a wide variety of head shapes (Figure 2). Positioning infants in hard wooden cradles created brachycephalic heads in some Middle Eastern cultures, due to inability of a swaddled infant to reposition itself, combined with constant supine positioning on a firm surface. Thus, CI is heavily influenced by infant sleep position, and constant supine positioning is a frequent cause of deformational brachycephaly during infancy.

The increasing prevalence of brachycephaly among American infants during recent years is a consequence of the success of efforts to prevent SIDS. The “Back to Sleep” campaign was initiated by the American Academy of Pediatrics in June of 1992 with the initial recommendation to place infants to sleep on their sides or backs to prevent SIDS. After 1996, the more stringent recommendation for only supine sleep positioning was made, when it was recognized that some side-sleeping infants were still dying from SIDS after assuming a prone sleeping position during the night. The end result of this public education effort has been a dramatic decline in the prevalence of prone sleeping position from 70% in 1992 to 10.5% in 1997, with a concordant reduction in the rate of SIDS from 2.6 per 1000 in 1986 to 1.0 per 1000 in 1998. When an infant remains in a persistently supine position without the developmental benefits of the head turning from side to side during consistent periods of tummy-time, the occiput becomes progressively flattened through the impact of gravity and persistent occipital mechanical pressure. A 1995 study of 7609 Dutch infants, who were screened for positional preference before age 6 months, revealed that 10% manifested occipital

| Table III. Initial and final means for age and CI in 92 brachycephalic infants (CI ≥ 90%) |
|---------------------------------------------|----------------------------------------|----------------------------------------|
| Reposition                                 | Helmet                                 | All                                    |
| No. of infants                             | Reposition                             | Helmet                                 | All                                    |
| Initial mean age (mo)                      | 37                                     | 55                                     | 92                                     |
| Initial mean CI (confidence interval)      | 94.0% (92.8%–95.0%)                    | 97.5% (96.1%–98.9%)                    | 96.1% (95.1%–97.1%)                    |
| Final mean age (mo)                        | 7.6                                    | 9.6                                    | 8.8                                    |
| Final mean CI (confidence interval)        | 91.4% (90.0%–92.9%)                    | 92.2% (91.2%–93.2%)                    | 91.9% (91.1%–92.7%)                    |
| Reduction of brachycephaly (confidence interval) | 2.5% (1.3%–3.8%)                   | 5.3% (4.3%–6.3%)                       | 4.2% (3.4%–5.0%)                       |
| P value for change in CI                   | .0003                                  | <.0001                                 | <.0001                                 |

<table>
<thead>
<tr>
<th>Table IV. Cephalic index in different populations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Population Group</td>
</tr>
<tr>
<td>Birth measurements</td>
</tr>
<tr>
<td>Italy (108 term infants by CIs)</td>
</tr>
<tr>
<td>India (20 neonates)</td>
</tr>
<tr>
<td>Supine sleepers</td>
</tr>
<tr>
<td>India (20 3-mo infants)</td>
</tr>
<tr>
<td>India (20 6-mo infants)</td>
</tr>
<tr>
<td>India (20 12-mo infants)</td>
</tr>
<tr>
<td>India (20 18-mo children)</td>
</tr>
<tr>
<td>India (20 24-mo children)</td>
</tr>
<tr>
<td>India (20 30-mo children)</td>
</tr>
<tr>
<td>India (20 36-mo children)</td>
</tr>
<tr>
<td>India (20 48-mo children)</td>
</tr>
<tr>
<td>Korea (430 7–10-y-old boys)</td>
</tr>
<tr>
<td>Korea (850 students)</td>
</tr>
<tr>
<td>Japan (Kyushu school girls)</td>
</tr>
<tr>
<td>Japan (62 boys &gt;40 y)</td>
</tr>
<tr>
<td>Japan (62 girls &gt;40 y)</td>
</tr>
<tr>
<td>Japan (Kyushu female adults)</td>
</tr>
<tr>
<td>Pakistan (757 Punjab adults)</td>
</tr>
<tr>
<td>Prone sleepers</td>
</tr>
<tr>
<td>Nigeria (45 1-y-olds)</td>
</tr>
<tr>
<td>Nigeria (50 2-y-olds)</td>
</tr>
<tr>
<td>Nigeria (67 3-y-olds)</td>
</tr>
<tr>
<td>Nigeria (85 4-y-olds)</td>
</tr>
<tr>
<td>USA (98 5–11-y-olds)</td>
</tr>
<tr>
<td>Canada (age 4-y students)</td>
</tr>
<tr>
<td>Canada (age 8-y students)</td>
</tr>
<tr>
<td>Canada (age 12-y students)</td>
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<tr>
<td>Canada (age 16-y students)</td>
</tr>
<tr>
<td>Hawaii (475 white)</td>
</tr>
<tr>
<td>Hawaii (548 Chinese)</td>
</tr>
<tr>
<td>Hawaii (721 Filipino)</td>
</tr>
<tr>
<td>Hawaii (3881 Japanese)</td>
</tr>
<tr>
<td>European/USA (4677 adults)</td>
</tr>
</tbody>
</table>

*Eighty-five percent of Burlington, Ontario 3-year olds (Anglo-Saxon) followed longitudinally.
flattening. Of these infants, 45% showed persistent asymmetric occipital flattening at age 2 to 3 years. An increase in prevalence of deformational posterior plagiocephaly has been documented, and our culture is gradually learning how to prevent this unintended consequence. The development of positional brachycephaly, with or without plagiocephaly, may indicate that parents are not providing their infants with tummy-time.

Cultures who put their infants to sleep in supine position are more brachycephalic CI than cultures who put their infants to sleep in prone position. The CI was 80% for 108 term neonates delivered by cesarean section, which is similar to that of prone-sleeping cultures. The CI during infancy in India (where infants sleep supine) is higher than later childhood. Of note, child-rearing practices in India promote frequent tummy-time when the infant is awake and under observation. Prone-sleeping cultures have a normocephalic CI (mean CI = 80%, with a range from 76% to 81%), whereas school children in Japan and Korea (supine-sleeping cultures) are brachycephalic (85% to 91%).

With the continued success of the “Back to Sleep Campaign,” infants will have a more rounded head shape than cultures that previously put their infants to sleep on their stomachs. The current normative CI is 86% to 88%, and it is relatively rare to encounter a dolichocephalic infant in cultures whose infants sleep supine. Parents who become concerned about their infant’s brachycephaly from prolonged supine positioning sometimes position their infants in a side-sleeping position, which is a dangerous sleep position for SIDS in an infant who has had little or no tummy-time. Infants who have no periods of consistent tummy-time become very distressed when placed in prone position, and their parents readily agree that their infants have never tolerated being on their tummies. The best management is to institute regular periods of tummy-time from early infancy while the infant is awake and under direct observation.

One prospective study from 1998 through 1999 randomized the treatment of 74 infants with positional plagiocephaly between repositioning (45 infants) and cranial orthotic therapy (29 infants). Some infants were initially repositioned and then later put into helmets when they failed to respond; their subsequent responses to helmets were included among the helmeted group. Outcomes were similar in both groups, but repositioning took 3 times as long as helmet therapy, and the groups were too small for statistical analysis. Cranial orthotic therapy has proven to be effective in correcting deformational posterior plagiocephaly, and it has also been used to correct brachycephaly. With extreme occipital flattening, a helmet can often be difficult to fit or may require prolonged treatment; thus, prevention is of paramount importance. At the first sign of occipital flattening, repositioning and tummy-time should be promptly initiated to correct brachycephaly, and routine use of these practices during the first 6 weeks and thereafter should prevent this deformity. For those infants who do not make progress with repositioning and tummy-time, with severe persistent brachycephaly (CI > 90%) at age 5 months, use of an orthotic helmet will correct the brachycephaly (Figure 1) and any associated plagiocephaly (Figure 3). Reassurance is appropriate for those infants with CI < 90 at age 5 months, since brachycephaly is unlikely to develop or worsen after this age. Limitations to this study (and all previous studies of infant head deformation) include the lack of long-term observations into later childhood. There are also no natural history studies of positional brachycephaly in supine-sleeping infants, which extend beyond age 2 to 3 years. Anthropologic data were analyzed in the absence of such naturalistic observations. It is important to distinguish deformational brachycephaly from craniosynostosis, since therapy and treatment are very different for each condition. Brachycephaly may also be associated with various syndromes that affect the pliability of the infant’s skull or an infant’s tendency to remain recumbent for prolonged periods of time. Thus, conditions associated with skull demineralization, such as osteogenesis imperfecta or hypophosphatasia, can lead to severe deformational brachycephaly because the cranium is demineralized. Conditions resulting in prolonged hypotonia, such as Down syndrome, will also result in brachycephaly, as will conditions associated with limited neck mobility, such as Klippel-Feil sequence. Infants with macrocephaly are at increased risk for positional head deformity, especially when the macrocephaly is accompanied by hypotonia. In all of these circumstances, variation of the infant’s supine sleeping position from one side of the occiput to the other, along with tummy-time, can prevent the need for further corrective therapy.

REFERENCES

MANAGEMENT OF DEFORMATIONAL PLAGIOCEPHALY: REPOSITIONING VERSUS ORTHOTIC THERAPY

JOHN M. GRAHAM, JR, MD, SCD, MAYELA GOMEZ, BS, ANDY HALBERG, BS, DAWN L. EARL, CPNP, MSN, JEANNE T. KREUTZMAN, CPNP, MSN, JINRUI CUI, MS, AND XIUQING GUO, PhD

Objectives We compare positioning with orthotic therapy in 298 consecutive infants referred for correction of head asymmetry.

Study design We evaluated 176 infants treated with repositioning, 159 treated with helmets, and 37 treated with initial repositioning followed by helmet therapy when treatment failed. We compared reductions in diagonal difference (RDD) between repositioning and cranial orthotic therapy. Helmets were routinely used for infants older than 6 months with DD >1 cm.

Results For infants treated with repositioning at a mean age of 4.8 months, the mean RDD was 0.55 cm (from an initial mean DD of 1.05 cm). For infants treated with cranial orthotics at a mean age of 6.6 months, the mean RDD was 0.71 cm (from an initial mean DD of 1.13 cm).

Conclusions Infants treated with orthotics were older and required a longer length of treatment (4.2 vs 3.5 months). Infants treated with orthosis had a mean final DD closer to the DD in unaffected infants (0.3 ± 0.1 cm), orthotic therapy was more effective than repositioning (61% decrease versus 52% decrease in DD), and early orthosis was significantly more effective than later orthosis (65% decrease versus 51% decrease in DD). (J Pediatr 2005;146:258-62)

Between 1981 and 1991, epidemiologic studies showed a strong association between infants sleeping on their stomachs and death from sudden infant death syndrome (SIDS). In 1992, infants who slept in prone position had as much as an 11.7 times higher risk for SIDS, and it was recommended that infants be positioned on their backs for sleep, except in cases of prematurity, gastroesophageal reflux, or obstructive sleep apnea. This decreased the prevalence of prone infant sleeping from 70% in 1992 to 10.5% in 1997 and decreased the incidence of SIDS from 2.6 per 1000 in 1986 to 1.0 per 1000 in 1998.

In 1974, plagiocephaly occurred once in every 300 live births among prone-sleeping infants. After the “Back to Sleep” campaign was initiated, the frequency of plagiocephaly increased to 1 in 60 in 1996. Among 7609 Dutch infants screened for positional plagiocephaly before the age of 6 months, the incidence of plagiocephaly was 8.2%, with brachycephaly noted in 10%. Deformity persisted in nearly one third when reexamined at age 2 to 3 years.

Among 1086 Hong Kong infants with congenital torticollis, 91% of infants improved when treated with manual stretching, and craniofacial asymmetry also resolved. Plagiocephaly has been treated with either early physical therapy and repositioning or helmet therapy, but no studies compared outcomes from both approaches with large enough numbers to provide evidence-based guidelines for treatment. Most authors agree that if there is little improvement in head shape in young infants being treated with repositioning and physical therapy, orthotic therapy should be initiated while there is still enough residual head growth to allow for correction. We compared repositioning with helmet therapy, demonstrating that both techniques work when used appropriately with neck physical therapy.

DD Diagonal differences
RDD Reductions in diagonal difference
SIDS Sudden infant death syndrome

See related article, p 253.
METHODS

Longitudinal data were collected on 298 consecutive normal infants, who were referred and treated for plagiocephaly at Cedars-Sinai Medical Center between January 1, 1994, and December 31, 2001. Cranial diagonal differences (DD) were compared before and after treatment. Institutional Review Board review was obtained to use anonymized patient care data. We compared size at birth and at the last treatment visit between the two sexes. Cranial diagonal measurements were taken by anthropometric metal cranial calipers (B. Braun Medical Products, Aesculap Division, Tuttingen, Germany), as shown in the diagram in Figure 1. Each measurement was taken 3 times and averaged by one of two pediatric nurse practitioners. There were not significant differences in measurement between practitioners when measuring the same patients. The normal DD in 36 healthy infants measured between 4 and 12 months (average age, 6.8 months) was 0.3 ± 0.1 cm, and this was considered to be our target DD. Since this was a clinical study of outcomes in infants referred for plagiocephaly, we routinely recommended helmets for all infants older than 6 months with a DD greater than 1 cm, since this was the previous standard of care.12,13 For infants referred at 4 months or younger, we began treatment with repositioning. For patients between 4 and 6 months of age, both treatment options were offered, and parents chose one option and were followed at monthly intervals to monitor progress and encourage compliance. Neonates who were referred for torticollis were treated with physical therapy and did not develop plagiocephaly, and they were not included in this study. Most infants referred for plagiocephaly were too old to detect sternomastoid tumors, and the presence or absence of such tumors was not routinely documented. All infants with plagiocephaly had some associated torticollis, and they were treated with physical therapy and followed at monthly intervals. Patients treated initially with physical therapy and repositioning, who failed to reduce their DD to less than 1.0 cm by 7.4 months, were treated with helmets. Their data were included in each treatment group for the period of time spent in each treatment modality. Plastic cranial orthotic helmets were fitted by using methods previously described by Clarren.12,13 Statistical t tests, using the SAS software package, were used to compare the effect of repositioning versus helmet therapy.

RESULTS

The mean age of 298 infants treated for plagiocephaly was 5.4 months; the mean length of treatment was 4.3 months; 70% were boys; 68% were left-sided. Size differences between boys and girls were not significant (Table I). Among 176 infants treated with repositioning, the mean RDD was 0.55 cm (±0.33), from a starting mean DD of 1.05 cm at 4.8 months. In 159 infants treated with helmets, the mean RDD was 0.71 cm (±0.36), from a mean starting DD of 1.13 cm at 6.6 months. The final DD for repositioning was 0.50 cm (±0.37), versus 0.42 cm (±0.28) for orthotic therapy (Table II). Despite no significant difference in starting DD, the final mean DD in the helmeted group (0.42 cm) was not significantly different from the target DD (0.30 ± 0.1 cm). The mean final DD for the repositioned group (0.50 cm) was significantly more asymmetric. The mean percentage decrease for the orthotic group (61%) was significantly greater than the mean percentage decrease for the repositioning group (52%). Severe cases that presented early were treated with initial repositioning, followed by helmet therapy if they still had a 1.0 cm DD at an average age of 7.3 months. This group reached a mean final DD of 0.40 cm after a mean of 5.1 months (1.94 months for repositioning and 3.14 months for helmet). Among 37 infants who failed initial treatment with repositioning, their initial mean DD was 1.46 cm when treatment started at mean age 5.06 months, with a mean RDD of 0.56 by mean age 7.4 months, at

Table I. Treatment group characteristics (mean ± SD) by sex

<table>
<thead>
<tr>
<th></th>
<th>Boys</th>
<th>Girls</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight (g)</td>
<td>3272 ± 694</td>
<td>3289 ± 724</td>
</tr>
<tr>
<td>Birth length (cm)</td>
<td>48 ± 4.5</td>
<td>48.5 ± 4.8</td>
</tr>
<tr>
<td>Weight at last visit (g)</td>
<td>9130 ± 1681</td>
<td>8516 ± 1990</td>
</tr>
<tr>
<td>Length at last visit (cm)</td>
<td>70.3 ± 6.6</td>
<td>69.1 ± 8.8</td>
</tr>
<tr>
<td>Age at last visit (mo)</td>
<td>8.2 ± 3.2</td>
<td>9.0 ± 4.8</td>
</tr>
</tbody>
</table>

Figure 1. This infant shows all key features of torticollis-plagiocephaly deformation sequence, with cranial measurements depicted on top of his skull, before initiation of helmet therapy at age 5 months.
which time helmet therapy was begun for another 2.7 months before reaching the target DD of 0.40 cm. Their improvement with repositioning was no different from our repositioning group, and parents were compliant, but the initial degree of plagiocephaly in this group was too severe to correct with repositioning alone.

