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THE EDITORS’ PERSPECTIVES

More intensity of care may not be good for preterms

It has been demonstrated repetitively that regionalization of care and maternal transfers improve outcomes for preterm infants. Outborns do not do as well when transferred to perinatal centers (obstetric plus neonatal care) as do inborns in those centers. Shah et al ask a more focused question: do outborn infants with gestational ages ≤32 weeks do better when transferred to perinatal centers or freestandard children’s hospitals with neonatal intensive unit services? The characteristics of the services differ: the perinatal center cares primarily for preterm infants, while the units in children’s hospitals specialize in the care of term infants with complex medical and surgical diseases. The preterms had a lower death rate, less nosocomial infection, and less bronchopulmonary dysplasia when cared for in a perinatal center. My preferred interpretation is that the care at the perinatal center will be less intensive in a less frenetic environment than at the children’s hospital, which seems to translate to better outcomes for preterms. Many neonatologists who care primarily for preterms are using fewer catheters, fewer drugs, and fewer other interventions than in the past—behaviors that differ from care strategies of surgical units. This article may result in a rethinking of regional referral preferences for the very preterm infant.

—Alan H. Jobe, MD, PhD
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Policy issues for newborn screening programs

Many states in America are contemplating expansion of their newborn screening programs to include cystic fibrosis or new testing for metabolic diseases made possible by tandem mass spectroscopy. The former is based on the recent publications from the Cystic Fibrosis pilot programs and from the Centers for Disease Control.

Wilfond and Gollust have completed a review of the current approaches to screening for cystic fibrosis in 12 programs offered in 11 states in 2002. Telephone interviews were conducted in the spring of 2003 and the results of this survey, and the issues that were raised, are presented in this issue of The Journal. The results show a considerable range of current approaches but also provide some general directions for the future.

—Robert W. Wilmott, MD
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Recognition of SLE

Children and adolescents with systemic lupus erythematosus (SLE) usually are cared for by a variety of subspecialists, depending upon their specific manifestations of the disorder and local custom. These could include immunologists, rheumatologists, nephrologists, hepatologists, dermatologists, and others. On the other hand, they may present for the first time to generalists.

Although various aspects of childhood SLE have been reviewed in the past few years, a comprehensive analysis of the initial presentation has not been published recently. For that reason, the very nice study of Bader-Meunier and associates in France is welcome. These authors examined the presenting features of 155 children under 16 years in a multicenter study in France. There are several take-home points in this study. Many of these children presented with very severe disease which initially did not meet the classical clinical criteria for SLE. Most physicians who deal with this disease regularly have seen children in whom a period of time elapsed before a definitive SLE diagnosis could be made; this study reinforces this observation. The study also highlights some unusual presenting features, including very severe abdominal pain and thrombophilia. This work also confirms the old clinical pearl that a discordance frequently exists between the ESR (markedly elevated) and CRP (often normal) in SLE.

—Thomas R. Welch, MD

Steroids and GI perforations in preterms – again

Bronchopulmonary Dysplasia (BPD) is a major complication of the postnatal management of very low birth weight infants. Because BPD is thought to be caused primarily by lung inflammation, postnatal corticosteroids have been frequently used very early in the clinical course to “prevent” BPD or later to “treat” BPD. Postnatal steroids (primarily dexamethasone) have been associated with poor neurodevelopmental outcomes, although their use can decrease mechanical ventilation and increase survival. To avoid toxicity, recent trials have used hydrocortisone in lower doses and for shorter durations than previous trials. Nevertheless, Watterberg et al (Pediatr 2004;114:1649-1657) recently reported that a trial of early hydrocortisone was stopped because the combination of hydrocortisone and indomethacin increased gut perforation. In this issue of The Journal, Peltonieni et al report another trial of early hydrocortisone treatment that was stopped because of gut perforation, again associated with indomethacin use. The most interesting aspect of the trial was the evaluation of cortisol levels and ACTH responses prior to the hydrocortisone treatments that were begun before 36h of age. Infants with high serum cortisol levels had a high risk of gut perforation, while infants with low cortisol values seemed to benefit from the hydrocortisone treatment. As in adults, a targeted and selective use of corticosteroids may be beneficial. The challenge is to identify those infants who may benefit.

—Alan H. Jobe, MD, PhD

Improving parenteral nutrition for low birth weight infants

Earlier reports, particularly in adults, have suggested that supplementing parenteral nutrition with glutamine leads to improved clinical outcomes. The results have been less conclusive in low birth weight infants. Kalhan and colleagues at Case Western University have completed a randomized, controlled trial of glutamine supplementation in 20 such infants. The results show that glutamine supplementation led to reduced rates of whole-body proteolysis, which should be clinically beneficial.

—Robert W. Wilmott, MD
Infection in ALL—a need for ongoing vigilance?

Most pediatricians are familiar with the risk of overwhelming infection in children with acute lymphocytic leukemia (ALL) during chemotherapy. The admission and treatment of febrile, neutropenic patients with ALL is commonplace in most pediatric hospitals.

Less is known, however, of the infection risk in children with ALL in remission and who are off chemotherapy. There have been some suggestions that defects in humoral immunity may be present in such children.

In the current issue of The Journal, Brodtman and associates in New York examined antibody titers for a variety of bacterial and viral pathogens in 100 children in remission of ALL. A number of children exhibited non-protective titers to one or more of the pathogens studied. The response to re-immunization was variable. There did not seem to be a correlation between non-protective titers and the type of chemotherapy employed; older age at diagnosis was more likely to be associated with protective titers for some but not all pathogens.

The authors extrapolate from these data to recommend routine monitoring of humoral immunity in children recovering from ALL. While this is a logical outgrowth of their observations, it may represent an overenthusiastic response to the data. Nonetheless, this is clearly a matter for further study, and something of which physicians caring for the many children now “cured” of ALL should be aware.

—Thomas R. Welch, MD

Classic and nonclassic cystic fibrosis

Over the last few years, it has become apparent that the diagnosis of cystic fibrosis (CF) applies to an expanded phenotype. The term “nonclassic” CF has been introduced to describe a group of patients who do not have the full spectrum of abnormalities seen in “classic” CF. In this issue of The Journal, Groman and others report extensive phenotypic characterization and genetic analysis on 158 patients with nonclassic CF. Patients referred with one common CF mutation—absence of the vas deferens or P. aeruginosa in the sputum—were very likely to have a second CFTR mutation on detailed genetic analysis. By contrast, the concentration of chloride in sweat, the presence of clinical diagnostic criteria for CF, and the presence or absence of steatorrhea were not predictive for having two CF mutations. This study suggests that men who present with clinical features of nonclassic CF should be checked for absence of the vas deferens. Secondly, it indicates that patients with nonclassic CF and steatorrhea should be studied for abnormalities in molecular mechanisms that do not affect CFTR.