To evaluate the effect of age on helmet treatment, we compared outcomes in 44 children who started treatment at an age of 8 months or greater with outcomes in 115 infants who started treatment before this age (Table III). The mean starting age in these younger infants was 5.8 months, with an initial DD of 1.14 cm. After a mean RDD of 0.76 cm (±0.36), the final mean DD was 0.37 cm (±0.22) after a mean treatment time of 4.4 months. The mean age of helmet initiation in older infants was 8.6 months, with a mean starting DD of 1.10 cm and a mean final DD of 0.51 cm (±0.28) after a mean of 3.73 months in treatment. In older infants, the mean final DD was larger, with a smaller RDD, and the percentage decrease in DD was significantly less (51% vs 65%).

### DISCUSSION

Before 1992, anterior deformational plagiocephaly predominated when infants slept on their stomachs. Among supine-sleeping infants with torticollis, one side of their occiput (more commonly the right side) becomes flattened, resulting in posterior deformational plagiocephaly. The incidence of torticollis was 1.3% among 250,000 Hong Kong infants followed in a prospective study of 1086 patients in infancy between affected boys and girls (Table I), suggesting that sex differences in the occurrence of torticollis may relate to some other factor, such as hormonal differences. Testosterone may accentuate muscular action in male fetuses, and relaxing hormones may affect female connective tissues. Girls may be protected from torticollis for the same reasons that make them more susceptible to developmental dysplasia of the hip.

Torticollis is more frequent in multiple births and usually affects the bottom fetus in vertex presentation. Findings that help to differentiate deformational plagiocephaly from craniosynostosis include forward ear placement, prominent mandibular sulcus with mandibular tilt, uplifted lower helix and smaller ear on the side of the torticollis, and unilateral epicanthal fold in cases with deformational plagiocephaly. Early signs of torticollis include an eye on the side of the torticollis that appears less open due to vertical displacement of the soft tissues of the cheek from pressure on the mandible (Figure 1). There may also be difficulty nursing from both breasts due to inability to turn the neck equally to both sides.

Early supervised tummy-time promotes prone motor skill development as well as neck range of motion. Prone positioning corrects positional preference, since positional preference was documented in only 2.4% of prone-sleeping Swedish infants, versus 19% of children who slept in supine position. Before the “Back to Sleep” campaign, many infants self-treated their own postural torticollis by turning their heads from side to side while sleeping in the prone position, and encouragement of tummy time has the same effect.

In a prospective study of 114 infants treated with either head repositioning (63 infants) or helmet therapy (51 infants), cranial symmetry was significantly better in infants treated with helmets than in those treated with positioning. Of 98 patients with positional head deformation, 3 had severe and progressive deformation requiring surgery, and the rest were successfully treated with changes in sleeping position or helmet therapy. Many infants with positional plagiocephaly will improve with repositioning and neck physical therapy, but severe cases may require helmet therapy to achieve adequate resolution.

### Table II. Mean values (SD) by treatment group

<table>
<thead>
<tr>
<th></th>
<th>Repositioning</th>
<th>Helmet</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>N</td>
<td>176</td>
<td>159</td>
<td></td>
</tr>
<tr>
<td>Starting DD (cm)</td>
<td>1.05 (0.45)</td>
<td>1.13 (0.38)</td>
<td>.076</td>
</tr>
<tr>
<td>Starting age (mo)</td>
<td>4.8 (1.7)</td>
<td>6.6 (1.7)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Final DD (cm)</td>
<td>0.5 (0.32)</td>
<td>0.42 (0.42)</td>
<td>.007</td>
</tr>
<tr>
<td>Final age (mo)</td>
<td>8.3 (3.7)</td>
<td>10.9 (2.7)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>RDD (cm)</td>
<td>0.55 (0.33)</td>
<td>0.71 (0.36)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>RDD/staring DD</td>
<td>0.52 (0.22)</td>
<td>0.61 (0.20)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Length of therapy (mo)</td>
<td>3.5 (3.5)</td>
<td>4.2 (2.2)</td>
<td>.024</td>
</tr>
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</table>

### Table III. Mean values (SD) for helmet therapy babies by age group

<table>
<thead>
<tr>
<th></th>
<th>Helmet before age 8 mo</th>
<th>Helmet at age 8 mo or older</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>115</td>
<td>44</td>
</tr>
<tr>
<td>Starting DD (cm)</td>
<td>1.14 (0.37)</td>
<td>1.10 (0.39)</td>
</tr>
<tr>
<td>Starting age (mo)</td>
<td>5.8 (1.1)</td>
<td>8.6 (1.3)</td>
</tr>
<tr>
<td>Final DD (cm)</td>
<td>0.38 (0.22)</td>
<td>0.51 (0.28)</td>
</tr>
<tr>
<td>Final age (mo)</td>
<td>10.3 (2.5)</td>
<td>12.4 (2.8)</td>
</tr>
<tr>
<td>RDD* (cm)</td>
<td>0.76 (0.36)</td>
<td>0.58 (0.33)</td>
</tr>
<tr>
<td>RDD/staring DD</td>
<td>0.65 (0.19)</td>
<td>0.51 (0.20)</td>
</tr>
<tr>
<td>Length of therapy (mo)</td>
<td>4.4 (2.4)</td>
<td>3.7 (1.7)</td>
</tr>
</tbody>
</table>

*Significant difference between two groups (P = .004 for RDD and .0001 for RDD/staring DD).
Among our 298 patients, the average length of treatment with helmet orthosis was 4.2 months for infants who averaged 6.6 months of age at the initiation of treatment. Younger infants with mild-moderate plagiocephaly were successfully treated with repositioning and physical therapy (Figure 2), and earlier treatment was significantly more successful than later treatment. There is no evidence that treatment after age 12 months of age provides significant benefit.11,20-24 Orthotic treatment does not restrict cranial growth but rather redirects subsequent cranial growth into a symmetric shape.24 Positional plagiocephaly can have permanent effects, as demonstrated by purposeful artificial cranial deformation in many primitive cultures during the first 6 to 8 months of life.25 Clinical reports26,27 confirm that altered head shapes can persist throughout life, and failure to treat muscular torticollis results in persistent facial asymmetry, cervical scoliosis, and persistent deformational plagiocephaly.26,27

Moss9 reported on 66 infants with mild to moderate plagiocephaly (defined as cranial vault asymmetry of 1.2 cm or less) who were treated with head repositioning and physical therapy. Cranial vault asymmetry was decreased by an average of 0.45 cm over an average of 4.5 months. These results are similar to those obtained by Ripley et al (1994),10 who reduced cranial asymmetry by an average of 0.49 cm in 46 infants treated with orthotic helmets for an average of 4.3 months. Pollack et al11 (1997) studied 69 infants with plagiocephaly (35 infants treated with repositioning alone and 34 infants who failed repositioning and required orthotic therapy). Both groups ultimately achieved normal head shapes, except for 5 infants who were older than 12 months when treatment was initiated. They recommended that if little improvement results from 6 to 8 weeks of repositioning in young infants receiving physical therapy for congenital muscular torticollis, then cranial orthotic therapy should be considered while there is still enough residual rapid head growth to allow for timely correction.11

We collected longitudinal data on 298 otherwise normal infants treated between 1994 and 2001. Cranial diagonal diameters were measured before and after treatment (Figure 1). All parents were instructed to provide neck physical therapy, and infants with a diagonal difference greater than 1.0 cm at 6 months of age were corrected with helmet orthoses. The effectiveness of treatment was measured by the reduction in diagonal difference (RDD). The normal diagonal difference in healthy infants measured between 4 and 12 months (average age, 6.8 months) is 0.3 ± 0.1 cm. Correction of the head shape, documented by a diagonal difference within this normal range, took an average of 4 to 5 months (longer in older or more severe cases).

For infants treated with repositioning, the mean RDD was 0.55 cm (from an initial mean DD of 1.05 cm), which is consistent with previous studies.22 For infants treated with cranial orthosis, the mean RDD was 0.71 (from an initial mean DD of 1.13 cm). Repositioning resulted in a 52% decrease in DD, whereas orthotic therapy resulted in a 61% decrease. Though these changes appear small in magnitude, they were statistically significant (Table II). Treatment with a helmet after age 10 months was not adequately evaluated. One case treated at almost 10 months had a reduction in diagonal difference of 0.9 cm, with a final diagonal difference of 0.3 cm after 7 months in helmet therapy. Another case treated at 15 months for 9 months had a 0.5-cm reduction in diagonal difference, reducing his starting diagonal difference from 2.0 to 1.5 cm. Orthotic treatment after age 12 months shows little promise for significant correction, and significant asymmetry can persist, but with consistent reshaping techniques during early infancy, a more symmetric head shape can be achieved.

Positioning devices that allow the infant to be positioned in a three-quarters turn, with the head resting on the occipital prominence, can be effective. With consistent repositioning, the bald spot migrates from the previously flattened site toward the occipital prominence (Figure 2). It is important to warn the parents not to simply position the infant on the side of his/her head to prevent further occipital flattening. This does not correct cranial asymmetry and may be a dangerous sleeping position if the infant turns over and becomes prone. If initiated by 3 months, conservative treatment of torticollis with physical therapy is very effective, resulting in full passive range of motion and no facial asymmetry in more than 90% of compliant families.8,15 The more severe the neck restriction, the longer the required duration of physical therapy to achieve full range of motion.8,15

Limitations to this study include lack of follow-up beyond the first year of life, lack of random assignment into treatment groups, and inability to quantify how the impact of small, statistically significant differences in outcome might relate to parental perceptions of cosmetic significance. Despite these limitations, we recommend that pediatricians attempt to identify infants with torticollis during early infancy and treat

Figure 2. This girl with torticollis-plagiocephaly deformation sequence was treated entirely with neck physical therapy and repositioning, using an infant positioning device between ages 3 and 9 months. She is shown here part way through her therapy in a three-quarters turn, resting on her left occiput, with her bald spot shifted from the right previously flattened occiput to the right occiput.
REFERENCES

TOXIC CLONIDINE INGESTION IN CHILDREN

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Objectives  We performed a prospective case series to seek dosage or clinical parameters to better identify patients who need direct medical evaluation.

Study design  All clonidine ingestions in children younger than 12 years of age reported to 6 poison centers were followed for a minimum of 24 hours. Exclusion criterion was polydrug ingestion.

Results  The study included 113 patients, of whom 63 were male. Mean age was 3.8 years (±2.4 SD). Clinical effects were common, but severe adverse effects occurred in <10% of patients. The dose ingested was reported for 90 patients (80%); 61 (68%) children ingested <0.3 mg and none had coma, respiratory depression, or hypotension. The lowest dose ingested by history with coma and respiratory depression was 0.3 mg (0.015 mg/kg). Prior clonidine therapy did not affect outcome. Onset of full clinical effects in all cases was complete within 4 hours of ingestion.

Conclusions  We recommend direct medical evaluation for (1) all children 4 years of age and younger with unintentional clonidine ingestion of ≥0.1 mg, (2) ingestion of >0.2 mg in children 5 to 8 years of age, and (3) ingestion of ≥0.4 mg in children older than 8 years of age. Observation for 4 hours may be sufficient to detect patients who will develop severe effects. (J Pediatr 2005;146:263-6)

Clonidine poisoning in children is a common event, with more than 1600 cases reported annually to poison centers in recent years. Of these, >50% were in children younger than 6 years of age. The majority of these ingestions (77%) resulted in minimal or no effects. Despite this, 84% were seen in a hospital emergency department and 50% were admitted overnight. The reason for this cautious approach is that clonidine has a very narrow therapeutic window. Reported clinical effects from overdose have included hypertension, hypotension, hypothermia, bradycardia, tachycardia, coma, respiratory depression, apnea, generalized hypotonia, hyporeflexia, and miosis. Death after overdose is rare, with only a single death reported in one study of more than 10,000 overdoses. The majority of publications on clonidine overdose are anecdotal reports. Most published studies are retrospective and provide no data on dosage response, toxicodynamic response in overdose, or other critical areas that might help guide the clinician. Review of the published case reports shows that prominent onset of adverse clinical effects in the severe cases was rapid, usually in <1 hour. However, the use of case reports as the sole source for decision-making can add significant bias. It is unclear if this rapid onset occurs in all cases and could be relied on as a diagnostic aid. The lack of a large study clarifying these issues leaves no clear guidance as to which, if any, cases can be managed outside of a hospital. Additionally, there is no clear guidance concerning those cases seen in the emergency department: which can be safely discharged after a short observation period and which need to be admitted for treatment or a longer observation period. We performed a prospective case series to evaluate if there were dosage or clinical data to better identify those patients who need direct medical evaluation from those who can be observed at home.

METHODS

We conducted a prospective case series of all patients with clonidine ingestion reported to participating poison centers from September 1, 2002, to April 30, 2003. Entrance criteria included clonidine ingestion, age 12 years or younger, and follow-up to a known outcome (minimum, 24 hours). Exclusion criterion was polypharmacy ingestion.
Each case was identified at the time of the initial contact with the poison center. A separate data collection sheet recording demographics, dose, neurologic, and hemodynamic changes was initiated. Definition of clinical effects used in this study are provided in Table I. The study was approved by the human studies committee of the appropriate institution for each participating poison center. To evaluate clinical effects, dose, and treatments by age, study subjects were categorized into 3 groups: 0 to 4 years, 5 to 8 years, and 9 to 12 years of age. Statistics were descriptive and, for analysis of significance, $\chi^2$ and Mann-Whitney tests were performed.

### RESULTS

The study population included 113 patients of whom 63 (56%) were male. The mean age of patients was 3.8 years (±2.4 SD), with a range of 6 months to 11 years. Ninety-seven patients (86%) were evaluated in a health care facility (HCF), and 63 of these children were admitted for medical care or observation.

The clinical effects reported are listed in Tables II and III. Clinical effects were common, and serious clinical effects resulting invasive measures such as intubation or pressors were infrequent but were reported more frequently in the very young children. Onset of full symptoms was complete within 4 hours of ingestion in all cases, with a mean time to maximum level of central nervous system depression of 1.7 hours (range, 1 to 3 hours).

The dose ingested was known for 90 patients (80%); 61 (68%) children ingested <0.3 mg, and none of these had coma, respiratory depression, or hypotension. The lowest dose ingested with coma and respiratory depression was 0.3 mg (0.015 mg/kg). This occurred in a 4-year-old, 20-kg boy with a history of cerebral palsy. He had a witnessed ingestion of 3 tablets of 0.1 mg of his own medication. Two hours before this, the child had been given his morning dose of 0.075 mg. The child’s medication schedule was 0.075 mg in the morning and 1 PM and 0.1 mg at hour of sleep, for a total of 0.25 mg/d. Within 20 minutes, the child was reported to be deeply somnolent and was transported to the hospital by emergency medical services. In the emergency department, the child was unarousable to voice and was intubated and placed on mechanical ventilation due to periods of apnea. His heart rate (HR) was 110 bpm and his blood pressure (BP) was 130/86 mm Hg. The HR decreased to 54 bpm within 3 hours of ingestion and BP decreased to 92/44 mm Hg. No specific therapy beyond mechanical ventilation and intravenous fluids was instituted. The child was extubated at 20 hours after ingestion and discharged after 48 hours.

In the 5- to 8-year-old group, one 5-year-old girl received intubation, mechanical ventilation, atropine, and fluid challenge after ingesting an unknown amount of 0.1 mg tablets from an open bottle of her own medication. The single child in the 9- to 12-year-old group with symptoms requiring intervention was a 10-year-old boy who was reportedly given 30 of his own 0.1 mg tablets by a grandparent who misunderstood directions while babysitting. The boy received a fluid challenge for a BP of 84/30 mm Hg with an HR of 80 bpm and was discharged after 24 hours of observation without further problems. The remaining children in this age group ingested between 0.2 and 0.5 mg, with only drowsiness or lethargy reported.

### Table I. Definitions of signs and clinical features

<table>
<thead>
<tr>
<th>Sign or clinical feature</th>
<th>Age 0 to 4 y</th>
<th>Age 5 to 12 y</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypotension</td>
<td>Systolic &lt;80 mm Hg</td>
<td>Systolic &lt;80 mm Hg</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>Heart rate &lt;75 beats per minute</td>
<td>Heart rate &lt;60 beats per minute</td>
</tr>
<tr>
<td>Coma</td>
<td>Unresponsive to verbal stimuli</td>
<td>Unresponsive to verbal stimuli</td>
</tr>
<tr>
<td>Hypertension</td>
<td>Systolic &gt;110 or diastolic &gt;75 mm Hg</td>
<td>Systolic &gt;120 or diastolic &gt;80 mm Hg</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>Temperature &lt;35.5°C</td>
<td>Temperature &lt;35.5°C</td>
</tr>
</tbody>
</table>

### Table II. Clinical effects of clonidine by age group

<table>
<thead>
<tr>
<th>Age 0 to 4 y (percent of age group)</th>
<th>Age 5 to 8 y (percent of age group)</th>
<th>Age 9 to 12 y (percent of age group)</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>All patients in age group</td>
<td>81</td>
<td>25</td>
<td>7</td>
</tr>
<tr>
<td>Drowsiness/lethargy</td>
<td>61 (75%)</td>
<td>16 (64%)</td>
<td>4 (57%)</td>
</tr>
<tr>
<td>Bradycardia</td>
<td>10 (14%)</td>
<td>2 (9%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Hypotension</td>
<td>7 (9%)</td>
<td>2 (8%)</td>
<td>1 (14%)</td>
</tr>
<tr>
<td>Respiratory depression with</td>
<td>7 (9%)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>mechanical ventilation</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Coma</td>
<td>6 (9%)</td>
<td>1 (4%)</td>
<td>0</td>
</tr>
<tr>
<td>Hypertension</td>
<td>4 (5%)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Hypothermia</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>
In 43 patients (39%), the ingestions involved their own medicine. Unintentional misdosing involving inadvertent double doses occurred in 17 patients (15%). This occurred in 4 patients 4 years old or younger, 9 patients 5 to 8 years old, and 4 patients 9 to 12 years old. Lethargy was reported in all patients 4 years old or younger and 5 to 8 years old, but no other symptoms were reported. In the 9- to 12-year-old patients, 2 reported no symptoms and 2 reported drowsiness. Prior clonidine therapy did not affect outcome, incidence of coma, hypotension, or respiratory depression ($P > .05$) (Table IV). However, dose ingested had a significant impact on outcome and clinical effects reported (Table V).

**DISCUSSION**

Clonidine is a recommended therapy for the treatment of attention deficit hyperactive disorder in children.\(^{10,11}\) As a result of this use in the pediatric population, there is widespread availability of clonidine to children, and frequent unintentional overdoses continue to occur.\(^1,9\) Despite this, there are limited data on outcomes of these sometimes low-dose exposures, with the majority of information derived from retrospective reviews of hospitalized patients. Our study design (prospective case series) allows for better capture of data regarding clinical effects, dose, and therapies. Severe symptoms resulting in invasive procedures were infrequent, with no fatalities (<10%, Table I). This finding is similar to previous reports. In 5 retrospective case series of children hospitalized for clonidine ingestion, intubation and mechanical ventilation were required in 8% to 12.7% of children.\(^9,12-15\) There were no fatalities in the total of 177 children in these cases series. In fact, only 2 fatalities, both in children, have been reported in the medical literature from isolated clonidine ingestion.\(^1,16\)

A concern over the risk of severe symptoms, such as respiratory depression or hypotension, was most likely responsible for the large number of cases evaluated in the emergency department in this series. Our data suggest that ingestion of 0.1 mg or 0.2 mg does not warrant direct medical evaluation in the emergency department in all children. This is consistent with previous reports, in which specific doses were reported. Fiser et al\(^{14}\) suggested in a review of 11 patients that toddlers with ingestion of <0.01 mg/kg had only mild symptoms, which is less than 2 times the recommended dosage. With ingestion of 0.01 to 0.02 mg/kg (2 to 4 times maximum dosage), bradycardia and hypotension tended to occur, and with ingestion of >0.02 mg/kg, patients were at risk for respiratory depression.\(^{14}\) However, the number of patients in our series and previous series in which doses were specifically reported may have been too small to catch the rare case of significant symptoms in a low-dose ingestion. Wedin et al\(^2\) reported a case of a 3-year-old boy (weight not reported) with hypotension who received a dopamine infusion after ingestion of a single 0.2 mg tablet. Intubation was not necessary, and the symptoms resolved approximately 10 hours after ingestion.

Unintentional misdosing involving inadvertent double doses occurred in 17 patients (15%) in our case series. Such cases present the clinician with a dilemma of whether the...
patient requires direct medical evaluation in the emergency department or can be treated safely outside of the hospital setting. Although the number of patients in this group is small, the data suggest that an inadvertent double dose does not put the child at risk for significant symptoms beyond the expected drowsiness.

At this time, it seems prudent to have all children 4 years old or younger to receive direct medical evaluation with unintentional clonidine ingestions of 2 times a therapeutic dose (0.005 mg/kg) or greater. Children 5 to 8 years old with ingestion of >0.2 mg may require direct medical evaluation. In children older than 8 years of age, >0.4 mg may require direct medical evaluation.

All patients in the series recovered fully with observation and/or supportive therapy. This is an experience similar to previous reports. Romano et al reported a documented overdose of >2800 mg/kg in a 5-year-old boy treated with supportive care and naloxone. Similar favorable outcomes with supportive therapy have occurred in adults with inadvertent 1000-fold overdoses. Finally, in our case series, all clinical effects were evident rapidly after ingestion, with a mean of 1.7 hours. This is consistent with the findings of Wiley et al in a case series of 47 patients.

Study limitations include the small number of patients, especially in the older age groups, and the possibility that in some cases dose information was inaccurate. Laboratory evaluation of plasma clonidine concentrations to confirm the history and dose are not available because therapeutic drug monitoring of clonidine concentrations is not routine. Since calls to poison centers are voluntary, sampling bias is possible. It is not possible to ascertain how cases not reported to poison centers differ from those reported to poison centers.

Table V. Mean dose ingested of patients with and without major symptoms

<table>
<thead>
<tr>
<th>Symptom</th>
<th>Mean (±SD) and median reported dose with clinical effect</th>
<th>Mean (±SD) and median reported dose without clinical effect</th>
<th>Two-sample test (Mann-Whitney)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Coma</td>
<td>1.2 mg (±0.59), 1.45 mg</td>
<td>0.25 mg (±0.21), 0.2 mg</td>
<td>( P &lt; .05 )</td>
</tr>
<tr>
<td>Respiratory depression</td>
<td>0.94 mg (±0.54), 0.8 mg</td>
<td>0.25 mg (±0.24), 0.2 mg</td>
<td>( P &lt; .05 )</td>
</tr>
<tr>
<td>Hypotension</td>
<td>0.66 mg (±0.64), 0.43</td>
<td>0.26 mg (±0.26), 0.2 mg</td>
<td>( P = .14 )</td>
</tr>
</tbody>
</table>

REFERENCES
A previously well 1-year-old boy, who was being treated with isoniazid for a latent tuberculosis infection diagnosed at 10 months of age, was admitted with a 10-day history of fever without a source.

HISTORY OF PRESENTING ILLNESS

The pediatrician had diagnosed right otitis media and started the patient on amoxicillin, but he continued to have fevers between 103°F and 104°F. Seven days before admission, in his pediatrician’s office, he was found to have an elevated white blood cell count (Table) and was given an intramuscular dose of ceftriaxone and sent home. Five days before admission, he was seen in a local emergency room for fever. At that time, his leukocytosis persisted (Table) and a chest radiograph revealed no infiltrates. A blood culture drawn that day was subsequently reported to be negative. The patient was given another intramuscular dose of ceftriaxone and discharged home.

Three days before admission, a pediatric infectious disease evaluation noted nonproductive cough and mildly decreased appetite, but there was no nasal congestion, decreased urine output, vomiting, or diarrhea. He had no weight loss and there was no history of recent travel, but he had a 50-year-old uncle who was being treated for pneumonia. Blood drawn that day revealed 31,300 white blood cells/mm³ (Table). Blood and urine cultures as well as titers for Epstein-Barr virus antibodies were negative.

One day before admission, he was seen in the emergency room for persistent fever. He was diagnosed with drug fever secondary to isoniazid and was sent home with instructions to discontinue the medication. The next day his fever spiked at 106.1°F with shaking chills, and he was sent to our hospital for admission.

MEDICAL HISTORY

He was born at term by vaginal delivery with no complications. The patient’s medical history was only significant for a positive PPD (tuberculin-purified protein derivative) of 10 mm induration placed at 9 months of age during a routine well baby check. A chest radiograph taken at that time was normal. The PPD was repeated at 10 months of age and was positive at 23-mm induration. He was diagnosed with latent tuberculosis infection and started on isoniazid. Family history was negative for exposure to tuberculosis or active tuberculosis infection. He did not have prior hospitalizations or other illnesses. He is an only child cared for at home by his parents and grandparents, who were all said to be well. His growth and developmental history were normal.

PHYSICAL EXAMINATION

On presentation to the emergency room, his temperature was 105.7°F, pulse was 190 bpm, blood pressure was 86/54 mm Hg, respiratory rate was 26 per minute, and oxygen saturation was 100% on room air. He was alert and irritable but consolable. His right tympanic membrane was mildly erythematous. Otherwise, the remainder of his examination was normal: There were no rashes, no nuchal rigidity, no lymphadenopathy, no murmurs, no crackles on lung auscultation, no retraction, and no hepatosplenomegaly or masses palpated in the abdomen.

HOSPITAL COURSE

A full workup for sepsis was performed, and intravenous ceftriaxone and vancomycin were started. Cerebrospinal fluid analysis revealed 14 white blood cells/mm³ with no
On hospital day 3, his fever persisted despite intravenous antibiotics and around-the-clock antipyretics. Blood and cerebrospinal fluid cultures were negative for growth. Computerized axial tomography (CT scan) of the abdomen revealed hepatomegaly, and the CT scan of the chest revealed multiple bilateral pulmonary nodules, mediastinal lymph nodes, and a mass that extended from the hilum to the mediastinum and the left lung apex (Figure 1, A and B). The persistently elevated white cell counts and the high fever led to this workup of our patient for fever of unknown origin. With the significant CT scan findings, the differential diagnosis included pulmonary tuberculosis, fungal infection, primary immunodeficiency with an opportunistic infection, and lymphoproliferative disease.

Three early morning gastric aspirates were obtained, and both cultures and stains were negative for tuberculosis. A bone marrow aspiration was performed and was normal. The following day, the patient underwent a left-sided thoracotomy with mediastinal lymph node and left upper lobe wedge biopsies. The pathology revealed a necrotizing granulomatous inflammation (Figure 2) with lipid-laden macrophages. Periodic acid–Schiff stain on the tissue showed the presence of polysaccharides. Tissue stains for fungi and acid-fast bacilli were negative and tissue stains for bacteria were noncontributory. Immunohistochemical stains for B-lymphocytes (L26) and T-lymphocytes (CD3) were positive, suggesting a mixed population and therefore excluding a lymphoproliferative disorder. Lymph node culture grew Acinetobacter lwoffi, and antibiotic coverage was changed to intravenous amikacin and meropenem.

An oxidative burst test revealed 0% oxidation (normal, >75%) and a phagocytic index of 1.2 (normal, >1.7), and a diagnosis of chronic granulomatous disease was confirmed. The patient was started on interferon-γ and itraconazole. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels reached a maximum of >2000 U/L and 2632 U/L, respectively, on hospital day 35. Interferon-γ, itraconazole, and isoniazid were held and oral acetylcysteine was started. After a 28-day course of meropenem, he was discharged home on hospital day 40 with a plan to continue acetylcysteine until his liver function tests decreased to <500. Over the next month, his AST/ALT returned to <100 U/L. Radiologic improvements were also noted.

**DISCUSSION**

Chronic granulomatous disease (CGD) was first described as a syndrome by Berendes et al (1957) in their paper titled “A Fatal Granulomatosis of Childhood” and in a follow-up paper by the same authors in 1959. In their initial paper, they report on four children who present with recurrent infections, lymphadenitis, hepatosplenomegaly, pulmonary infiltrates, and periorbital eczematoid skin changes. A decade after the first literature description of the disease, a decrease in the bactericidal ability of the polymorphonuclear leukocytes was demonstrated by Quie et al as the likely pathologic mechanism for the syndrome. The pathogenesis was further shown to be due to a failure of the respiratory burst by phagocytes.

It is now known that CGD is a rare genetic immunodeficiency syndrome characterized by the inability of phagocytes to kill certain pathogenic organisms. The phagocytes are unable to generate the required reactive oxygen species (ROS) needed for the killing of certain bacteria and fungi. Those ROS are generated by the enzyme NADPH oxidase, found in the phagocytes. Specific mutations lead to defective components of this enzyme and its resulting inability to generate ROS. The active form of NADPH consists of two plasma membrane subunits (gp91phox and p22phox) and three cytosolic components (p47phox, p67phox and p40phox). Genetic defects in four of these subunits, namely gp91phox, p22phox, p47phox and p67phox, have been shown to significantly reduce sor support (dopamine), and furosemide, the patient improved with resolution of shock and anasarca.

He also had elevated liver function tests after interferon-γ and itraconazole were introduced. Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels reached a maximum of >2000 U/L and 2632 U/L, respectively, on hospital day 35. Interferon-γ, itraconazole, and isoniazid were held and oral acetylcysteine was started. After a 28-day course of meropenem, he was discharged home on hospital day 40 with a plan to continue acetylcysteine until his liver function tests decreased to <500. Over the next month, his AST/ALT returned to <100 U/L. Radiologic improvements were also noted.

### Table. Timeline of some laboratory values

<table>
<thead>
<tr>
<th></th>
<th>7 d PTA</th>
<th>5 d PTA</th>
<th>3 d PTA</th>
<th>Admission</th>
<th>Discharge</th>
</tr>
</thead>
<tbody>
<tr>
<td>WBC/mm³</td>
<td>18,600</td>
<td>22,700</td>
<td>31,300</td>
<td>28,600</td>
<td>12,800</td>
</tr>
<tr>
<td>Neutrophils (%)</td>
<td>53</td>
<td>57</td>
<td>42</td>
<td>62</td>
<td>27</td>
</tr>
<tr>
<td>Lymphocytes (%)</td>
<td>37</td>
<td>35</td>
<td>43</td>
<td>24</td>
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<tr>
<td>Monocytes (%)</td>
<td>10</td>
<td>6</td>
<td>8</td>
<td>10</td>
<td>9</td>
</tr>
<tr>
<td>Bands (%)</td>
<td></td>
<td></td>
<td></td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>CRP (mg/dL)</td>
<td></td>
<td></td>
<td>45</td>
<td>56</td>
<td>19</td>
</tr>
</tbody>
</table>

*PTA, Prior to admission; WBC, white blood cells; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein.*
or eliminate the activity of the oxidase, thus leading to the clinical symptoms of CGD. Catalase negative organisms are less likely to cause problems in patients with CGD, since their production of hydrogen peroxide compensates for the lack of a phagocyte source for ROS. This hydrogen peroxide gets converted to hypochlorous acid by the neutrophil and consequently leads to the killing of the organism. Catalase-positive bacteria do not demonstrate a net production of hydrogen peroxide, since catalase degrades the small amounts of hydrogen peroxide that these organisms produce and thus are the pathogenic organisms most commonly isolated in patients with CGD. In addition to catalase-positive bacteria, fungi, especially Aspergillus, are also frequently implicated in CGD infections.

The incidence of CGD in the United States is estimated to be approximately 1:250,000 without predilection for any specific ethnic group. A National Registry of patients with CGD was created beginning in 1993 by the Immune Deficiency Foundation, and a total of 368 patients were reported in the Registry by September 1997. The most common form of the disease is X-linked, accounting for approximately 70% (259/368) of the Registry cases. Approximately 22% of the cases are autosomal recessive, with the remaining 8% eluding genetic identification. The X-linked cases are due to a mutation on the CYBB gene, on the X-chromosome, coding for gp91phox. The majority of these mutations lead to complete lack of this NADPH component and consequently to complete deficiency of ROS production. Of the reported autosomal recessive cases, 56% have been with a deficiency in the p47phox component, 12% in the p67phox component, and 8% in the p22phox component. The remaining 24% of the autosomal recessive cases remain of an unknown subtype. No defects in the p40phox cytosolic protein have been detected to date.