—Robert W. Wilmott, MD

Insufficient intubation opportunities for resident training

The Pediatric Resident Review Committee of the Accreditation Council for Graduate Medical Education expects that pediatric residents will be competent to intubate infants at completion of training. However, with changes in the indications for intubation for meconium at birth, an increasing use of ventilation strategies that avoid endotracheal intubation, and decreased work hours for residents on neonatal services, is it realistic to expect competence in intubation? Leone, Rich, and Finer document what neonatologists who train residents understand—the answer is no. The mean number of newborn intubation attempts over the 3 years of training decreased from 38 in 1994 to 12 in 2002. Anesthesiologists consider that more than 40 successful intubations are necessary to assure competence, and pediatric residents having 10 to 20 attempts were successful only 38% of the time. What is the solution? Different training programs use animal labs, manikins, or simulators to provide resident training experience. However, it is not clear that such training strategies can, in fact, replace real time intubation experiences. The importance of procedural skills training for pediatric residents is an important problem that is not acknowledged or not being adequately addressed.

—Alan H. Jobe, MD, PhD

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CHILDREN AS CLINICAL RESEARCH SUBJECTS

RUSSELL W. CHESNEY, MD

For many years there existed a concept, especially in the United States, that children needed to be safeguarded from use as subjects in clinical research. This notion arose from good intentions and posited that children were a distinct class that needed protection. More recently, pediatricians and others have vigorously argued that, as was the case for women and minorities, children have not benefited equally from biomedical advances as compared with adults (especially adult Caucasian men). Children remained “therapeutic orphans,” with the usage of most drugs in children based upon extrapolation of adult data to younger groups. The manifest differences in drug metabolism, pharmacokinetic profiles, age- and weight-related factors, and excretion patterns between child and adult could lead to overdosing, underdosing, or particular adverse events not found in adults.

The Best Pharmaceuticals for Children Act of 2002 (Public Law 107-109) called for the Institute of Medicine (IOM) to prepare a report that reviewed federal regulations, reports, and research and made recommendations concerning desirable practices in clinical research in children. This law called for commentary in seven areas: (1) the value of regulations in children of various ages; (2) the interpretation of regulatory criteria for approving research; (3) the issue of consent; (4) the expectations and comprehension of children and parents participating in research; (5) the appropriateness of payments related to a child’s participation in research; (6) compliance with and enforcement of federal regulations; and (7) the unique tasks of institutional review boards (IRBs).

The report represents the work of a 14-member committee of the IOM. It emphasizes clinical research pertaining to preventive, diagnostic, therapeutic, or similar interventions or to direct interactions in children. The report focuses on three broad themes: (1) the need for well-designed and well-executed clinical research involving children in the United States and worldwide; (2) a robust system for protecting human research participants, in general, is a necessary foundation for protecting child research participants, in particular; and (3) effective implementation of policies to protect child participants in research requires appropriate expertise in child health at all stages in the design, review, and conduct of such research.

The IOM committee stated that the federal regulations (subpart D of 45 CFR 46 which defines categories of research involving children) providing special protection for child participants are, in general, appropriate for children of different ages. The main problems with federal regulations involve insufficient government guidance covering interpretation, shortfalls in data about compliance, and variability in investigator and IRB interpretations.

The IOM report examines the interpretation of research risks and recommends that minimal risk should be interpreted in light of “the normal experiences of average, healthy, normal children.” It also suggests that one should “consider the risk of harm or discomfort in relation to the ages of the children to be studied.” Existing federal laws permit research that involves a minor increase over minimal risk without the prospect of direct benefit if the research involves children with a disorder or condition. The report, which focuses upon the term condition, refers to a specific physical, psychological, neuro-developmental, or social characteristic that an established body of scientific evidence or clinical knowledge has shown to negatively affect children’s health.

The issue of understanding and agreeing to children’s participation in research involves the concepts of parental permission and child assent. The IOM committee felt that IRBs should focus more on the adequacy of the process for securing permission and assent. A sensitive issue is to provide children the opportunity to express and discuss their willingness or unwillingness to participate.
Payments related to research participation are controversial and should involve IRB policies that adapt explicit written policies on acceptable and unacceptable amounts of payments related to research participation. Payments, including reimbursement for reasonable expense, should be allowed, but paying the parents for allowing the child to be exposed to a greater risk or more painful procedure should not be allowed.

The roles and responsibilities in protecting children involved in research also are of major concern. An emerging concept is that parents are central in protecting the child. They need to be given information about the study so that they can protect and articulate the details to their child. The principal investigator has ultimate responsibility for ensuring the safety, rights, and welfare of persons participating in research, and for assuring that all members of the research team adhere to the requirements for valid, ethical research. Among the factors to be considered are the disclosure of potential conflicts of interest, the support of appropriate safety monitoring, the reporting of adverse events, and the disclosure of research results to the scientific community and the public.

The IRB also has a role in the protection of child subjects. This can include the use of IRB pediatric consults concerning ethical, legal, and scientific issues or the provision of child-relevant reference materials and resources concerning child-centered research. A final recommendation stated that

IRBs should have at least three persons with adequate expertise in child health and research.

Finally, the IOM report recommended strongly that the Office of Human Research Protection, the Food and Drug Administration, and the National Institutes of Health should provide, in a set of linked documents, comprehensive, consistent, and updated guidance to the interpretation and application of federal regulations for the protection of children in research for investigators, IRBs, and others.

This IOM report should be of interest, and hopefully valuable, to all pediatricians and clinical researchers engaged in child-centered research.

REFERENCES

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n this issue of The Journal, Cloutier et al describe their efforts to improve quality of care delivered to a poor, urban, pediatric asthma population. Cloutier et al have outlined the impact of a locally developed guideline and associated treatment plan in six primary care sites that care for 85% of the children in Hartford, Connecticut. The primary outcome measures were hospital admissions and emergency department visits. They also tracked inhaled corticosteroid usage. Using Medicaid claims data, children were compared before and after enrollment in the program. Several significant differences were noted after enrollment: a 35% decrease in hospitalizations, a 27% decrease in emergency department visits, and a 25% increase in claims for inhaled corticosteroids. The study was neither randomized nor masked; however, the use of each child as his or her own control and the use of a time-adjusted control group mitigate these concerns. In addition, because the evidence is fairly convincing and the risk of this intervention is minimal, it is reasonable for the authors to take this approach to improve the care delivery system. The results are effectively displayed in a 4-year run chart. We expect to see run charts and statistical process control charts used more commonly as part of delivery system improvement.

Flores et al have documented a wide range of willingness among pediatricians to use evidence-based guidelines developed at the national level. Muething et al previously showed that guidelines with clear diagnostic criteria and simple point-of-care tools developed locally decrease unwarranted variation, increase adherence to evidence-based practice, and improve outcomes. This study by Cloutier et al confirms these findings and extends the experience to an outpatient setting for a high-risk population.