The majority of patients with CGD in one study were diagnosed by the age of 2 years, although the oldest patient in the National Registry was diagnosed at age 69 with the X-linked recessive form of the disease. The mean age at diagnosis for the X-linked recessive form is 3 years, whereas for the autosomal recessive group it is 7.8 years. The X-linked recessive group also has a more severe clinical course and a higher mortality rate (21.2% compared with 8.6% for the autosomal recessive patients). Overall, after the first decade of life, the clinical picture tends to improve with infections that are less severe and less frequent.

In a European follow-up study of 39 patients with CGD, lymphadenitis was the most common presenting symptom followed by skin infection, pneumonia, and hepatic abscess. The spectrum of different infections of variable severity may contribute to difficulties in the diagnosis and treatment of CGD. Of the more serious infections in CGD, pneumonia is the most common in all age groups, and Aspergillus species are currently the number-one cause and also the most common cause of death. Gastrointestinal infections and lymphadenitis with or without abscess formation are the next most common infections noted in patients with CGD, along with skin infections and skin abscesses.

Another typical finding with CGD is the presence of granulomas resulting from the chronic response to the inflammation caused by the offending organisms. Granulomas can be found in patients with CGD in a number of organ systems, including the gastrointestinal tract and the lung. They can complicate the clinical picture by causing pain or obstruction, for example, dysphagia, dysuria, and hydro nephrosis, all most commonly manifesting in the X-linked recessive group of patients. Under microscopic examination, these granulomas are notable for the presence of lipid-laden macrophages. In addition, granulomas may have golden brown–pigmented histiocytes. This material is made of glycoproteins and phospholipids, which are demonstrated by the use of periodic acid–Schiff stain.

Thoracic findings of chronic granulomatous disease on plain radiographs and CT include nonresolving and recurrent pneumonias as well as lung abscesses. Hilar and mediastinal
lymphadenopathy is often present, which may be massive. In the abdomen, hepatomegaly may occur along with splenomegaly. Hepatic and splenic abscesses are common. Calcifications are found at the sites of prior infection, including the lungs, liver, spleen, and lymph nodes. Gastric antral narrowing, although rare, is characteristic and may be the initial presentation. This narrowing corresponds to edema and fibrosis of the gastric submucosa, with granulomatous infiltration that disrupts the muscularis propria. The same process may also occur in the esophagus, and esophageal and gastric dysmotility have been described on upper gastrointestinal series.

A number of tests are available to diagnose CGD, and the majority are based on detecting the phagocytes’ inability to produce ROS and a respiratory burst. The phorbol myristate acetate–stimulated nitroblue tetrazodium test (NBT) is the one most commonly used. This test can also be used to determine carrier status of the X-linked CGD by observing the population of NBT-positive and NBT-negative cells. In addition to the NBT test, a flow cytometry test is also commercially available that measures NADPH activity in whole blood. Furthermore, the location of the responsible mutation can be identified by immunoblotting techniques. Finally, prenatal diagnosis is possible for parents who are carriers, but the existing methods are limited and of variable reliability.

Because of the nature of the deficiency that leads to CGD, catalase-positive organisms are primarily responsible for the majority of the infections. Those frequently isolated include Staphylococcus aureus, Salmonella, Klebsiella, Acinetobacter, and Serratia. Additionally, Burkholderia (Pseudomonas) cepacia and Nocardia are commonly found, especially as the cause of lung infections. Among the fungi, Aspergillus spp (most often Aspergillus fumigatus) are currently the most common causative agents of severe pulmonary infections in CGD. Other causes of pneumonia are S aureus, B cepacia, and enteric Gram-negative bacteria. S aureus is the most frequently encountered organism in patients who have development of abscesses, with the exception of lung abscesses, in which Aspergillus spp are again the most common cause. Osteomyelitis is another complication encountered in patients with CGD and can be the result of the hematogenous spread of an organism or can develop as a result of localized spread. An example of the latter is Aspergillus osteomyelitis of the ribs or the thoracic vertebrae secondary to Aspergillus pneumonia. Overall, the most common cause of osteomyelitis among the patients in the US CGD registry was the Serratia spp.

Because of the difficulty in isolating some of the causative organisms in these infections and because of the immunocompromised status of the patient with CGD, empiric treatment is often required before the identification of the pathogen. In pulmonary infections, bronchoscopy and/or biopsy may be indicated, since sputum cultures have a low yield or may be impractical, secondary to the age of the patient. Early diagnosis through a high index of suspicion and aggressive and timely management of the frequent infections is the basis for a good outcome and improved prognosis in patients with CGD. Currently, prevention of infections has become the key to the successful treatment of patients with this disease. The yearly influenza vaccine should also be promoted. Proper oral, dental, and perianal hygiene as well as thorough cleaning techniques of minor injuries with antiseptic solutions are important.

Prophylactic administration of antibiotics has been shown to significantly improve the incidence of serious infections. In the previously mentioned European follow-up study of 39 patients, trimethoprim-sulfamethoxazole (TMP-SMX), at a dose of 5 mg/kg daily, was shown to decrease the mean incidence of severe bacterial infections from 4.8 severe infections per 100 patient-months (PM) to 1.6 severe infections per 100 PM. In a 1990 study analyzing the data of 43 patients admitted to the National Institutes of Health between the years of 1970 and 1988, the authors report that TMP-SMX prophylaxis decreased the incidence of nonfungal infections from 7.1 to 2.4 per 100 PM in patients with the autosomal type of CGD, whereas the incidence of infection fell from 15.8 per 100 PM to 6.9 per PM for the X-linked recessive subgroup. TMP-SMX is well tolerated in children and is the first choice for antibiotic prophylaxis in CGD.

Prophylactic use of itraconazole, a highly lipophilic oral triazole agent effective against Aspergillus at doses of 5 to 10 mg/kg per day, has been demonstrated to significantly decrease the incidence of fungal lung infections. No significant side effects were observed in either the adult or the pediatric population, and the treatment was well tolerated. The role of newer antifungals (eg, voriconazole, posaconazole) in the treatment and prevention of fungal infections in patients with CGD is currently being investigated. Currently, Amphotericin B is the drug of choice for established...
Aspergillus infections, requiring a course of several months to eradicate the fungus and postpone the development of restrictive lung disease, followed by itraconazole therapy to prevent recurrence of a potentially lethal pulmonary infection.

Interferon-γ, a macrophage-activating factor, conferred significant protection from serious infections and was well tolerated when administered subcutaneously, 3 times per week for up to 1 year, in 128 patients with CGD. Interferon-γ does not improve superoxide production by the neutrophils but rather boosts alternate defense mechanisms.

Bone marrow transplantation (BMT) has successfully been used as a possible cure for CGD. In a 2002 European study of 27 patients who received hematopoietic stem cell transplantation from a perfectly matched HLA donor, a cure rate of 81% was reported (22 of 27 patients). The 4 patients who died (15%) all had preexisting fungal infections. In 1 patient, the disease persisted after the transplant. BMT is a high-risk alternative to conventional treatment.

Gene therapy is also being investigated as a cure for CGD or as a method of temporarily boosting the defense mechanisms of a patient during episodes of severe infections. In a study of 5 patients with the p47phox-deficient form of the disease, peripheral blood CD34+ cells were collected, transduced with a retroviral vector carrying a p47phox DNA, and reintroduced to the patients. A low percentage of corrected peripheral blood neutrophils were noted after approximately 3 weeks, and the improved function persisted for several months. More work is under way for improving the efficiency of gene transduction techniques as well as the outcome of gene therapy trials.

Because of the nature of the disease, presenting symptoms can vary, as mentioned before. Our patient presented first at the age of 9 to 10 months with a positive PPD but with no other symptoms at the time. Cases of Bacillus Calmette-Guerin (BCG) infections after BCG vaccination have been reported in the literature, and the BCG vaccine is currently not indicated for patients with confirmed CGD. However, no case of CGD is reported in the English literature with a positive PPD at presentation. Secondary to this unlikely association between PPD positivity and CGD, and because our patient was asymptomatic at the time, the diagnosis of CGD was not considered until a later time, when persistent febrile episodes developed. As an additional unique finding, our patient’s workup revealed A. laevis as the pathogenic organism isolated from mediastinal lymph nodes. Only 1 patient from the CGD National Registry of 368 patients was reported to have a subcutaneous abscess with Acinetobacter as the causative pathogen without further classification mentioned. A. laevis is a Gram-negative, catalase-positive, oxidase-negative, nonsaccharolytic organism that microscopically resembles Moraxella and Kingella. It is resistant to penicillin, ampicillin, the cephalosporins, and chloramphenicol. Many strains of Acinetobacter are susceptible to doxycycline, minocycline, and aminoglycosides. Acinetobacter is commonly found in the environment and can usually be considered a contaminant or a colonizer when found in culture. It rarely causes infection in healthy individuals and should be considered a pathogen, primarily in immunocompromised hosts.

The prognosis has greatly improved over the past few decades, and the future looks more promising for patients with CGD. Both BMT and gene therapy have demonstrated success toward curing the disease. Additional trials and studies probably will be beneficial and improve morbidity and mortality rates and even lead to a possible cure in the not-so-distant future.

REFERENCES


SODIUM TRANSPORT IN AIRWAY EPITHELIUM CORRELATES WITH LUNG COMPLIANCE IN HEALTHY NEWBORN INFANTS

OTTO HELVE, MD, OLLI PITKANEN, MD, PHD, TURKKI KIRJAVAINEN, MD, PHD, AND STURE ANDERSSON, MD, PHD

To study the relation between sodium transport in airway epithelium and postnatal pulmonary adaptation, we measured nasal potential difference at 1 to 4 hours and lung compliance at 21 to 48 hours after birth in 20 healthy infants. Sodium transport correlated with lung compliance ($r^2 = 0.40, P < .003$). Airway sodium transport plays a role in postnatal pulmonary adaptation. (J Pediatr 2005;146:273-6)

During fetal life, vectorial secretion of Cl$^-$ from the airway epithelium to the luminal space by the airway epithelium is vital for lung growth. At birth, a rapid change to net Na$^+$ absorption is crucial for facilitating a rapid clearance of lung fluid. The amiloride-sensitive epithelial sodium channel (ENaC) may be a pivotal pathway for transepithelial movement of Na$^+$ from the apical to the basolateral direction, resulting in osmotic lung fluid absorption. This concept was demonstrated in experiments in which laboratory animals had respiratory distress after injection of amiloride into the airways at birth. In addition, mice that are homozygous for mutations in the ENaC gene, causing loss of function of the $\alpha$-subunit, died soon after birth as the result of respiratory distress associated with increased wet-to-dry lung weight ratio. In human respiratory distress syndrome (RDS), lower transepithelial sodium transport and lower expression of ENaC subunits were measured in preterm infants than in healthy term infants. Therefore, in this pilot study we hypothesized that because perinatal liquid clearance is important for normal human perinatal lung adaptation, there is a temporal relation between static lung compliance and airway epithelial sodium transport.

METHODS

The study protocol was approved by the local ethics committee. We studied 20 healthy newborn infants (Table). Informed consent was obtained from the parents. The initial measurements were performed as soon after birth as possible, at 1 to 4 hours of age. The second set of measurements took place at 21 to 48 hours of age. Sodium transport in airway epithelium was quantified as potential difference over the nasal epithelium. The measurement was based on methods described earlier. A silver wire electrode was inserted into a 3-lumen central catheter (23 G, COOK, Bjaeverskov, Denmark) to allow accurate administration of the perfusion fluids. The system for nasal potential difference (N-PD) measurements included a voltmeter and recording device for recording and saving data (Logan Research Ltd, Maidstone, UK). Before the measurement, the circuit was checked by confirming that the negative potential difference of the exocrine glands on the infant’s skin was $\leq 35$ mV or less. The circuit was re-checked, or the electrode replaced, if the readings did not reach $\leq 30$ mV or were below $\leq 60$ mV. Measurements were performed on the floor of the nose. Initially, N-PD of both nostrils was recorded and the maximal stable N-PD was measured for 10 seconds before perfusion of physiologic saline was commenced. The function of the amiloride-sensitive sodium channel was determined by perfusion with amiloride ($10^{-4}$ mol/L). To measure chloride transport, the perfusion was continued with a chloride-free perfusion solution that included $10^{-4}$ mol/L amiloride. Perfusion was continued for 2 minutes for each solution, during which a stable N-PD for 10 to 20 seconds was achieved. Achievement of amiloride sensitivity was used as the criterion for a successful measurement. The measurement was discontinued if the infant was restless and there was an obvious change of position of the electrode.

In separate experiments, the stability and compatibility from readings of the silver wire exploring electrode was validated in 5 adult volunteers against a conventional method with

<table>
<thead>
<tr>
<th>ENaC</th>
<th>Epithelial sodium channel</th>
<th>N-PD</th>
<th>Nasal potential difference</th>
</tr>
</thead>
</table>

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Lung compliance

N-PD, N-PD 21–48 h, mV (Germany).9 Sleep stage of 9 subjects was determined by device (Labmanager 4.52i, Erich Jaeger GmbH, Hoechberg, Germany). Static lung compliance was measured with the double occlusion technique during quiet non-REM sleep with regular respiration, using a computerized pulmonary function testing device (Labmanager 4.52i, Erich Jaeger GmbH, Hoechberg, Germany).9 Sleep stage of 9 subjects was determined by direct observation of eye movements, muscle tension, and regularity of breathing assessed by using airflow signal. Airflow for polysomnogram and lung compliance measurements was measured by using a pressure transducer and a full face mask.

Clinical data are presented as mean ± SD. Study data are expressed as mean ± SEM. Comparisons were performed with the paired t test or the Mann-Whitney U test. The Pearson test was used for correlations.

RESULTS

A successful N-PD measurement and sodium transport quantification was possible in all infants within 4 hours of birth (range, 1 to 4 hours; median, 2.0 hours) and in 16 infants at 21 to 48 hours of birth (median, 26.5 hours). Recording of N-PD lasted for 6.2 (±0.5) minutes; prolongation of the measurement often induced restlessness in the infants. Accordingly, measurement of N-PD during perfusion of the chloride-free solution was possible in 11 infants within 4 hours and 9 infants within 21 to 48 hours. The lung compliance measurement was possible in 19 and 20 infants, respectively.

The maximal stable N-PD was −15.4 (±1.5) mV and −14.4 (±1.5) mV (n = 36, NS) during perfusion with saline. At the initial <4-hour time point after birth, amiloride in the perfusion solution reduced the potential difference by 44.0% (±4.2) (Table, P < .0001 vs saline). The N-PD response was similar at 21 to 48 hours after birth, as amiloride in the perfusion solution reduced the potential difference by 43.4% (±3.7) (P < .0001 vs saline) and amiloride-sensitive sodium transport had lung compliance of 15.1 (±3.6) mV (n = 19). The 6 newborn infants with the highest initial amiloride-sensitive potential difference at <4 hours and lung compliance at 21 to 48 hours after birth (r² = 0.40, P < .003; Figure). Importantly, a correlation existed between the initial amiloride-sensitive nasal potential and the change in lung compliance between the two time points (r² = 0.31, P = .013; n = 19). The 6 newborn infants with the highest initial amiloride-sensitive sodium transport had lung compliance of 27.3 (±3.2) mL/kPa per kilogram at 21 to 48 hours after birth.