Asthma is the most common chronic condition affecting children. The condition is particularly prevalent in poor, urban, minority populations, affecting as many as one-third of such children. Cloutier’s findings have added significance for immediate application. Mannino et al, analyzing Centers for Disease Control data, reported a national rate for asthma hospitalizations of 55.4/10,000 children 0 to 4 years of age. For urban populations, Blaisdell et al reported an asthma hospitalization rate of 134/10,000 children 0 to 4 years of age in Baltimore. Although the exact cost of the program described in Cloutier et al’s study is not reported, the intervention costs appear to be relatively low. The potential for a significant reduction in hospitalizations and emergency department visits for such a high-risk group makes the findings even more compelling.

Of note, however, is what the results also highlight: the reliability of the delivery system in place. Three years after introducing this program to the providers in these six clinics approximately half of the children believed to have asthma had been enrolled. This finding is consistent with measures of reliability of the US healthcare delivery system in general. In a national survey, McGlynn et al reported patients received only 55% of recommended care and preventative therapy. Although this study focused on adults, it is reasonable to believe the reliability of care delivery for children is similar. Berwick and Nolan defined reliability for healthcare as, “the capability of a process, procedure or health service to perform its intended function in the required time under existing conditions.” The Cloutier et al and McGlynn et al studies demonstrate delivery systems with a reliability of approximately 50%, or error rates of 5 of 10.

Weick and Sutcliffe have described the attributes of “High Reliability Organizations” and have outlined the key principles of the system design required to achieve the desired level of performance reliability. Different levels of reliability will require different design principles. Providers working to improve a delivery system for evidence-based asthma care in children may have a goal of 90% reliability, or an error rate of 1 of 10 (Level 1 reliability). A system designed to prevent postoperative infections may establish a goal of 99% reliability, equal to an infection or error in 1 of 100 patients (Level 2 reliability). Hospital systems designed to prevent adverse drug events will seek error rates of 1 of 1,000 patients or reliability of 99.9% (Level 3 reliability). This concept of system design linked to system reliability is fundamental to reliability science. Berwick and Nolan have outlined the design principles necessary to reach Level 1 or 90% reliability in healthcare delivery systems: standardization of approach, awareness and training, feedback of data, and reminders. A Level 1 evidence-based system that includes agreement by the providers to use the standard protocol whenever appropriate, monthly or quarterly feedback of results to providers, reminder stickers on charts of children with asthma, and knowledge of the protocol by all staff, as well as patients and families, will attain 90% reliability.

Of note, however, is what the results also highlight: the reliability of the delivery system in place. Three years after introducing this program to the providers in these six clinics approximately half of the children believed to have asthma had been enrolled. This finding is consistent with measures of reliability of the US healthcare delivery system in general. In a national survey, McGlynn et al reported patients received only 55% of recommended care and preventative therapy. Although this study focused on adults, it is reasonable to believe the reliability of care delivery for children is similar.

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Higher levels of reliability will not be achieved by merely expecting more of the current system; it will require redesign. Level 2 reliability principles include: checklists, redundancy, making the desired action the default, and real-time identification of errors. A practice achieving 97% reliability on an evidence-based asthma protocol will likely have widespread use of standard order sets or prescriptions, staff tracking all asthma patients using an electronic registry, and patients automatically receiving appropriate education unless otherwise ordered by the provider.

As knowledge of reliability science increases in the healthcare field, we can expect to see a growing body of work demonstrating improved outcomes for patients based on redesign of systems. The addition of reliability science to the underlying principles of evidence-based care already outlined in this manuscript will help further reduce unnecessary hospitalizations. We call on pediatric organizations and practices to use these concepts in care for children with asthma.

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DIET, IMMUNITY, AND AUTISTIC SPECTRUM DISORDERS

Autistic spectrum disorders present many challenging issues for families and those involved in the care of autistic children. The etiology of these disorders has become an area of significant controversy, largely due to uncertainties about temporal change in their incidence. There has been difficulty in reconciling prevailing largely genetic theories of causation with evidence suggesting a marked increase in incidence in the last 20 years. However, diagnostic criteria and thresholds have been altered during that time. Others argue that the true overall incidence is in fact unchanged.

Areas of uncertainty include the possibility of extracranial abnormalities in autism, notably within the gastrointestinal (GI) and immune systems. There have been several reports of gut abnormality in children with autism, and at least a proportion of autistic children show excess lymphocytic infiltration throughout the upper and lower GI tract. Several studies have suggested that circulating lymphocytes and monocytes may show excess activation status and dysregulated cytokine production in autism, although some have shown increased lymphocyte production of T_{H}2 cytokines and others a contrasting T_{H}1 dominance. Whether therapeutic modulation of GI or immune function may be beneficial, and for whom, remains uncertain. Despite a variety of anecdotal reports, there have been few properly controlled trials.

There also have been anecdotal reports and one small controlled trial to suggest that exclusion of cow’s milk and wheat from the diet may exert beneficial effects on behavior and cognition in some children. The study by Jyonouchi and colleagues in this issue of The Journal contributes to the debate by suggesting that lymphocyte responses to dietary antigens may be abnormal in autistic children, overlapping with those seen in children with non-immunoglobulin(Ig)-E-mediated food allergy. The non-IgE-mediated food allergies themselves represent a rather poorly characterized disease group, lacking the precision in diagnosis made possible in IgE-mediated allergy by specific IgE and skin prick testing, and the unequivocal answers on food challenge testing. However, recent reports point toward a more stereotyped, and thus recognizable, clinical presentation in non-IgE-mediated food allergy than previously thought.

Thus future studies may determine whether there is an area of true overlap between non-IgE-mediated food allergy and autistic spectrum disorders. One important clinical issue is the need for appropriate dietetic

GI  Gastrointestinal
Ig  Immunoglobulin

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J Pediatr 2005;146:582-4. 0022-3476/ $ - see front matter

See related article, p 605.
supervision of any exclusion diet, as significant nutritional inadequacy may occur with unsupervised exclusion.\textsuperscript{16}

There are several factors that need to be considered in assessing what this study adds to the literature. First, it is important to recognize heterogeneity among patients with autistic spectrum disorders, and an apparent over-representation of a regressive history in those with GI symptoms.\textsuperscript{3-6,8} This study of 109 children contained 75 with GI symptoms, which represents a much higher proportion than reported in other population-based studies. Thus more detail about the mode of recruitment and clinical presentation of these patients would be helpful in providing context. The 16-fold male dominance in the group without GI symptoms is unusual, and the relative frequency of \textit{Candida albicans} overgrowth contrasts with findings by others.\textsuperscript{3,17} The extent of overlap of these cases with those reported in the authors' previous publication of T\textsubscript{H}1 and T\textsubscript{H}2 responses to these antigens\textsuperscript{9} also is important, as similar findings in a separate cohort of more than 100 children would support the specificity of these findings in autistic spectrum disorders. In addition to patient selection, the methodology used for cytokine analysis is relevant. The technique used in this study, assaying supernatant fluid from peripheral blood lymphocytes cultured for several days with dietary antigens, has previously given conflicting results and remains far from being considered a "gold standard." Recent methodological advance, by loading lymphocytes with carboxyfluorescein succinimidyl ester before culture, allows detection of cytokine production by proliferating cells using flow cytometric analysis and offers much greater specificity for future studies in determining true antigen-specific T\textsubscript{H}1/T\textsubscript{H}2 skewing.\textsuperscript{18} With these reservations, and particularly mindful of the fact that in vitro reactions may have very little relevance to clinical responses, these findings do support the rather "gray" literature on unusual dietary responses in autism.

The development of non-IgE-mediated food allergies is associated with relatively subtle immunological abnormalities, including maturational delay in IgA, IgG subclass, CD8, and NK cell responses.\textsuperscript{14} In particular, there may be impaired development of regulatory lymphocytes, notable those producing transforming growth factor-\beta.\textsuperscript{19,20} which may reflect a low transforming growth factor -\beta producer genotype.\textsuperscript{21} These findings point to a suboptimal innate immune response to early infectious exposures, likely due to a combination of genetic predisposition to low innate responses and altered infectious exposures.\textsuperscript{22} A family history of allergy is common in autistic children with GI symptoms.\textsuperscript{4-6} This begs the question whether shared susceptibility simply represents shared genetic predisposition to unrelated disorders, or whether genes involved in immune processes may be relevant in the development of autism. There is some evidence favoring the latter, and the null allele of complement C4B (C4BQ0) was identified in 42% of autistic children in Oregon and Utah against 14% controls.\textsuperscript{23} Importantly, the C4B null allele status was inherited with a non-uniform genotypic basis, with some showing a monomodular deletion of C4 gene, others a C4BQ0 associated duplication of C4A.\textsuperscript{23} Thus deficient C4B expression, however determined, appears to be a risk for autism. The relative risk of C4B null status was a substantial 4.33 in this study,\textsuperscript{23} suggesting that immunological responses may be significant in the development of autism for at least some children. The significance of C4B null status is the predisposition it confers to the development of autoimmunity, which occurs in excess in families of autistic children\textsuperscript{24} and indeed in non-IgE-mediated food allergy.\textsuperscript{14} There are reports suggesting cerebral or intestinal autoimmunity as possible disease mechanisms in autism,\textsuperscript{5,25} but as yet no confirmatory evidence. Recent data suggest that immunological factors may indeed play a role in the development of autism, but findings of activated neuroglial cells occurred without focal Ig deposition within brain tissue of autistic children, studied post-mortem after dying of unrelated causes.\textsuperscript{26}

The area of systemic immunity in autism clearly remains understudied. There are as yet no hard data showing whether systemic immune dysregulation is truly common in autism, with cognitive abnormality essentially the tip of an iceberg of subtle multi-system abnormality, or whether the children reported in this and other studies represent atypical subgroups largely irrelevant to the main body of autistic children. It is notable that several of the genes linked to autistic spectrum disorders exert effects in immune and intestinal development,\textsuperscript{27} and it is possible that subtle impairment of cellular interaction may show up most clearly in the developing central nervous system. This is shown in Rett syndrome, where DNA methylation is globally impaired by MECP2 mutation,\textsuperscript{28} but cognitive effects are more recognizable than the subtle lymphocyte abnormalities.\textsuperscript{29} The real imperative for further work on immunity in autism comes from animal studies, in which the development of immune mediated neuropathology can be prevented by early immunosuppression.\textsuperscript{30} Dietary exclusion may or may not have beneficial effects in the manifestation of autistic symptomatology; it will not prevent its development. We need to know more about the links between the immune and GI systems and the brain in autism, to determine whether these are legitimate avenues for novel therapy in selected cases or even for attempted primary prevention in genetically at-risk subgroups.

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In this issue of The Journal, Vrakking et al report the results of a survey of Dutch physicians regarding their willingness to hasten death for some children. The law in The Netherlands now permits euthanasia when a 16-year-old requests it, with parental notification, and when a 12- to 15-year-old requests it and the parents agree. The authors of the current study sought to understand if Dutch physicians might also agree to administer life-shortening medication to children falling outside of the boundaries of existing law. The authors note that nearly a quarter of neonatal and infant deaths in their country occur after use of drugs that could shorten life when given for the purposes of symptom relief—a figure that might not differ much from what happens in the United States, though we lack comparable data—and 8% of the Dutch infant deaths follow “the use of lethal drugs.”

The report from the Netherlands raises two quite different kinds of issues. The first concerns the authors’ research method and the second regards the ethics of assisted dying/euthanasia in pediatrics.

Although the opinions of physicians responding with fixed-choice answers to hypothetical situations can provide some evidence of intent, belief, and predisposition to act, such results quite imperfectly predict actual behavior. One cannot reasonably hope to generalize from Vrakking’s survey results—however well

SURVEYING EUTANASIA PRACTICES: METHODS AND MORALITY

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they do or do not map onto real-world actions—beyond the
borders of The Netherlands. Even with the survey results, do
we know what Dutch physicians would do when faced with
the request of an 11-year-old with relapsed myeloid leukemia
and increasingly distressing symptoms to administer a lethal
injection, with parental approval? We hope they strive more
assiduously to achieve adequate palliation, including psy-
chologic counseling, psychoactive medication, and pastoral
care. The physicians in the study—some of whom primarily care for
adults—might say they would use barbiturates and neu-
muscular blockers but feel uncomfortable completing the task.
Or, they might really do it because Dutch culture permits
euthanasia. In other words, this survey, like similar studies,
provides limited insight into behavior, and even the best
survey from The Netherlands sheds little light on opinions,
much less actions, outside of The Netherlands.

The medical community needs to broaden its view of
worthwhile social science. Qualitative methods accepted in
anthropology and sociology could give much more substantive
insight into what is going on than surveys. Acknowledging
some difficult, though surmountable, issues surrounding
anonymity when studying illegal activity, both ethnography
and in-depth semi-structured interviews could lead to a much
better understanding of euthanasia in The Netherlands, or
anywhere else. Ethnography involves direct observation by
trained researchers, whereas semi-structured interviews allow
fuller exploration of reasoning, attitudes, and beliefs than do
most surveys. Despite the historic importance of nonquan-
titative clinical and pathologic observations to modern medi-
cine, physicians often scoff at the efforts of qualitative social
scientists to study our profession, dismissing the research as
“anecdotal.” Yet, qualitative studies about euthanasia would
tell us a great deal more than surveys.