Table. Clinical characteristics, transepithelial nasal potential difference, and static lung compliance of 20 healthy newborn infants at <4 and 21 to 48 hours after birth

<table>
<thead>
<tr>
<th>Subjects</th>
<th>All (n = 20)</th>
<th>Vaginal delivery (n = 13)</th>
<th>Cesarian section (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male/female</td>
<td>10/10</td>
<td>5/8</td>
<td>5/2</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>39.1 ± 1.5</td>
<td>39.6 ± 1.4</td>
<td>38.9 ± 1.8</td>
</tr>
<tr>
<td>Birth weight, kg</td>
<td>3.7 ± 0.6</td>
<td>3.6 ± 0.4</td>
<td>3.8 ± 0.8</td>
</tr>
<tr>
<td>N-PD &lt;4 h, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>−14.3 ± 1.9 (n = 20)</td>
<td>−14.8 ± 2.8 (n = 13)</td>
<td>−13.3 ± 2.2 (n = 7)</td>
</tr>
<tr>
<td>Amiloride</td>
<td>−8.8 ± 1.7 (n = 20)</td>
<td>−8.8 ± 2.3 (n = 13)</td>
<td>−8.7 ± 2.4 (n = 7)</td>
</tr>
<tr>
<td>Chloride-free</td>
<td>−15.6 ± 3.0 (n = 11)</td>
<td>−14.9 ± 3.7 (n = 8)</td>
<td>−17.3 ± 5.8 (n = 3)</td>
</tr>
<tr>
<td>N-PD 21–48 h, mV</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Basal</td>
<td>−14.6 ± 2.3 (n = 16)</td>
<td>−13.9 ± 3.0 (n = 10)</td>
<td>−15.7 ± 3.8 (n = 6)</td>
</tr>
<tr>
<td>Amiloride</td>
<td>−9.0 ± 1.9 (n = 16)</td>
<td>−8.5 ± 2.5 (n = 10)</td>
<td>−10.0 ± 3.0 (n = 6)</td>
</tr>
<tr>
<td>Chloride-free</td>
<td>−15.1 ± 2.7 (n = 9)</td>
<td>−12.0 ± 2.4 (n = 6)</td>
<td>−15.6 ± 5.2 (n = 3)</td>
</tr>
<tr>
<td>Lung compliance &lt;4 h, mL/kPa per kg</td>
<td>17.3 ± 1.4 (n = 19)</td>
<td>15.9 ± 1.3 (n = 12)</td>
<td>18.0 ± 2.8 (n = 7)</td>
</tr>
<tr>
<td>Lung compliance 21–48 h, mL/kPa per kg</td>
<td>22.8 ± 2.2 (n = 20)</td>
<td>23.1 ± 2.4 (n = 13)</td>
<td>22.1 ± 4.6 (n = 7)</td>
</tr>
</tbody>
</table>

N-PD, transepithelial nasal potential difference; LC, static lung compliance. Statistical comparisons were performed within the subject category (*P < .0001, †P < .005, ‡P < .05).
and the measurements for quantification of amiloride-sensitive sodium transport characteristics between infants born vaginally and by cesarean section are corroborated by a recent report but are in conflict with an earlier observation by Gowen et al. However, irrespective of the method of delivery, the infants in the present work were comparable in terms of the initial lung compliance values, suggesting that the extent of perinatal lung adaptation was similar. Lung compliance was measured during quiet non-REM sleep, and the values observed are consistent with the few previous measurements in human beings and laboratory animals, suggesting an inverse relation between compliance and the content of lung water. This fact is important and underscores the significance of our finding of a correlation of amiloride-sensitive sodium transport and the perinatal increase in lung compliance.

In animal models, intact perinatal sodium transport is critical for pulmonary adaptation and survival. On the other hand, in the newborn human being, excess lung fluid is a characteristic feature of respiratory distress, such as transient tachypnea of the term infant and respiratory distress syndrome of the preterm infant. The low ENaC expression in the airway epithelium of preterm infants can be induced by hormonal stimuli. We speculate that sodium transport is a potential target for therapeutic interventions aimed at improving clearance of lung fluid in the newborn infant. In conclusion, our present results indicate that sodium transport in the airway epithelium is a characteristic property in healthy term newborn infants undergoing successful postnatal pulmonary adaptation.

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Sodium Transport In Airway Epithelium Correlates With Lung Compliance In Healthy Newborn Infants

**Figure.** Postnatal amiloride-sensitive sodium transport of nasal epithelium at 1 to 4 hours and static lung compliance at 21 to 48 hours in 20 newborn infants. Sodium transport is expressed as the percent inhibition of potential difference caused by amiloride. Regression line \((r^2 = 0.40, P < .003)\) and 95% confidence intervals are shown.

No significant difference in N-PD and lung compliance existed between infants delivered by cesarian section and those delivered vaginally.

**DISCUSSION**

The important observation of the present study was the correlation of amiloride-sensitive sodium transport by the early perinatal airway epithelium with the improvement in lung compliance during the subsequent 2 postnatal days. Since the timeline of the perinatal lung fluid transport is rapid, we were not surprised to find that only the early amiloride-sensitive Na⁺ transport showed correlation with lung compliance. First, it is possible that the electrophysiologic measurements of the nasal epithelium at <4 hours represent a response induced by humoral factors just before birth, or result from significant changes in physiologic and physical factors to which the newborn infant is exposed at birth. Accordingly, the later bioelectric measurement of the proximal airways in a healthy newborn infant in a stable clinical condition may represent successful adaptation and not a state of a net fluid absorption. Second, previous investigations have demonstrated that in adult human beings without respiratory distress, transepithelial PD and airway ENaC subunit gene expression change along the course of the respiratory tract. This may complicate the use of nasal epithelium as a sole surrogate of amiloride-sensitive Na⁺ transport of a healthy pulmonary epithelium after successful lung adaptation.

The present values of the transepithelial baseline N-PD and the measurements for quantification of amiloride-sensitive Na⁺ and Cl⁻ currents are within ranges detected in previous studies. Of technical note regarding the neonatal population, at times the restlessness of the infants increased as the measurement proceeded. Consequently, a precise topical administration of Cl⁻ free medium was not feasible in every infant. Measuring the response of Cl⁻ secretion to isoprenaline was not attempted. Our finding of no difference in airway sodium transport characteristics between infants born vaginally and by cesarean section are corroborated by a recent report but are in conflict with an earlier observation by Gowen et al. However, irrespective of the method of delivery, the infants in the present work were comparable in terms of the initial lung compliance values, suggesting that the extent of perinatal lung adaptation was similar. Lung compliance was measured during quiet non-REM sleep, and the values observed are consistent with the few previous measurements in human beings and laboratory animals, suggesting an inverse relation between compliance and the content of lung water. This fact is important and underscores the significance of our finding of a correlation of amiloride-sensitive ion transport and the perinatal increase in lung compliance.
We evaluated the consequence of different types of fetal arrhythmia in the development of neonatal cholestasis. The charts of 38 children born at St. Justine Hospital between 1993 and 2001 with sustained and hemodynamically significant fetal arrhythmias were studied: 19 with supraventricular tachycardia, 14 with atrial flutter, and 5 with atrio-ventricular (AV) block. Six of these 38 children presented with cholestasis. The average duration of arrhythmia was 15.7 days in the noncholestatic group, compared with 40.3 days in the cholestatic group \((P < .05)\). The three infants with supraventricular tachycardia who developed cholestasis survived and resolved their cholestasis, whereas 2 of 3 infants with AV block died. No infant with atrial flutter developed cholestasis. We conclude that newborns who developed tachyarrhythmia during their fetal life can show transient neonatal cholestasis. In patients with AV block, severe and irreversible liver failure could be observed. In addition, extensive collapse of the stroma and the absence of hepatocytes (foie vide) also were observed in a patient with anti-Ro antibodies. (J Pediatr 2005;146:277-80)

Neonatal cholestasis, defined as a decrease in bile flow in the first months of life, is a result of impairment of bile formation or/and obstruction of bile flow through the biliary tree. The clinical features are jaundice, dark urine, and pale stools, with elevated serum levels of conjugated bilirubin. The incidence of prolonged neonatal cholestasis (>14–21 days) is approximately 1 in 2500 live births.\(^1\) The largest diagnostic groups are biliary atresia and multifactorial cholestasis seen in premature infants; in some cases the cause cannot be found, and the cholestasis resolves spontaneously. Several studies have reported that factors such as perinatal asphyxia,\(^2,3\) prematurity, and circulatory failure are implicated in the pathogenesis of cholestasis.\(^4\) Fetal arrhythmia is frequently associated with perinatal distress and, in theory, could be another etiologic factor. A MEDLINE search of the literature from January 1990 to June 2003 did not find any study correlating fetal arrhythmia with neonatal cholestasis. The objective of the present study was, therefore, to evaluate the incidence and significance of neonatal cholestasis following fetal arrhythmia.

**MATERIALS AND METHODS**

**Cohort**

The cases of patients with sustained fetal tachy- and bradyarrhythmia who presented between 1993 and 2001 at our Fetal Cardiology Unit were analyzed retrospectively. The diagnosis of neonatal cholestasis was based on jaundiced newborns with dark urine and pale, clay-colored stools associated with conjugated bilirubin levels >2.0 mg/dL or >10% of the total bilirubin.\(^5\) Sustained tachycardia and bradycardia were defined as heart rates constantly >180 or <100 beats/minute, respectively. The type of supraventricular tachycardia could be identified by M-mode and/or Doppler echographic criteria as published previously.\(^6,7,8\) Only fetuses who were delivered in our institution were included in this study. The following variables were analyzed: type of arrhythmia, gestational age at the beginning of arrhythmia and at the time of delivery, duration of arrhythmia, conversion to sinus rhythm before or after birth, type and duration of antiarrhythmic drugs used, presence of hydrops during pregnancy, persistence of hydrops after birth, and final outcome after birth (alive vs deceased).
RESULTS

Of the 55 fetuses followed for sustained arrhythmia during this period, 4 died in utero and 38 were delivered in our institution, constituting the final study group. Cholestasis was diagnosed in six neonates. Comparative analysis of the cholestatic and noncholestatic cases is reported in Table I. Gestational age at diagnosis of arrhythmia and at delivery was 37.3 weeks earlier for the cholestatic group, compared with 40.3 days in the noncholestatic group, demonstrating a tendency for more premature babies in this group. All babies with cholestasis and tachyarrhythmia had hydrops (n = 3) and did not convert to sinus rhythm before delivery. The average duration of arrhythmia was 15.7 days in the noncholestatic group, compared with 40.5 days in the cholestatic group (P < .05). None of the newborns from the noncholestatic group presented with infection, persistence of ductus venosus, or necrotizing enterocolitis, or received parenteral nutrition. One infant had omphalocele; three other infants had respiratory distress syndrome of the newborn. Nine infants presented an intra-atrial communication, with no hemodynamic repercussion. One had pulmonary atresia, one pulmonary valve dysplasia, and one cardiac complex malformation. In the cholestatic group, only two infants received total parenteral nutrition (TPN) (started after the beginning of the cholestasis) during an average of 20 days. Only one child in the cholestatic group received ursodeoxycholic acid because of TPN, without significant improvement of the biochemical tests.

### Tachyarrhythmia

Three case patients with cholestasis presented maximum conjugated bilirubin levels of 18 mg/dL, 3.8 mg/dL, and 2.5 mg/dL, respectively. They all recovered with complete normalization of the bilirubin (conjugated bilirubin <1.0 mg/dL), aminotransferases (alanine (ALT) <25; asparate (AST) <50), and gammaglutamyl transferase (GGT) (<115 U/L; age = 1 year) serum levels, varying from within 12 days to 7 months. Their main characteristics are presented in Table II.

### SHORT VA TACHYCARDIA

Twelve fetuses had short ventricular-atrial (VA) tachycardia. Arrhythmia started at a median age of 30.5 ± 3.6 weeks. Hydrops fetalis was observed in four fetuses, and all of them were still hydropic at birth. Two case patients developed neonatal cholestasis. Conversion to sinus rhythm was documented before delivery in seven case patients. Digoxin (83%) and sotalol (58%) were the most common medications used.

### LONG VA TACHYCARDIA

Seven fetuses had long VA tachycardia, with arrhythmia starting at a median of 33 ± 3.3 weeks. Hydrops fetalis was observed in two fetuses, but only one was still hydropic at birth and developed neonatal cholestasis. Conversion to sinus rhythm was documented before delivery in four case patients. Digoxin (71%) and sotalol (71%) were the most common medications administered.

### ATRIAL FLUTTER

Fourteen neonates were born in our institution with sustained atrial flutter during their fetal period. Arrhythmia was diagnosed at a median of 32 ± 8.5 weeks of gestation. Only two fetuses presented with hydrops that continued until delivery. All patients received digoxin; sotalol was added in four cases. Conversion to sinus rhythm was achieved in seven instances before delivery. None of these neonates presented signs of cholestasis.

### AV Block

Of the 10 fetuses initially diagnosed with AV block, 3 were not delivered in our institution and 2 died in utero. In the remaining 5, bradycardia was noted at a median age of 26 ± 11.3 weeks of gestation, and the fetuses were delivered at a median of 34 ± 2.6 weeks. Two fetuses presented with hydrops during pregnancy and after delivery. A pacemaker had to be installed in all 5 neonates during the first days of life. Three newborns showed signs of neonatal cholestasis, presenting maximum conjugated bilirubin levels of 8.9 mg/dL, 26 mg/dL, and 3.5 mg/dL, respectively. The third newborn was the only one who recovered (the two others

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Table I. Comparative analysis of cholestasis and noncholestatic cases

<table>
<thead>
<tr>
<th></th>
<th>No cholestasis (N = 32)</th>
<th>Cholestasis (N = 6)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean gestational age from diagnosis of arrhythmia (weeks)</td>
<td>31.9</td>
<td>28.3</td>
<td>0.054</td>
</tr>
<tr>
<td>Mean gestational age at delivery (weeks)</td>
<td>37.3</td>
<td>34.2</td>
<td>0.003</td>
</tr>
<tr>
<td>Tachyarrhythmias</td>
<td>30</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>AV block</td>
<td>2</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Hydrops during pregnancy</td>
<td>6 (18.8%)</td>
<td>4 (66.7%)</td>
<td>0.014</td>
</tr>
<tr>
<td>Tachyarrhythmias (n = 33)</td>
<td>5 (16.7%)</td>
<td>3 (100%)</td>
<td></td>
</tr>
<tr>
<td>AV block (n = 5)</td>
<td>1 (50%)</td>
<td>1 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>Hydrops during delivery (n = 33)</td>
<td>5 (15.6%)</td>
<td>4 (66.7%)</td>
<td>0.007</td>
</tr>
<tr>
<td>Tachyarrhythmias</td>
<td>4 (13.3%)</td>
<td>3 (100%)</td>
<td></td>
</tr>
<tr>
<td>AV block (n = 5)</td>
<td>1 (50%)</td>
<td>1 (33.3%)</td>
<td></td>
</tr>
<tr>
<td>Conversion before delivery (n = 5)</td>
<td>18 (56.3%)</td>
<td>0</td>
<td>0.011</td>
</tr>
<tr>
<td>Duration of arrhythmia (days)</td>
<td>15.7</td>
<td>40.3</td>
<td>0.014</td>
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<tr>
<td>Tachyarrhythmias (n = 33)</td>
<td>12.3</td>
<td>18.7</td>
<td>0.44</td>
</tr>
<tr>
<td>AV block (n = 5)</td>
<td>66.5</td>
<td>63</td>
<td></td>
</tr>
</tbody>
</table>

Statistical Methods

Chi-square analysis (for categorical variables) and t test (for continuous variables) determined if there were differences between fetuses who developed cholestasis and the rest of the group. Statistical significance was set at $P < .05$. 

---

Sant'Anna, Fournon, and Alvarez  The Journal of Pediatrics • February 2005
died), with complete resolution of the cholestasis in 1 month (Table II). One showed at the liver biopsy an extensive fibrosis with scarce hepatocytes (foie vide); her mother had anti-Ro and anti-La circulating autoantibodies.9 Hepatic failure was diagnosed at 3 days of age, and in this case, the neonate died 46 days later. The second neonate with cholestasis died at 1 month of age from multisystemic failure; hemochromatosis was found at liver histology. In this patient, the diagnosis of neonatal lupus erythematosus is highly probably, despite the absence of serological markers. In both cases, there was no reason to believe that these infants had shock liver syndrome or ischemic liver. The third neonate with cholestasis had a complex cardiac malformation characterized by severe pulmonary stenosis, interatrial communication, patent ductus arteriosus, dextrocardia, and persistence of fetal circulation. In this last instance, resolution of cholestasis was documented at 1 month of age.

**DISCUSSION**

The present study shows that as many as 15% of neonates who experienced sustained tachy- or bradyarrhythmia in utero present signs of cholestasis. The prenatal clinical picture was, however, not similar for all children with arrhythmias. The
number of cases was obviously too small to reach a definitive conclusion, but two potential physiopathological mechanisms can be involved: congestion or ischemia. It appears that among fetuses with reentrant tachycardia (short VA tachycardia), only those with hydrops fetalis developed postnatal signs of cholestasis. Furthermore, no hepatic complications were observed among fetuses or neonates with atrial flutter. These findings suggest that significant venous congestion classically seen in cardiac failure and secondary hydrops is a major element for the appearance of postnatal cholestasis. It must be emphasized that in tachycardia with a fast conducting reentrant pathway, as classically observed in the pediatric age group, atrial contractions occur during ventricular ejection, thus against closed AV valves. Doppler flow velocity investigations have provided evidence that tall reverse “A” waves are present in the venous system of fetuses with reentrant supraventricular tachycardia. It is, therefore, conceivable that this type of arrhythmia could induce backward venous congestion and hydrops, even in absence of myocardial failure. In a fetus with atrial flutter who did not develop cholestasis, there is no venous congestion. This feature could explain why they did not develop liver disease. Study of a greater number of patients will be necessary to confirm this hypothesis. The duration of the process also may play a significant role in the development of impaired bile flow, as suggested by the observation of hydrops in all the neonates with cholestasis.