As to the ethics of physician assistance of dying in
pediatrics—restricting that phrase to mean actions beyond
withholding/withdrawing life-sustaining treatments or vigor-
ous pursuit of symptom control that might, secondarily, hasten
death—one hardly knows where to begin. Many in the United
States who concern themselves with ethics or palliative care in
pediatrics would agree that physicians have a primary duty to
alleviate, if not eliminate, pain and other troubling symptoms
affecting dying children. Most would agree that symptom
relief, indeed comfort, can almost always be achieved, though
sometimes only by paying the price of unconsciousness. In a
very small number of situations, some might argue, acting to
cause death could find justification.

The first sort of case might involve an adolescent, or in
rare instances a sophisticated preteen, with considerable
experience with his or her condition, who articulates substantial
and enduring psychologic or spiritual suffering despite expert
and sustained efforts to relieve the distress. The second sort of
case might involve a child at any age with minimal conscious-
ness whose behavior suggests continuing discomfort or irrita-
bility despite appropriate treatment and for whom removal of
life-sustaining measures, including artificially administered
fluids and nutrition, probably would not produce death in a
short time—48 to 72 hours, for sake of discussion. Under such
circumstances, one could understand a parental request to
hasten death directly.

Putting aside worry about the law (ie, murder charges), it
does not seem clearly wrong to take seriously patient or
family requests to administer lethal measures in these kinds of
cases. Although we have made many advances in palliative and
hospice care, our treatments sometimes fail, especially in the
psychosocial and spiritual realms. Refusal to engage claims
that doctors should help the patient die faster seems arrogant.
Physicians need to ask themselves what they hope to
accomplish by insisting on the prolongation of suffering they
cannot relieve. And how can we judge the suffering of another
in the first place?

Inevitably, such considerations lead to worries about
slippery slopes.2,3 Many have discounted such concerns,
confident of preventing abuses. However, reports in the
popular and medical press4,5 suggest that some euthanasia in
The Netherlands, contrary to current law, involves infants
with “severe multiple handicaps.”5 Without knowing the
specifics, one must worry whether the newborn infants
involved are clearly and imminently dying or whether
decisions to cause their deaths may reflect morally problem-
atic, perhaps repugnant, intolerance of disability.

In any case, the results of the survey reported by
Vrakking, et al should prompt us to think seriously about
end-of-life practices. Can the North American reliance on
distinguishing between withholding/withdrawing life support
and euthanasia stand up to logical challenges? If one accepts,
even welcomes the death of some patients, can one consist-
tently and validly defend practices that result in prolonged
patient or family suffering until the death occurs? On the other
hand, directly bringing about death of some through use of
lethal drugs may extend the practice into situations most of us
would find ethically unacceptable. Great caution and care
seem necessary.

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ARE SOFT DRINKS A SCAPEGOAT FOR CHILDHOOD OBESITY?

ROBERT MURRAY, MD, FAAP, BARBARA FRANKOWSKI, MD, MPH, FAAP, AND HOWARD TARAS, MD, FAAP

In an editorial accompanying the study by Mrdjenovic and Levitsky on the topic of sweetened drinks and childhood nutrition, Schwartz proposed that Americans “reduce the availability and limit portion sizes of sugar-sweetened drinks sold at school and provided at home.” In the study, 30 children 7 to 13 years of age acted as their own controls, collecting daily diet records and weighing food to estimate intake from a baseline period through 4 to 8 weeks of follow-up. Body mass index was measured regularly. The data showed that not only were those children with the highest intake of sweetened drinks consuming greater daily energy, but also that sweet drinks displaced milk from their diet. The resulting trade-off resulted in lower daily protein, calcium, phosphorus, magnesium, and vitamin A. The authors concluded that excessive sweetened-drink consumption associated with decreased milk intake may be one important risk factor for childhood obesity and nutrient deficiencies.

In his editorial, Schwartz recommended teaching children to drink water for thirst, promoting healthier choices in vending machines in schools and communities, prohibiting advertising of sweetened beverages in schools and daycare centers, and even levying small taxes on soft drinks and snack foods to be used to educate children and fund extra-curricular activities. In light of the accumulating evidence, Schwartz’s recommendations to limit sweetened drinks would seem reasonable. But have we gone too far in making soft drinks the fall guy for obesity?

Some think so. Subsequent letters to the editor, as well as press releases from representatives of the soft drink industry, disagreed with the conclusions of the authors of the study. They objected to recommendations from the healthcare community that supported curbing soft drink consumption. The respondents felt that the designation of sweetened drinks as a cause for such a complex, multi-factorial problem as obesity was simplistic. They cited declining physical activity and increasing television and screen time as etiologies with a greater base of research evidence than that for soft drinks. Likewise, frequency of fast food, extreme portion sizes, and other unbalanced patterns of food consumption have had an equal or greater impact than that of soft drinks. They stated that the data on the increase in soft drink consumption are contradictory. Despite several studies based on the USDA’s National Health and Nutrition Examination Survey and the Continuing Food Survey of Food Intakes by Individuals methodologies that showed an increasing trend, not all studies demonstrated this trend. In their public and private communications, representatives of the soft drink industry cited a study by Park et al that suggests that carbonated soft drink consumption has not increased, nor has milk consumption fallen, over the past decade, whereas obesity has accelerated. In addition, they state that research has failed to confirm the displacement of milk intake with rising soft drink consumption. Ultimately, they cite evidence that consumption of carbonated soft drinks from vending machines at school is minimal, only 2.5 oz per week, certainly insufficient to account for the obesity crisis.

Many of their objections are valid. Certainly, soft drinks are not the root cause of obesity. The healthcare community has convinced Americans that there is an obesity epidemic. Now the public wants health professionals to identify the villain. Finding a single culprit would be convenient, but it will not happen with obesity. This disease is wrapped in genetics and culture, behavior and psychology. It is important to recognize that even if soft drink consumption was eliminated through a zealous, latter-day prohibition movement, it is unlikely to eliminate obesity, given the many factors that contribute. However, among those many factors, soft drinks have a prominent place. Although obesity prevention will require many interventions that affect all aspects of children’s lives, curbing the current intake of sugar consumed in the form of sweetened drinks will be one of the most
important strategies. The objections of the soft drink industry sidestep the intent of the recommendations being made by pediatricians. And in doing so, the industry may miss an opportunity to play a central role in helping to unravel childhood obesity.

**IN A CROWDED FIELD, WHY ARE SOFT DRINKS SINGLED OUT?**

Far from picking on soft drinks as the sole cause for obesity, the American Academy of Pediatrics (AAP) has issued several policy statements on a broad array of issues surrounding childhood obesity. The AAP Committee on Nutrition statement on “The Prevention of Pediatric Overweight and Obesity” was issued in August 2003, addressing genetics, family dynamics and the home environment, recent societal changes, the decline in physical activity and concomitant rise in television viewing, as well as dietary factors that promote obesity. 14 Previously, the AAP Committee on Nutrition recommended a limitation on juice intake by babies to lessen the risk of overweight. 15 In a clinical report on the prevention and treatment of diabetes type II, the AAP cited the role of the community in helping to prevent the advent of obesity-related diabetes through improved physical activity and nutrition, especially among high-risk populations. 16 The Committee on Sports Medicine called for healthy weight-management practices for children participating in athletics. 17 The Committee on School Health and the Committee on Sports Medicine jointly called on schools to increase opportunities for daily physical activity and recommended several ways to achieve it. 18 The Committee on Public Education urged avoidance of television and media viewing for children under the age of 2 years and urged limiting viewing to 1 to 2 hours for all children above the age of 2 years. 19 It was only in January 2004 that the Committee on School Health (COSH) issued the statement “Soft Drinks in Schools,” adding it to the list of statements on obesity. 20

Why were soft drinks singled out in the AAP COSH statement? American children over-consume foods that constitute the tip of the USDA Food Guide Pyramid, the section labeled “discretionary fats and sugars,” a term that connotes fats and sugars added to the diet by choice, not inherent in the foods themselves. 9,12,21-23 Currently, American children and adults consume nearly one third of their daily energy from this class of foods; ie, energy-dense, nutrient-poor foods, also termed snack foods. 24,25 For the US population as a whole, added sweeteners account for 16% of total daily energy. 9 Soft drinks are the number one source of added sugars in the American diet and account for 33%. When the number two source, fruit drinks, is added, together they account for 43% of the total added sugars. 12 For children, especially teens, the impact of soft drinks is even greater. As a food group, added sugars constitute 18% to 20% of a child’s daily energy. 9 Sweetened soft drinks amount to 40% of all added sugars in a child’s diet and with the addition of fruit drinks, more than 50%. 9 Americans have been warned to moderate their sugar intake. 22 A limit of 6% to 10% of daily calories from the combined added sugars in the diet has been recommended. 12,26 With many common food products, such as ketchup and spaghetti sauce, containing added sugars, dietary recommendations are easily exceeded. For a child consuming 1300 kcal per day, this represents only 6 teaspoons of added sugars, and for a teenager consuming 2800 kcal per day, 18 teaspoons of sugars, excluding sugars inherent in fruits and dairy products. Such a modest amount can be consumed at a single sitting in the form of a large soft drink at a fast food restaurant. Daily adolescent intake of sweetened soft drinks averages nearly two 12-oz servings per day, or the equivalent of 20 teaspoons of sugar and 300 kcal. 10,27 This would be less of a problem if soft drink intakes were offset by decreases in energy intake elsewhere in the diet. However, studies suggest that such calories are added to the daily total. 28,29

**IS THE PROPOSED CONNECTION BETWEEN OVER-COMSUMPTION OF SOFT DRINKS AND OBESITY SUPPORTED BY RESEARCH?**

Besides the study by Mrdjenovic and Levitsky already cited, 1 two other longitudinal, randomized studies have been done using late elementary school and middle-school children. Both showed an effect of soft drink consumption on weight. 30,31 In the first study, Ludwig et al 30 followed 548 children 11 to 12 years of age prospectively for 19 months. Soft drink consumption increased 57% as obesity rose 9.3%. When diet, activity, television, and anthropometrics were controlled for, each soft drink consumed daily was shown to increase the child’s risk of obesity by 60%. In the second study, James et al 11 sought to determine whether reducing consumption of carbonated beverages had an impact on weight gain. The authors followed 644 children 7 to 11 years of age for 1 school year. Using a focused intervention program meant to promote alternate drinks, the control group increased 0.2 glasses (50 mL), whereas the treatment group decreased carbonated soft drink intake by 0.6 glasses per day (150 mL). At 12 months, the percent overweight and obese children among controls increased by 7.5% but decreased by 0.2% in the intervention group. Eliminating just a few ounces of sweetened drinks is likely to affect the risk of childhood obesity substantially. Investigators suggest that small alterations in daily energy consumption may have significant implications. Even small amounts of sweetened drinks consumed at home, at school, and in the community may have a cumulative effect. Hill and colleagues 12 calculated that altering the energy gap by only 0.42 MJ/day (100 kcal/day)—which, ironically, is the equivalent of one 8-oz serving of sweetened soft drink—would prevent excessive weight gain in most Americans. In a review of sugar consumption in the American diet, Krebs-Smith 12 concluded that even though added sugars are not the sole cause of obesity and that both energy intake and expenditure also are suspects, the fact that large segments of the population are experiencing an energy surplus makes it difficult to justify regular intake of soft drinks because of the additional, nutritionally empty calories.
Soft drink industry representatives cite the National Family Opinion World Group Share of Intake Panel (SIP) study, which they funded, as evidence for their claim that soft drinks are not over-consumed and are rarely consumed in schools, certainly not in amounts that would contribute to obesity.3-5,13 When children 1 to 5 years of age in the study were examined as a group, only 3.7 oz of carbonated soft drinks were consumed per child per day, a fall from 5.2 oz in the 1987-88 survey, according to their data. However, even the Park survey data showed that teen females consumed 16 oz per day and teen males 23 oz per day, amounts similar to those reported in other surveys.8,10 The SIP survey is a mail marketing survey that utilized 2-week family diaries from 12,000 persons per year to monitor total beverage intakes. The data were weighted quarterly to be representative of the US population, but it was not a randomized sampling. The study group was further biased by self-selection. Instructions were given in writing and not reviewed with participants by trained research assistants using multiple pass methodologies through onsite or phone contact, as was the case for larger, randomized national surveys. Park13 cited a single decade of data, eliminating the acceleration of soft drink consumption that occurred in the 1970s and 1980s. Park's frequently cited statistics on consumption of carbonated soft drinks neglects other, faster growing segments of the sweetened drink market.13 Park's data on fruit drinks, eg, showed an increase across all age groups and both genders.13 Irrespective of the validity of the SIP study, the question for the AAP COSH was whether soft drink contracts in schools promoted further soft drink consumption by students. No study to date has examined the effects on student consumption patterns of introducing a soft drink contract into a school district. But current levels of consumption suggest that students do not need further encouragement.

ARE THERE NUTRITIONAL CONSEQUENCES FROM OVER-CONSUMPTION OF SOFT DRINKS?