The situation seems to be different with congenital AV block because only 1 of the 3 fetuses in this group who presented with cholestasis showed evidence of hydrops. Two elements deserve mention: first, the arrhythmia lasted until delivery and persisted after birth, increasing the duration of hemodynamic disturbances; second, cholestasis has been described in patients with neonatal lupus erythematosus without AV block. Both venous congestion and liver inflammation could cause cholestasis. In this disease, liver injury probably develops during fetal life. In fact, hepatic failure was present at birth in one of our patients who died as a consequence of AV block. Another patient presented with hemochromatosis at liver biopsy, a finding already described in a previous report. These results lead to the conclusion that newborns who develop an AV block or a tachyarrhythmia during their fetal life need close follow-up after birth with liver function tests. In patients with AV block, severe and irreversible liver failure could be observed. In addition, extensive collapse of the stroma and the absence of hepatocytes (foie vide) also were associated in a patient with the presence of anti-Ro antibodies.

REFERENCES

HIGH PROTEIN DIET MIMICS HYPERTYROSINEMIA IN NEWBORN INFANTS

CHULALUCK TECHARITTIROJ, MD, AMY CUNNINGHAM, MS, LDN, RD; PLEASANT F. HOOPER, MD, HANS C. ANDERSSON, MD, AND JESS THOENE, MD

Tyrosinemia resulting from administration of protein-dense infant diets was detected by newborn screening in two infants. Change of formula resulted in rapid resolution of the hypertyrosinemia. These cases identify nonstandard infant diets as a benign and reversible cause of tyrosinemia and a potential cause of positive newborn phenylketonuria screening. (J Pediatr 2005;146:281-2)

Tyrosinemia is a group of inborn errors of metabolism that presents with a variable phenotype. There are many causes of hypertyrosinemia in the newborn and infancy periods, including tyrosinemia type 1, transient tyrosinemia of the newborn infant, and cytomegalovirus infection. Tyrosinemia type 1, caused by fumarylacetoacetate hydrolase deficiency, presents in infancy with jaundice, progressive cirrhosis, and generalized symptoms of hepatic dysfunction such as clotting abnormalities and/or the renal Fanconi syndrome. Treatment includes tyrosine (protein) restriction, therapy with NTBC [2-(2-Nitro-4-Trifluoromethylbenzoyl)-1,3-Cyclohexanedione (Nitisinone)], and/or liver transplantation. Transient tyrosinemia of the newborn infant results from a combination of immature function of 4-hydroxyphenylpyruvate dioxygenase (4HPPD), high protein diet, and ascorbate deficiency. Treatment with oral ascorbate and/or protein restriction usually results in normal plasma tyrosine within weeks to months. The incidence of transient tyrosinemia has decreased with more breast-feeding and the use of lower-protein commercial formulas.

We report two cases of tyrosinemia caused by inappropriately high dietary protein intake with nonstandard infant diets that resolved after dietary normalization.

METHODS

Case 1

A term male infant was reported to have a presumptive positive phenylketonuria (PKU) newborn screen. The birth weight and length were 3.5 kg and 50 cm, respectively. He was initially fed on Enfamil (Mead Johnson Nutritional, Evansville, Ind), and his first newborn screen (at 24 to 48 hours of life) was normal. Because of spitting up and jaundice, he was fed with on Shaklee Slim Plan Drink Mix (Shaklee Corporation Pleasanton, Calif) at 5 days of age by his mother, providing about 7 g/kg per day of dietary protein (Table). A routine second newborn screen at 7 days of age was positive for PKU. At 2 weeks, he was a healthy, normal infant with normal growth parameters. Plasma tyrosine was elevated to 2330 μmol/L (normal value <148) and plasma phenylalanine was increased to 220 μmol/L (normal value <138), resulting in the positive newborn PKU screen. Large amounts of p-hydroxyphenyllactic acid and p-hydroxyphenylactic acid were in the urine. Transient tyrosinemia was considered, and he was given Isomil (Ross Products, Columbus, Ohio) (providing 2 g of protein/kg per day) and vitamin C (100 mg/kg per day). The plasma tyrosine and phenylalanine was normal within 5 days. Vitamin C was discontinued at 3 months of age and plasma tyrosine remained normal at follow-up at 1 year of age. At 1 year of age, his growth parameters were at the 50 percentile and his development was normal.

Case 2

A term 2.7-kg female infant was fed with Similac Advance (Ross Products, Columbus, Ohio) with iron from birth, and her first newborn screen at 24 hours was...
Because of constipation, the mother switched the formula at 4 days of age to Pet evaporated milk (J.M. Smucker Co., Minneapolis, Minn) 2 cans per day, which provided approximately 10 g/kg (Table). A routine second newborn screen performed at 1 week of age (because the first screen was done at <2 days of age) was positive for PKU. The infant appeared healthy and normal at 3 weeks of age, with weight and height at the 25 percentile. The plasma tyrosine and phenylalanine were markedly elevated (tyrosine, 1822 μmol/L; phenylalanine 306 μmol/L). Urine organic acids showed large amounts of 4-hydroxyphenyllactic acid and 4-hydroxyphenylacetic. Liver function tests were normal, including α-fetoprotein (490 μmol/L; normal value <10,000 μmol/L).4

The infant was switched to Similac advance with iron at 5 weeks of age. Her tyrosine and phenylalanine returned to normal within 4 days, with no other treatment.

**DISCUSSION**

These cases demonstrate the effect of an extremely high protein diet on plasma phenylalanine and tyrosine concentrations. In each case, the infant had a presumptive positive PKU screen. The phenylalanine in both cases was higher (2 to 3 times) than normal but not as high as the tyrosine concentration (>10 times normal). The urine organic acids in both cases demonstrated compounds commonly found in tyrosinemia. Both patients were being fed nonstandard high protein diets at the time of high plasma tyrosine, and the plasma tyrosine returned to normal within a short period of time after a change to an appropriate infant formula. The usual protein requirement in infancy is 2.2 g/kg per day,5 and the infants received 3 to 4 times more protein than normally recommended. These 2 patients would not have been identified if newborn screening had not been repeated during the high protein administration. Both patients were normal at the time of diagnosis, but the long-term effects of high protein intake in infants have not been systemically studied. These cases highlight the importance of following infant nutritional guidelines.

A high protein diet is a known risk factor for transient tyrosinemia in the newborn infant. Most patients with transient tyrosinemia have no complications or long-term effects from a short period of high plasma tyrosine.1,2 However, Rice et al6 and Mamunes et al7 reported learning disabilities without gross motor developmental effects after 8-year follow-up, especially in a group having very high tyrosine levels (>1100 μmol/L). Corneal crystals were reported by Driscoll et al8 in one patient with transient tyrosinemia. Some patients with transient tyrosinemia will not be diagnosed, and those diagnosed with transient tyrosinemia are not followed for long-term effects.

**REFERENCES**


<table>
<thead>
<tr>
<th>Table. Nutritional analysis and phenylalanine/tyrosine content of formula per kilogram per 24 hours of administration</th>
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</thead>
<tbody>
<tr>
<td><strong>Formula</strong></td>
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<tr>
<td><strong>Formula</strong></td>
</tr>
<tr>
<td>Calories (kcal/kg)</td>
</tr>
<tr>
<td>Protein (g/kg)</td>
</tr>
<tr>
<td>Phenylalanine (mg/kg)</td>
</tr>
<tr>
<td>Tyrosine (mg/kg)</td>
</tr>
<tr>
<td><em>Formula recipe: 2 cup (60 g) Shaklee Slim Plan powder with 1 tsp olive oil and enough water to make 20 oz per batch. Formula intake: averaged 5 oz every 4 hours or 30 oz every 24 hours.</em>*</td>
</tr>
<tr>
<td>†Formula recipe: 12 oz evaporated milk, 2 tsp corn syrup with 18 oz water, and boiled. Formula intake: averaged 4 oz every 2 hours or 48 oz every 24 hours.</td>
</tr>
</tbody>
</table>
TUMOR NECROSIS FACTOR RECEPTOR–ASSOCIATED PERIODIC SYNDROME IN A YOUNG ADULT WHO HAD FEATURES OF PERIODIC FEVER, APHTHOUS STOMATITIS, PHARYNGITIS, AND ADENITIS AS A CHILD
FRANK T. SAULSBURY, MD, AND BRIAN WISPELWEY, MD

Tumor necrosis factor receptor–associated periodic syndrome (TRAPS) was diagnosed in a 22-year-old man after a 1-year history of periodic fever, myalgia, conjunctivitis, cervical lymphadenopathy, and oral ulcers. As a child he had signs and symptoms suggestive of periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome. This report indicates the importance of considering TRAPS as a cause of periodic fever in older children and adults and that TRAPS may present with signs and symptoms suggestive of periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome in young children. (J Pediatr 2005;146:283-5)

The periodic fever syndromes are a heterogeneous group of disorders characterized by repeated attacks of fever and localized inflammation primarily affecting serosal surfaces, skin, and the musculoskeletal system. To date, five periodic fever syndromes have been well characterized on the basis of distinct clinical features and in four of the five on the basis of the underlying genetic abnormality. Two disorders have autosomal recessive inheritance: familial Mediterranean fever, caused by mutations in the Mediterranean fever (MEFV) gene, and the hyperimmunoglobulinemia D syndrome, caused by mutations in the mevalonate kinase gene. Two others are autosomal dominant disorders: Muckle Wells syndrome, caused by mutations in the cold-induced autoinflammatory syndrome 1/PYD containing protein 3 (CIAS1/NALP3) gene, and tumor necrosis factor receptor–associated periodic syndrome (TRAPS), caused by mutations in the tumor necrosis factor receptor superfamily 1A (TNFRSF1A) gene. The periodic fever, aphthous stomatitis, pharyngitis, and adenitis syndrome (PFAPA) has no known genetic basis.

This report describes a 22-year-old man diagnosed with TRAPS who had a history compatible with PFAPA.

CASE REPORT

The patient presented for evaluation of periodic fevers. As a child, he had recurrent episodes of pharyngitis. The childhood episodes were characterized by fever, sore throat, and tender cervical lymphadenopathy. Aphthous ulcers occasionally accompanied these episodes. There was no fixed interval between the febrile episodes. He had a tonsillectomy at 8 years of age, and the frequency of fevers and pharyngitis diminished greatly after the tonsillectomy. His health was excellent until 21 years of age. There was no family history of periodic fevers.

At 21 years of age, he had an illness characterized by daily fever ranging from 39°C to 40°C, sore throat, tender cervical lymphadenopathy, and multiple painful oral ulcers, severe flank pain, and myalgia of the leg muscles. The episode lasted 2 weeks. Thereafter, he had periodic illnesses that occurred every 2 to 3 weeks. With each attack, he had fever of 39°C to 40°C, sore throat, tender cervical lymphadenopathy, and painful oral ulcers. The episodes were also associated with flank pain, myalgia, and frequently photophobia and conjunctivitis. Some episodes were associated with abdominal pain and vomiting, but these were
not constant features. Likewise, arthralgia occurred occasionally, but at no time did he have objective arthritis. None of the episodes were associated with skin rash, orchitis, genital ulcers, or neurologic symptoms. The attacks generally lasted 7 days, but occasionally symptoms persisted for 2 weeks or longer.

The patient was initially evaluated 6 months after the onset of the febrile episodes. At that time, he was asymptomatic and the physical examination was normal. Laboratory studies revealed white blood cell count of 4500 cells/mm³ with 47% neutrophils and 43% lymphocytes, hemoglobin of 13 g/dL, platelet count of 326,000/μL, erythrocyte sedimentation rate of 10 mm/h, and C-reactive protein of 0.7 mg/dL. Tests for antinuclear antibody and rheumatoid factor were negative. Serum immunoglobulin concentrations were normal. For treatment, he was instructed to take prednisone at the onset of fever in a dosage of 60 mg/d for 2 days, then 40 mg/d for 1 day, then 20 mg/d for 1 day. In addition, because of concern for the diagnosis of PFAPA, he was started on cimetidine in a dosage of 800 mg twice each day.

He was reevaluated 6 months later. In the interim, he continued to have febrile episodes every 2 to 4 weeks; however, prednisone was effective in aborting the attacks after 2 to 3 days. The cimetidine had been discontinued 2 months previously. He was healthy between attacks. Physical examination was again normal except for a few small oral ulcers. Laboratory studies again were normal except for a few small oral ulcers.

DISCUSSION

The patient in this report was diagnosed with TRAPS at 22 years of age, 1 year after he presented with periodic fever, myalgia, and conjunctivitis. In addition, his febrile episodes were frequently associated with cervical lymphadenopathy and oral ulcers. As a child, he had multiple episodes of fever, pharyngitis, and adenopathy. The episodes ceased after a tonsillectomy at 8 years of age, and he remained healthy until 21 years of age.

TRAPS was first described in 1982 in a large Irish family. It was originally called familial Hibernian fever. Affected patients had recurrent episodes of fever, abdominal pain, arthralgia, and rash. In 1997, McDermott et al. confirmed the autosomal dominant inheritance of Hibernian fever and expanded the clinical features and natural history of the condition. Subsequent work demonstrated that the condition is not confined to individuals of Irish descent; it has been reported in patients from virtually all ethnic origins.1 The genetic basis for TRAPS was discovered in 1999 by McDermott et al. They showed that patients with TRAPS had mutations in the gene encoding the 55 kDa TNF-α receptor (TNFRSF1A). The mutations occurred predominantly in exons 2, 3, and 4 of the extracellular domain and resulted in impaired shedding of the TNF-α receptor from cell surfaces and decreased plasma levels of soluble TNF-α receptor between attacks in most but not all patients.4 The condition is now referred to as tumor necrosis factor receptor–associated periodic syndrome to reflect the molecular basis of the condition.

The patient in this report had the R92Q mutation in the TNFRSF1A gene, the most common mutation in patients with TRAPS.5 The R92Q mutation has been reported in 1% of seemingly healthy individuals, indicating incomplete penetrance. Most patients with TRAPS who have the R92Q mutation do not have impaired TNF-α receptor shedding.6 Moreover, patients with the R92Q mutation have a more heterogeneous clinical presentation, including adult-onset disease, compared with patients with other mutations in the TNFRSF1A gene.7 The clinical variability associated with the R92Q mutation may explain the late onset of typical TRAPS symptoms in the current patient and suggests that the signs and symptoms of PFAPA that he had as a child were probably a consequence of TRAPS.

The age of onset of symptoms in patients with TRAPS is highly variable, ranging from less than 1 year to more than 60 years of age.7 Nevertheless, the vast majority of patients have symptoms before 20 years of age. The duration of attacks may be as short as 1 to 2 days, but they typically last for more than 7 days, and occasionally they persist for several weeks.5 The fever is high grade, usually 40°C or higher. Severe localized myalgia is a characteristic feature of an attack. Many patients have an erythematous maculopapular rash overlying the areas of myalgia, and the rash tends to migrate centrifugally. Painful conjunctivitis associated with periorbital edema and colicky abdominal pain are also common features. Arthralgia is frequent, but objective arthritis (usually monoarticular) is uncommon. Testicular pain and orchitis have been reported in a substantial proportion of affected men. Chest pain, pleuritis, and pericarditis are uncommon manifestations of TRAPS. Tender cervical lymphadenopathy has been reported in a substantial minority of patients. However, unlike in PFAPA, pharyngitis and oral ulcers are not prominent features of TRAPS. There is no fixed periodicity to the occurrence of the attacks. The interval between attacks ranges from 1 to 2 weeks to months or years.5 The patients are asymptomatic between attacks. During febrile episodes, there is often an exuberant acute-phase response. The major long-term consequence of TRAPS is amyloidosis, which occurs in approximately 15% of patients.5 Corticosteroids administered at the onset of symptoms are effective in shortening the duration and severity of attacks, but they do not prevent subsequent attacks. Recent reports indicate that etanercept may be an effective form of therapy for patients with TRAPS.5,8 Etanercept is a recombinant fusion protein containing two copies of the soluble extracellular ligand-binding domain of the type 2 TNF receptor linked to the Fc portion of human IgG1. It binds effectively to both soluble and cell-bound TNF-α and TNF-β and it attenuates the biological effects of TNF.
Our patient was diagnosed with PFAPA in childhood and had apparent favorable response to tonsillectomy. He lacked the fixed periodicity of PFAPA, however. The cause of PFAPA is unknown. Prednisone therapy, cimetidine therapy, and tonsillectomy have had variable effectiveness.