Besides excess sugar consumption as a source of unnecessary daily energy, the AAP COSH also considered the corresponding decline in milk consumption as soft drink intake increased and its effect on the daily nutrient profile of children. Milk is a nutrient-dense food. Its elimination from a child's diet is not without consequences. Risk of future osteoporosis and bone fractures because of inadequate daily calcium is only the most prominent clinical issue associated with declining milk consumption. Based on National Health and Nutrition Examination Survey data from 1999-2000, several nutrients have become “problem nutrients” because of consumption rates falling below the daily recommended intakes.33 Corresponding to this phenomenon, milk intake also has fallen as the intake of sweetened drinks has risen. Several studies have shown that the two are closely connected.12,22,34-36 This reciprocal relationship suggests that displacement of milk by sweetened drinks is one of the principle factors that fuels some nutritional deficiencies noted in children.6,37,38 When soft drinks are chosen in place of milk in school lunches, intake of protein, calcium, zinc, vitamins A and C fall, a finding seen in several previous studies34,39 and reiterated by Mrdjenovic and Levitsky.1 In addition, those with the highest soft drink intakes in the diet also have the highest energy intakes.40 Guthrie et al12 looked at the displacement phenomenon a different way. By examining women with adequate calcium intakes, they were able to show that these women had lower intakes of sweetened soft drinks and greater intakes of milk. Displacement can even be identified in the first 24 months of life. In the Feeding Infants and Toddler Study, Skinner et al41 found that as fruit juice, fruit drink, and carbonated drinks increased, calcium density in the diet fell. So, rather than being a consolation that fruit drinks represent the second greatest source of vitamin C among children of all ages as Park suggests, this fact is distressing.13 Fortification of a sugared drink is no substitute for fruit consumption.

IS IT THE DRINK OR THE MARKETING THAT IS THE PROBLEM?

When they wrote the policy statement, the members of the AAP COSH were faced with a new marketing strategy aimed at children termed “exclusive soft drink contracts,” contracts between representatives of the soft drink industry and school authorities. Fearing that one consequence of these lucrative school contracts was going to be the promotion of even greater over-consumption of sugar, the COSH recommended that pediatricians work with their local school districts to eliminate sweetened drinks from schools, substituting instead water, milk, and fruit and vegetable juices.20 The committee’s fears proved well founded. The recent School Health Policies and Programs Study, conducted in 2000, surveyed all 51 state education agencies, 523 school districts, 841 school food service representatives, and 927 schools about current policies. The survey showed that 49.9% of districts had a soft drink contract and that of these, nearly 80% received a specified percentage of the sales receipts. Almost two thirds of the schools were given incentives once sales achieved a specified amount. One third of the schools allowed advertising in their buildings. Of elementary schools, 58% allowed students to purchase beverages from vending machines, of middle schools 83%, and of high schools 93%.42 Despite current USDA guidelines to discourage sales of “foods of minimal nutritional value,” which includes soft drinks, the survey found that 70% of schools allowed students to purchase them during the lunch period.

The public looks to physicians as their most trusted source for nutrition guidance.43,44 With their statement, the AAP COSH urged pediatricians to take an active role in shaping the second most important environment for child development, the schools. Every day 55 million children attend school, offering society an unparalleled opportunity to address their nutrition and fitness in an efficient and cost-effective way.45 By strengthening existing programs such as the school breakfast program, the national school lunch program,
classroom nutrition instruction, daily physical fitness instruction, intramural sports, and after-school programs, we can improve the health of the nation’s children. Any practices and policies that dilute these programs need to be reconsidered. 7,45,46 To ensure that the school promotes the health of its students, each school district should draft a nutrition policy with the guidance of parents and health professionals, especially pediatricians, dietitians, and dentists. Vended and a lá carte foods, school stores, fund-raisers, school parties, and booster sales at sporting events should all conform to the stated goals of the policy.

WHAT CAN WE CONCLUDE?

Obesity is a multi-factorial problem. Any recommendation that singles out one activity or dietary change can be criticized as “simplistic” and is unlikely to be effective in isolation. Yet that does not mean that factors should be ignored; the cumulative effect of many small changes across a child’s environment can be synergistic. This is as true for attempts to create societal change through a public health agency, a professional organization, or a school as it is for personal change, such as that directed from a physician to a patient. It is only by making such changes, one at a time if necessary and more if possible, that we are likely to contain a problem of the magnitude of obesity.

Obesity is America’s biggest threat to child health. 47 One study found that as many as 25% of obese children already showed signs of early glucose intolerance, a precursor to type II diabetes. 48 Further, it has been estimated that a child who is diagnosed with type II diabetes mellitus at age 10 years will lose between 17 and 26 life-years to the disease, depending on gender and ethnic background. 49 Not only will the duration of their lives be cut short, but the quality of their lives also will be drastically worsened by chronic disease. The psychological ramifications of early obesity, important in the near-term for young children, may last a lifetime. 50,51 With the public now aware that obesity-related morbidity and mortality is poised to exceed that from tobacco, pediatricians have a responsibility to develop effective solutions and aggressively advocate for them.

Soft drinks are not tobacco. The majority of Americans drink them. Like other energy-dense, nutrient-poor foods, they may have a place in everyday nutrition, albeit only in moderation and, in the opinion of the AAP COSHI, not in schools. To be successful in our efforts to prevent childhood obesity, we need the cooperation of the beverage, restaurant, and vended and snack foods industries. We should not make any one of them the scapegoat for obesity. On the other hand, with obesity assuming the mantle of one preventable disease in the nation, these industries should expect pediatricians and parents to hold them accountable for marketing practices that worsen an already deleterious health situation for children.

REFERENCES


USE OF ASTHMA GUIDELINES BY PRIMARY CARE PROVIDERS TO REDUCE HOSPITALIZATIONS AND EMERGENCY DEPARTMENT VISITS IN POOR, MINORITY, URBAN CHILDREN

MICHÈLE M. CLOUTIER, MD, CHARLES B. HALL, PHD, DOROTHY B. WAKEFIELD, MS, AND HOWARD BAILIT, DMD, PhD

Objectives To determine whether an organized, citywide asthma management program delivered by primary care providers (PCPs) increases adherence to the National Asthma Education and Prevention Program (NAEPP) Asthma Guidelines and whether adherence to the guidelines by PCPs decreases medical services utilization in low-income, minority children.

Study design Analysis of the utilization of medical services for a cohort of 3748 children with asthma who presented for care at one of six primary care urban clinics in Hartford, Connecticut, and who were enrolled in a disease management program (Easy Breathing™) between June 1, 1998 and August 31, 2002.

Results Of the 3748 children with physician-confirmed asthma, 48% had persistent disease. Paid claims for inhaled corticosteroids increased 25% (P <.0001) after enrollment in Easy Breathing. Provider adherence to the NAEPP guidelines for anti-inflammatory therapy increased from 38% to 96%. Easy Breathing children with asthma experienced a 35% decrease in overall hospitalization rates (P <.006), a 27% decrease in asthma emergency department (ED) visits (P <.01), and a 19% decrease in outpatient visits (P <.0001).