The natural history of PFAPA is unclear. Spontaneous remissions have been reported in approximately 50% of patients after a mean duration of symptoms of 5 years, but 30% of patients continued to have typical symptoms after follow-up of 5 years. We are unaware of any reports of PFAPA with onset of symptoms in adulthood, and there is only one report of an adult with PFAPA in whom symptoms began in childhood. That patient had the onset of PFAPA symptoms in early childhood; the symptoms ceased after a tonsillectomy at 4 years of age but recurred at 15 years of age and persisted into adulthood.

No genetic basis for PFAPA has been discovered. To date, few patients reported with PFAPA have a family history of periodic fevers, making an autosomal dominant inheritance unlikely. Two studies have excluded mutations in the MEFV gene (responsible for familial Mediterranean fever) as an important factor in the underlying cause of PFAPA, but there has been no systematic investigation of patients with PFAPA for mutations in other genes associated with periodic fever syndromes.

SUMMARY

This young adult patient had periodic fever caused by TRAPS. Typical features of TRAPS did not appear until adulthood. He had signs and symptoms of PFAPA as a young child that abated after tonsillectomy. Nevertheless, the variable interval between febrile attacks as a child would be atypical for PFAPA, indicating that his childhood symptoms were probably caused by TRAPS. We suggest that TRAPS should be considered in patients of any age with periodic fever and other characteristic features. Moreover, TRAPS should be considered when evaluating young children presenting with apparent PFAPA, particularly if the clinical presentation is atypical or if symptoms become more characteristic of TRAPS over time.

REFERENCES

Prior fracture was associated with increased risk of new fracture in 601 members of a cohort studied between birth and 18 years. Hazard ratios for new fracture in data adjusted for age and sex were 1.90 (95% CI 1.51-2.39) after first fracture and 3.04 (95% CI 2.23-4.15) after second fracture. (J Pediatr 2005;146:286-8)

The influence of prior fracture on risk of new fracture during childhood and adolescence has received little attention, although it is well established that a history of prior fracture at any site is associated with an increased risk of future fracture in adults.1,2 To date, only one short, longitudinal study in girls has shown that children with fractures had a greater risk of breaking more bones than those who were initially fracture-free.3 Yet fractures are common adverse events during growth, and many children and adolescents break bones repeatedly.4 It is a concern that fractures in youngsters appear to be increasing.5

This study had two aims: first, to ascertain to what extent prior fracture increases risk of new fracture during childhood and adolescence by calculating the hazard ratios for new fracture in children presenting with earlier fractures, and second: to determine how much of the total fracture burden throughout growth is borne by subjects who have fractured on a single occasion and what proportion of the total fracture burden is accounted for by youngsters who fracture on more than one occasion.

METHODS

Data from the Dunedin Multidisciplinary Health and Development Study, a birth cohort study of 1037 New Zealand youngsters born in 1972/3 was used. Injury histories covering the period from birth to the age of 18 years were collected at regular intervals by questionnaire from participants who were seen as close as possible to their birthdays at ages 3, 5, 7, 9, 11, 13, 15, and 18 years, a period covering total growth.6 This assessment was one of a number related to health and development. Fracture information was part of a comprehensive question on injuries. Each phase of this study received ethics approval. A complete history was available for 601 participants. The fracture patterns of these participants with complete data did not differ from other cohort members who were not seen at every phase of the study.6 Hazard ratios for new fracture in persons presenting with previous fractures were calculated using survival analysis methods for discrete data. Adjustments were made for sex and age in 5-year age groups.

RESULTS

The Table shows that of the 601 study members investigated at every phase (10,818 person years of risk), 310 participants (51.6%) experienced no fractures, whereas 291(48.4%) reported fractures, with approximately 1 in 3 participants having a single fracture and 1 in 5, more than one. The mean (SD) ages in years for initial fracture, second fracture, third fracture, and fourth fracture were: 10.2 (4.3) n = 122, 12.3 (3.6) n = 46, 13.5 (4.0) n = 16, and 14.3 (2.5) n = 3 in girls and 10.5 (4.6) n = 169, 12.5 (3.2) n = 73, 14.4 (2.7) n = 41, and 14.9 (2.8) n = 18 in boys, respectively. The incidence rates of second and third fractures to age 18 years were substantially increased in participants with prior fractures. Hazard ratios for a further fracture after adjusting for age and sex were 1.90 (95% CI 1.51-2.39) at the first fracture and 3.04 (95% CI 2.23-4.15) after a second fracture.

The majority of the fractures occurred in the 119 participants who broke bones more than once. Thus the small proportion of the total sample population (19.8%) who sustained two or more fractures over the course of the study accounted for two-thirds (66%) of all the 498 fractures reported in the whole sample (Figure). By contrast, the 172 participants who...
broke a single bone during growth explained only 34% of the total fracture load between birth and age 18 years.

**DISCUSSION**

These results establish that prior fracture was associated with an increased risk of further fractures during growth. The findings confirm our earlier observations in another smaller sample and extend the age range over which any prior fracture has been shown to be associated with elevated risk for new fracture to include childhood and adolescence, in both girls and boys. Risk associated with prior fracture is much greater than that associated with measures of birth size and early growth or personal smoking between 15 and 18 years in the same sample. The magnitude of the increase of hazard ratios we record now is similar to that observed in adults, where persons with prior fractures have approximately twice the risk of future fractures compared with those without previous fractures. Reasons underlying this exacerbation of risk for fracture warrant further study but have not yet been elucidated even in adults. The elevated fracture risk could reflect inherent skeletal weakness or lower bone mineralization associated with genetic, hormonal, or nutritional factors or lower physical activity. High body weight also can favor fracture after falls. Alternatively, the explanation could lie in greater exposure to higher risk-taking behavior or greater trauma during sports and play. Poor muscular coordination or balance also might increase the propensity to fracture.

Study strengths include evaluation of a birth cohort for 18 years and regular collection of fracture information throughout growth. Lack of information regarding trauma exposure and the circumstances of initial and recurrent fractures were limitations. However, events precipitating fracture are similar for single and repeat fractures.

**Table. Fracture characteristics of the 601 children with complete fracture data available from birth to age 18 years**

<table>
<thead>
<tr>
<th></th>
<th>Girls (n=289)</th>
<th>Boys (n=312)</th>
<th>Total (n=601)</th>
</tr>
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<tbody>
<tr>
<td>Total fractures reported (n)</td>
<td>188</td>
<td>310</td>
<td>498</td>
</tr>
<tr>
<td>Total fractures reported by children with multiple fractures (n)</td>
<td>112</td>
<td>214</td>
<td>326</td>
</tr>
<tr>
<td>Participants who did not report any fractures*</td>
<td>167 (57.8%)</td>
<td>143 (45.8%)</td>
<td>310 (51.6%)</td>
</tr>
<tr>
<td>Participants reporting a single fracture</td>
<td>76 (26.3%)</td>
<td>96 (30.8%)</td>
<td>172 (28.6%)</td>
</tr>
<tr>
<td>Participants reporting multiple fractures*</td>
<td>46 (15.9%)</td>
<td>73 (23.4%)</td>
<td>119 (19.8%)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th></th>
<th>After first fracture</th>
<th>After second fracture</th>
</tr>
</thead>
<tbody>
<tr>
<td>All participants to first fracture</td>
<td>2.87 (2.38, 3.42)</td>
<td>3.88 (3.32, 4.51)</td>
</tr>
<tr>
<td>After initial fracture to second fracture</td>
<td>6.70 (4.90, 8.93)</td>
<td>8.47 (6.64, 10.66)</td>
</tr>
<tr>
<td>After second fracture to third fracture</td>
<td>8.42 (4.81, 13.67)</td>
<td>16.27 (11.67, 22.07)</td>
</tr>
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<table>
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<tr>
<th></th>
<th>After first fracture</th>
<th>After second fracture</th>
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<td>Incidence rate of fracture (95% CI)†</td>
<td>1.90 (1.15-2.39)</td>
<td>3.04 (2.23-4.15)</td>
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<th>After first fracture</th>
<th>After second fracture</th>
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<tr>
<td>Hazard ratio (95% CI)</td>
<td>1.90 (1.15-2.39)</td>
<td>3.04 (2.23-4.15)</td>
</tr>
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* Number and percentage of total sample.
† Number of participants fracturing divided by years of exposure before developing the fracture of interest expressed per 100 person years.
‡ Versus children who have not yet had a fracture, in data adjusted for age and sex.

Although the view that fracture is a normal event in childhood and adolescence is prevalent, we found that the majority of fractures occurred in children who fractured repeatedly. This subset of the population appears to have a higher fracture risk. It is therefore important to identify early in life youngsters who have a propensity for multiple fractures. Strategies to reduce pediatric fractures should perhaps be focussed on every child presenting with a first fracture because children with fractures have an increased risk of breaking more bones during growth. These youngsters should be encouraged to follow good nutrition, safeguard vitamin D status, maintain healthy body weight, undertake daily weight-bearing exercise, avoid smoking, and play sports in a safe environment. Such measures would seem worthwhile as intervention trials in
children have demonstrated that improved nutrition and load-bearing physical activity can strengthen bone.9,10

REFERENCES


FATAL ACUTE FIBRINOUS AND ORGANIZING PNEUMONIA IN A CHILD WITH JUVENILE DERMATOMYOSITIS
SAMPATH PRAHALAD, MD, MSc, JOHN F. BOHNSACK, MD, CHRISTOPHER G. MALONEY, MD, AND KEVIN O. LESLIE, MD

Acute fibrinous and organizing pneumonia, a recently described form of diffuse acute lung injury, sometimes affects adults with inflammatory myopathy. We describe a child with juvenile dermatomyositis who had development of acute fibrinous and organizing pneumonia. There does not appear to be a successful method of treatment, particularly in severe cases with respiratory failure. (J Pediatr 2005;146:289-292)

Juvenile dermatomyositis (JDM) is an uncommon disease of childhood. Acute pulmonary disease is known to occur in adult dermatomyositis, but it is not well described in JDM. A histologic pattern of acute lung injury termed acute fibrinous and organizing pneumonia (AFOP) has recently been described in adults and appears to be particularly associated with inflammatory myopathies. AFOP is a form of acute lung injury and possibly a variant of diffuse alveolar damage (DAD). We describe a fatal case of AFOP in a child with JDM and review other reports of acute lung injury in JDM.

CASE REPORT

A 14-year-old Hispanic girl was evaluated because of a photosensitive rash, joint pain, and muscle weakness. She had been treated with 3 weeks of prednisone therapy for possible systemic lupus erythematosus. She was born at 28 weeks’ gestation and underwent 1 month of care in the neonatal unit but subsequently was healthy without a history of bronchopulmonary dysplasia, reactive airway disease, or other respiratory symptoms. Her mother died at 24 years of age from complications of systemic lupus erythematosus. Examination revealed Gottron papules, heliotrope rash, and a rash over the left cheek, pinnae, neck, and forearms. Musculoskeletal examination revealed proximal muscle weakness, diffuse muscle tenderness, and symmetric polyarthritis.

Laboratory testing revealed elevated of some muscle-derived enzymes, von Willebrand factor antigen, and positive antibody to nuclear antigen (Table I). The chest radiogram was normal. Electromyography revealed extensive myopathic changes. JDM was diagnosed on the basis of the characteristic rash, weakness, elevated muscle-derived enzymes, and electromyographic findings.

Prednisone (30 mg prednisone bid, 1 mg/kg) and naproxen were begun. Over the next 8 weeks, rash, arthritis, and strength improved, but she had development of steroid-induced hyperglycemia and was started on insulin and metformin. Methotrexate was added as a steroid-sparing agent. She received 2 weekly doses of 10 mg (6 mg/m²) and a dose of 15 mg (8.9 mg/m²). This was stopped because aspartate aminotransferase and alanine aminotransferase remained abnormal and gamma glutamyl transferase (GGT) was elevated. Over the next 10 weeks she had development of cutaneous ulcerations. Hydroxychloroquine was added.

Two weeks later she had acute dyspnea. Computed tomography revealed extensive parenchymal process with patchy densities prominent in both lower lobes. An atypical pneumonia was diagnosed. She was treated with azithromycin and increased doses of corticosteroids. Four days later she was transferred to our institution, with persistent hypoxemia. On day 6 of her illness, bronchoalveolar lavage (BAL) was performed; testing for infectious pathologies, including Pneumocystis jirovecii (previously carinii), respiratory viruses, Mycoplasma, fungi, and bacteria was negative. Blood, BAL, and tissue cultures were negative.

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negative for bacterial, fungal, and mycobacterial organisms. She received 3 consecutive days of methylprednisolone (1 g/d) and intravenous immunoglobulin (2 g/kg) without improvement. Video-assisted thoracoscopic surgical lung biopsy revealed AFOP (Figure). Special stains for infectious organisms (acid-fast bacillus, Grocott methenamine silver, Warthin Starry, tissue Gram stain, and immunohistochemical stains for cytomegalovirus, adenovirus, and herpesvirus types I and II) were performed, all with negative results. Needle biopsy of the liver showed mild steatosis. On day 7, Mycoplasma immunoglobulin M was positive (1.09 [negative or equivocal <0.95]), but on day 11, both culture and polymerase chain reaction for Mycoplasma was negative on the lung biopsy tissue. Other laboratory tests were unrevealing (Table I).

She was treated with azithromycin, but the respiratory failure worsened over the next 5 days, with increased oxygen requirements and dyspnea. A repeat BAL and extensive evaluation again failed to demonstrate an infectious agent. Intravenous cyclosporine was started at 5 mg/kg per day, and a dose of cyclophosphamide at 500 mg/kg was administered intravenously. She was placed on conventional and then oscillating mechanical ventilation but had persistent pleural air leak and massive subcutaneous emphysema. She died 2 weeks after admission to our institution.

Autopsy confirmed the diffuse acute pathologic process in the lungs, with edema, fibrinous exudates, hyaline membrane formation (not identified on initial biopsy), type 2 pneumocyte hyperplasia, and areas of organization. This evolution over time to a more classic DAD pattern was possibly a consequence of terminal events rather than a manifestation of the natural history of AFOP.

**DISCUSSION**

Juvenile dermatomyositis is characterized by vasculopathy of the skin and muscles, proximal muscle weakness, and typical skin rash. Pulmonary involvement has been reported less frequently in children with JDM than in adult patients. Abnormalities of pulmonary function in the absence of respiratory symptoms have been described in JDM, suggesting that subclinical involvement might be more common than is generally appreciated. Several case reports describe the
occurrence of interstitial pneumonia (IP) and/or pneumothorax in JDM. 4-6 The IP in these cases was not well defined, and pneumothorax often dominated the presentation, sometimes with acute respiratory failure.

In 1975, Park and Nyhan 7 described a 12-year-old boy who had respiratory symptoms 4 months after the onset of JDM. Radiographs revealed bilateral basal infiltrates. He died after massive subcutaneous emphysema and pneumothorax. Histopathology of the lungs showed organizing DAD with proteinaceous exudates and hyaline membranes, expected findings for the organizing phase of DAD. His course was complicated by *Staphylococcus aureus* septicemia.

There are reports of fatal DAD in adults with dermatomyositis/polymyositis. 8-11 Tazelaar et al 8 reviewed lung biopsy specimens in patients with dermatomyositis/polymyositis and classified the observed IP into three groups: usual IP, bronchiolitis obliterans organizing pneumonia, and DAD. All three cases with DAD died after 1 to 6 weeks of respiratory failure. Lee et al 9 reported 5 patients with inflammatory myopathy and DAD. Despite intensive immunosuppressive therapy, all of them died. In a recent series, adults with AFOP who had respiratory failure requiring mechanical ventilation had a poor outcome, as did our patient. 1

Dyspnea developed in our patient 4 months after the onset of JDM, while receiving high doses of prednisone and hydroxychloroquine. Therapy with high-dose methyprednisolone, cyclosporine, intravenous immunoglobulin, and cyclophosphamide was insufficient to treat the pulmonary process. The patient had received 3 doses of methotrexate (a described cause of lung toxicity) approximately 12 weeks before the onset of pulmonary symptoms, but the lack of typical changes on lung biopsy, absence of peripheral eosinophilia, and failure to improve with immunosuppressive therapy argue against methotrexate-induced pneumonitis as a contributing factor. It is possible but unlikely that *Mycoplasma* initiated her acute lung disease but was rapidly eradicated by treatment with azithromycin. It is unclear what role, if any, prematurity or metformin therapy played in the development of the pulmonary process.

All examples of acute lung injury (including DAD) invoke the same differential diagnosis: infection, drug reaction, systemic connective tissue disease with pulmonary manifestation (as in our patient), and an idiopathic form (“acute IP”). The lung reactions encompassed by the terms AFOP and DAD probably represent forms of acute lung injury existing along a spectrum related to severity and mechanism of injury (Table II). 12-14 AFOP describes a relatively distinctive pattern of acute lung injury (eg, edema, fibrin exudation, and diffuse pneumocyte injury) lacking the classic hyaline membranes of DAD and sometimes affecting the lung in a more patchy distribution. Both imply a potentially adverse prognosis.

### REFERENCES

COARCTATION OF THE AORTA PRESENTING AS CEREBRAL HEMORRHAGE

A previously well 31-day-old boy presented to our hospital with agitation, tachypnea, ophistotonus, tensed fontanel, body temperature 39.5°C and a 2/6 heart murmur. Femoral pulses were not palpable, a blood pressure gradient between the upper and lower limb was determined (right arm 174/95 mm Hg, right leg 70/35 mm Hg). Cerebral hemorrhage was found on ultrasound examination, echocardiography showed a severe coarctation of the aorta (maximum blood flow velocity 5 m/s, Figure 1). Treatment was started with esmolol and surgical repair with end-to-end-anastomosis was carried out the next day. Magnetic resonance angiography revealed a right-sided intracerebral hematoma with origin in the nucleus caudatus (Figure 2). The boy recovered well from heart surgery but needed a ventriculoperitoneal shunt because he developed high cerebral pressure at the age of 8 weeks. The follow-up at the age of 21 months did not show any developmental disorders. No bleeding disorders were found. The family history was negative for congenital heart diseases.

Cerebral hemorrhage is an uncommon event in term infants after the neonatal period. Systemic hypertension may occur as one of the most striking symptoms. However, in our patient, brachiocephalic hypertension was most likely the trigger of the cerebrovascular event. Thus, coarctation of the aorta should be ruled out in hypertensive infants with cerebral bleeding.

There are multiple risk factors for cerebral hemorrhage in children. The cause of the bleeding can usually be found if the children are fully evaluated. The most likely cause of cerebral hemorrhage is an arteriovenous malformation that can be detected by cerebral angiography. Atypical localization of the intracerebral hemorrhage and the absence of arteriovenous malformation in MRI angiography are important findings to suggest other causes.

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REFERENCES


The role of leuprolide acetate therapy in triggering auto-immune thyroiditis

To the Editor:

We read with interest the report by Eyal and Rose regarding the occurrence of autoimmune hypothyroidism in a 9-year-old girl with precocious puberty who was treated with the gonadotropin-releasing hormone (GnRH) agonist, leuprolide acetate, for approximately 8 months.1 From this single case report, the authors suggested an association of leuprolide acetate with autoimmune thyroiditis and recommended thyroid stimulating hormone (TSH) screening before and 6 to 8 months into treatment. We believe the authors' conclusions are overstated.

The authors suggest that autoimmune disease is “related to potential immunostimulatory properties of GnRH agonists independent of gonadal steroids,” as stated in the paper. The reference cited in support of the authors' suggestion states “there is little evidence for direct immune actions of gonadotropins” and “it appears that the studies demonstrating immunostimulatory properties of GnRH were done exclusively in female rats.”2 No other accompanying organ specific or non-organ specific autoimmune disorders were reported. In the case presented, there was no documentation of autoantibody status before the start of leuprolide therapy. In addition, the patient had a sibling with primary hypothyroidism and a family member with type 1 diabetes (T1DM). Clustering of organ specific autoimmune disorders were reported. In the case report, the authors suggested an association of leuprolide acetate with autoimmune thyroiditis and recommended thyroid stimulating hormone (TSH) screening before and 6 to 8 months into treatment. We believe the authors' conclusions are overstated.

Based on this single case report, we therefore disagree with the suggestion that leuprolide acetate treatment requires thyroid screening. The use of primary autoantibody screening vs primary thyroid function screening also must be addressed. Screening always involves additional cost and, often, additional stress. Well-designed studies should be conducted before such recommendations are made. Outside a research setting, patients should be screened for autoimmune thyroid disease only on the basis of well-established risk factors and clinical presentation.

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10.1016/j.jpeds.2004.07.032

REFERENCES

Reply

To the Editor:

We greatly appreciate the knowledgeable comments regarding our paper. However, we believe that there is more than just a coincidence between the onset of hypothyroidism and the initiation of leuprolide acetate treatment in our patient, even though a positive family history of autoimmunity plays a role in the development of her autoimmune thyroiditis, as stated in our paper. The relation between gonadotropin-releasing hormone (GnRH) agonists and autoimmunity has been shown in animal and human studies and is supported by a few case reports in adults.

The responders quote a statement from one of the references. This reference gives strong evidence to support the possible role of GnRH in the exacerbation of autoimmune diseases. It summarizes that “GnRH is produced by lymphocytes and exerts potent immunomodulatory actions. GnRH has been shown to exert gender restricted immune actions in vitro and in vivo.”

There are a few case reports in adults that suggest the possible relation between GnRH agonists and autoimmune diseases. One 45-year-old woman developed transient autoimmune thyroiditis after initiation of leuprolide acetate treatment. Five months after initiation of the treatment, her thyroid function returned to normal with a decrease in her thyroid autoantibodies levels. Another case report describes a 37-year-old woman with lupus nephritis who developed exacerbation of her disease after initiation of leuprolide acetate treatment.

It is well known that gonadal failure, regardless of etiology, is associated with a high incidence of autoimmune
diseases. Gonadal failure is associated with elevated GnRH as a result of loss of negative feedback at the level of the hypothalamus. Patients with Turner’s syndrome and with Klinefelter’s syndrome have increased incidence of autoimmune thyroiditis.

Patients with systemic lupus erythematosus (SLE) display significantly elevated gonadotropin levels compared with controls. In a mouse model of SLE, GnRH agonist administration exerted effects on autoantibody levels in females.

It is certainly possible that our patient had euthyroid autoimmune thyroiditis as a background disorder and that the administration of leuprolide acetate triggered the development of hypothyroidism. We agree that thyroid function tests should not be performed routinely in every patient treated with GnRH agonist. However, we believe that there should be careful attention to thyroid status when GnRH agonists are given to patients who are at risk for autoimmune thyroid disease or when symptoms develop that suggest thyroid disease.

REFERENCES

Craniofacial features with growth hormone treatment

To the Editor:

In a cross-sectional study, Segal et al1 analyzed the effects of growth hormone (GH) treatment of variable lengths (0.2-15.5 years) on craniofacial growth with photographic facial morphometrics, head circumference, and hand and foot size in 52 children with GH deficiency (GHD), compared with untreated GHD children and normal first-degree relatives. They concluded that conventional GH doses partially correct craniofacial deficits and do not adversely affect hand and foot growth but appear to result in excessive head circumference growth. There was a trend indicating longer mandibular ramus length compared with normal relatives in the GH-treated patients who had been treated for the longest time or who had received the greatest cumulative GH doses.

We had reported acromegalic features at the end of GH therapy among 21 patients (9 girls) with GHD who had achieved final height. Although hand size was within the normal range, foot length for height was greater than the 97th percentile in 8 of 21 patients (5/9 girls), and linear jaw length, at lateral radiographs of the face, was greater than +2 SD in 4 of 21 patients (1/9 girls). Foot size percentile exceeded final height percentile in 11 of 21 patients (7/9 girls). During GH treatment, insulin-like growth factor I (IGF-I) and IGFBP-3 levels ranged from −3 to +2 SD. We concluded that long-term treatment with standard GH doses might be associated with acromegalic features, especially in girls, and that IGF-I and IGFBP-3 levels were not useful to predict these side-effects.

Have the authors noticed differences in head circumference, foot size, or hand size in relation to sex? Were IGF-I and IGFBP3 levels measured during GH treatment and related to outcome?

Prospective longitudinal studies measuring head circumference, lower jaw, and hands and feet, before and during GH therapy, are necessary to further elucidate the frequency and extent of these side effects.

REFERENCES

Reply

To the Editor:

We appreciate the comments of Carvalho et al. Our data do not demonstrate any sex differences between boys and girls in foot and hand lengths for age and height age. Foot lengths for age for girls ranged from less than the 3rd percentile to greater than the 97th percentile, with a mean between the 25th and 50th percentiles. For boys, foot lengths for age ranged from the 3rd percentile to greater than the 99th percentile, with a mean near the 50th percentile. When analyzed for height age, girls had foot lengths that ranged from the 10th
percentile to greater than the 97th percentile, with a mean between the 50th and 75th percentile. Only 2 girls had foot lengths for height age greater than the 97th percentile. Boys had foot lengths for height age that ranged from the 25th to greater than the 97th percentile, with a mean near the 75th percentile. Only one boy had a foot length greater than the 97th percentile. Unfortunately, IGF1 and IGFBP3 levels were not measured consistently during treatment. We agree that longitudinal prospective studies should be conducted.

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YMPD1152
10.1016/j.jpeds.2004.08.033

Effects of exercise training on vascular function in obese children

To the Editor:

We read with great interest the article, “Effects of exercise training on vascular function in obese children.” The authors examined the influence of 8 weeks exercise training on endothelial function in 14 obese subjects, 8.9 ± 0.4 years of age, with high resolution ultrasonography and flow-mediated dilatation of the brachial artery. We reported the effects of obesity on the stiffness of the abdominal aorta using trans-thoracic echocardiography in 50 obese pediatric patients (25 normotensive obese, 25 hypertensive obese), mean age 12.2 ± 1.3 years. In the group with obesity, aortic strain, pressure strain elastic modulus, and normalized elastic modulus measurements were significantly different than in the control group. These findings may be important in determining the relationship between obesity and endothelial or arterial function in children. In both studies, the overweight children had impaired endothelial function. These abnormalities of obesity and arteriosclerosis have traditionally been viewed as problems of adulthood, but they may begin in childhood or adolescence. We did not investigate the effect of exercise training or other therapies on impaired vascular function.

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YMPD1197
10.1016/j.jpeds.2004.08.078

REFERENCES


Reply

To the Editor:

We thank Dr Levent and colleagues for drawing our attention to their paper, which indicates that obese hypertensive children possess evidence of increased aortic stiffness. Although no data on endothelial function were provided in their study, endothelium-derived nitric oxide can modulate large artery stiffness, at least in adults. Their results therefore compliment our own, which indicate that obesity, in the absence of other recognized cardiovascular risk factors, impairs endothelial function in obese children and adolescents.

It would be interesting to determine whether interventions could improve arterial stiffness in obese children, including those with hypertension. Levent and colleagues did not investigate this possibility, but in our studies, exercise training improved the impaired endothelial function evident in obese children and adolescents. Levent and colleagues also emphasize the importance, in intervention studies, of investigating relationships between changes in cardiovascular risk factors. It is possible that the beneficial effect of exercise training we observed was mediated through risk factor modulation, but we observed no changes in blood pressure, plasma lipids, or body mass index as a result of training. Furthermore, based on our previous studies of adult groups in whom short-term exercise training improved vascular function without changing cardiovascular risk factors, we strongly suspect an important role for direct effects of exercise on the vasculature, mediated through episodic increases in wall shear stress during acute bouts of exercise. Apart from the obvious advantage that it is a nonpharmacological intervention in obese children and adolescents, exercise training may therefore exert direct shear stress-mediated beneficial vascular effects, as well as the well-described indirect effects on cardiovascular risk factors.

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REFERENCES


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**Abbreviations.** Complex terms used frequently in the manuscript may be abbreviated. The manuscript should include a list of all abbreviations used. Abbreviations are placed in parentheses at first use in the abstract, and again at first use in the text.

**Medical Progress**

Authors considering preparation of a review article for the Medical Progress section should submit a proposal letter and outline to the Editors for approval before submitting the full paper.

**Invited Commentaries**

Commentaries are generally invited only. Authors who wish to propose a commentary can submit a proposal letter and outline to the Editors for approval before submitting the full paper.

**Clinical and Laboratory Observations**

Papers in this format should fill 3 journal pages or less, the text 1000 words or less with a brief abstract of 50 words or less. A combined total of 2 illustrations and tables, and approximately 10 references, is recommended.

**Insights**

Submissions to the Insights section of *The Journal* should succinctly illuminate clinical problems or solutions of interest to readers and must fit on one published page. Captioned photographs, brief anecdotes or analyses, or even cartoons are welcome; however, a fresh, useful clinical insight must be offered. All material must be original. Text must not exceed 300 words and is subject to shortening if the text and figure(s) do not fit on one published page. Figure(s) and references may be placed in the online only version of *The Journal* if the piece exceeds one published page. Photograph(s) must be original glossy prints, and artwork must be in the original form (see “Illustrations” above). Original, signed, written permission from the patient, or parent or guardian of a minor child, is required for publication of recognizable photographs in all forms and media. (See “Permissions” above.) Contributors will be required to sign a standard copyright transfer agreement; therefore, all submissions must have a title. Submissions will undergo review by the Editors, and their decision to accept or reject will be final (figures from rejected contributions will be returned).

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Authors for Grand Rounds should send a proposal letter before submission to ensure that their topic is not covered by a manuscript already in process. Manuscripts for the Grand Rounds section may be prepared in traditional clinico-pathologic conference (CPC) style or as a didactic discussion.

**Editorial Correspondence**

Letters pertaining to papers published in *The Journal* within the past year or to related topics should not exceed 300 words. Letters must be double–spaced. References, including reference to
Inquiries Regarding Decisions

All inquiries concerning manuscript decisions should be in writing; please see contact information above. The complete manuscript file will be forwarded to the appropriate Associate Editor for response to the inquiry. The Editors are not available for telephone calls regarding decisions.

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Pages of The Journal are reserved for the Association of Medical School Pediatric Department Chairs, Inc., (AMSPDC) which is solely responsible for their content. Only authors interested in this section should contact AMSPDC directly. All other papers must be submitted as detailed above.

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News Items

Announcements of scheduled meetings, symposia, or postgraduate courses of interest to the pediatric readership may be sent to the publisher for consideration at least 5 months in advance of the meeting date. News items of general interest to pediatricians and related specialists will also be considered.

Books for Review


Checklist

- Letter of submission
  - Names and complete contact information for 5-7 suggested reviewers
- Statements that all authors take full responsibility for the paper and that the paper is not nor will be submitted to any other journal while being considered by The Journal of Pediatrics
- Disclosures of any conflicts of interests and prior publications
- Title page
  - Title of article
  - Full name(s), academic degrees, and affiliations of authors
  - Name, address, e-mail address, telephone and fax numbers of corresponding author
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  - List of key words not in the title
  - Source of funding and conflict of interest statement, if applicable
  - Short running title (<12 words)
- Abstract (double-spaced), structured (200 words) for Original Article or unstructured (50 words) for Clinical and Laboratory Observations
- Article proper (double-spaced), including
  - List of abbreviations (double-spaced)
  - References (double-spaced), on a separate page
  - Figure legends (double-spaced), on a separate page
- Tables including title (double-spaced), each on a separate page, saved as a separate file
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February 2005

American Heart Association’s Scientific Councils on Cardiovascular Disease in the Young, Clinical Cardiology, and Cardiovascular Radiology and Intervention, and co-sponsored by the Kawasaki Disease Foundation, the Japan Kawasaki Disease Research Center, and the Japan Heart Foundation, “8th International Symposium on Kawasaki Disease,” February 17-20, 2005, Omni San Diego Hotel, San Diego, California. This 3-day conference intends to identify areas of progress in Kawasaki disease research, address controversies surrounding the modification of the current clinical case definition, and introduce new therapies for treatment of acute vasculitis. For further information, please contact: Andrea Zabkar, Conference Coordinator, American Heart Association, E-mail: scientificconferences@heart.org; Web site: www.myamericanheart.org.


March 2005


April 2005


May 2005

Programme for Global Paediatric Research Symposium, “Birth Asphyxia: A Review of the Clinical Problem” and “Platform Presentations from Selected Abstracts: A Global View on Birth Asphyxia,” May 17-18, 2005, Washington, D.C. For further information, please contact: Professor Alvin Zipursky, Chairman and Scientific Director, The Programme for Global Paediatric Research, The Hospital for Sick Children, 555 University Avenue, Toronto, ON M5G 1X8 Canada; phone; 416-813-8762; Email: Alvin.Zipursky@sickkids.ca.