Conclusions An organized, disease management program increased adherence to the NAEPP guidelines for anti-inflammatory use by PCPs in urban clinics. Adherence to this element of the guidelines by PCPs reduced hospitalizations, ED visits, and outpatient visits for children with asthma. (J Pediatr 2005;146:591-7)

In the United States, asthma disproportionately affects poor, minority populations, especially children living in urban areas. Asthma prevalence rates as high as 36.8% have been reported in minority, urban, low-socioeconomic populations, and the rates are rising.¹⁻⁵

Reducing asthma morbidity is a national healthcare objective. Despite the wide dissemination of national guidelines for the management of asthma from the National Asthma Education and Prevention Program (NAEPP), anti-inflammatory drugs, first line therapy for chronic asthma, are under-prescribed.⁶⁻¹² In addition, the effectiveness of these guidelines in reducing asthma morbidity and hospitalizations, when used in primary care settings by primary care providers (PCPs), has not been established.¹³

We conducted a study to determine whether a systematic, standardized, asthma disease management program would increase adherence by PCPs to the 1997 NAEPP guidelines for anti-inflammatory therapy and whether greater adherence was associated with a decrease in hospitalizations and emergency department (ED) visits in low-income, minority children who reside in Hartford, Connecticut, a medium-sized city, with a large low-income population.

METHODS

Subjects All children between 6 months and 18 years of age who presented for medical care regardless of payer or chief complaint at any of the six primary care clinics in Hartford,
Connecticut, between June 1, 1998 and August 31, 2002 constituted the eligible sample. Other than age, there were no exclusion criteria. The study was approved by the Institutional Review Board at Connecticut Children’s Medical Center.

The Easy Breathing Program

Easy Breathing™ is an asthma management program for primary care clinicians. The Easy Breathing program focuses on four elements of care: diagnosing asthma, determining asthma severity, prescribing therapy appropriate for the asthma severity, and developing a written Asthma Treatment Plan that is given to the family. The Easy Breathing Survey consists of four previously validated questions that aid clinicians in diagnosing asthma. The Survey is completed by the parent of any child (6 months to 18 years of age) who presents for care, regardless of chief complaint, at a participating clinic. The Survey responses and information from the medical record are reviewed by the clinician, who responds to the question: “Does this child have asthma?” Clinicians consider a diagnosis of asthma for children with a history of recurrent (>2) episodes of wheezing, cough, and/or shortness of breath in response to known asthma triggers, when other diseases have been excluded. Clinical criteria have been successfully used to predict asthma even in young children.

If the clinician determines that the child has asthma, the clinician uses a separate instrument (the Provider Assessment) to ask a series of five questions about the frequency of daytime and nocturnal symptoms, exercise impairment, as needed bronchodilator use, corticosteroid prescriptions, and missed school days for asthma. Asthma severity is then determined by the clinician using the symptom frequency associated with the highest disease severity according to the NAEPP guidelines.

Once asthma severity has been determined, the clinician chooses the child’s therapy using the Asthma Treatment Selection Guide, a list of drugs and dosages appropriate for asthma of that severity and of the HMOs that cover those drugs. For each child with asthma, a field-tested, written Asthma Treatment Plan is developed and is given to the child’s caregiver. A copy of the treatment plan also is placed in the child’s medical record and is given to the program. The Asthma Treatment Plan instructs the family in how to manage the child’s asthma daily; how, when, and how long to treat an asthma exacerbation; what to do in an emergency; and when to call the doctor. Changes in therapy are noted through the development and submission to the Easy Breathing program of new treatment plans.

If the clinician determines that the child does not have asthma, the clinician answers “no” to the question “Does this child have asthma?” and signs the form. No further evaluation is necessary, and these children have physician-confirmed “no asthma.”

A child was considered “enrolled” in Easy Breathing if a Survey, Provider Assessment, and Asthma Treatment Plan (if the child had asthma) were completed. Ninety-three percent of all children who were surveyed were enrolled.

Providers and Clinics

Between June 1, 1998 and August 31, 2002, 33 physicians, 28 mid-level (Advanced Practice Registered Nurse, Pediatric Nurse Practitioner, Physician Assistant) practitioners, and more than 90 pediatric residents and medical students were trained in the Easy Breathing program. Newly hired clinicians were trained each year. The Easy Breathing curriculum has been previously described. Briefly, the curriculum consisted of a presentation of what is known about asthma in Hartford, the definition of asthma, the clinical diagnosis of asthma, especially in young children, a brief overview of the NAEPP guidelines, and the role of inhaled corticosteroids in asthma management, including the risks.

Clinical criteria were introduced to the various record forms and instructed in how to use them. Finally, a series of cases were presented, and clinicians used the forms to determine the asthma diagnosis and severity and to develop a severity-specific Asthma Treatment Plan. More than 95% of the clinicians attended the training session; for the few who missed the training, the program coordinator discussed how to use the forms with the clinicians. During the first year, a pediatric pulmonologist visited the clinics for 2 hours per week to provide on-site consultation and program-related education as needed.

Of the six primary care clinics, two were hospital-based, two were federally funded health centers, and two were university-affiliated clinics. Four clinics were part of the pediatric or family medicine residency program at the University of Connecticut Health Center. These six clinics provide care for most (85%) of Hartford’s children.

Sources of Data

Patient demographic information and exposure histories were obtained from the Easy Breathing Survey. Demographic data for children residing in Hartford were obtained from the 2000 United States Census. Claims data and eligibility files were obtained from Connecticut’s Peer Review Organization, Qualidigm, Inc., for all Medicaid and State Children’s Health Insurance Plan (S-CHIP) enrollees residing in Hartford. Asthma drugs were identified using National Drug Codes and were grouped into four categories: bronchodilators, inhaled corticosteroids, oral corticosteroids, and nonsteroidal anti-inflammatory drugs (including leukotriene modifiers).

Statistical Analyses

Univariate and multivariate logistic regression and χ² analysis were used to compare demographic and exposure information for children with and without asthma who were enrolled in Easy Breathing.

For the primary efficacy analyses, we examined utilization of medical services including hospitalizations, ED visits, outpatient visits, and prescriptions through analysis of paid Medicaid/S-CHIP claims. We examined the relative rates of utilization (in events/child months) of these services by children after enrollment into Easy Breathing vs the rates for the same children before enrollment. Claims data were therefore obtained for an entire year (July 1997-June 1998)
before the beginning of the program in order to have sufficient pre-enrollment follow-up data.

Children were continuously enrolled into the Easy Breathing program during years 2, 3, and 4 of the analysis period. Each child contributed to the analysis for every month of claims data that were available during the 4 years of data analysis. Thus, during years 2, 3, and 4, at any point in time, there were children already enrolled in Easy Breathing and children not yet enrolled. Utilization was determined by pooling the time and events of all children after enrollment and comparing them with the utilization rates calculated in the same way before enrollment. Therefore, the primary efficacy comparisons are both historical (using each child's entire utilization experience after and before enrollment) and contemporaneous, (using the utilization of all children enrolled at a given time along with all children yet to be enrolled).

To control for demographics, asthma severity at the time of enrollment into Easy Breathing, seasonal and longer-term secular trends, and the effect of the aging of the cohort, the efficacy analyses were performed using multivariate marginal binary and Poisson regression models, with generalized estimating equations used to fit the models. This approach takes into account the fact that children contribute multiple observations to the dataset.

**RESULTS**

**Study Population**

Between June 1, 1998 and August 31, 2002, 9339 children who resided in Hartford were enrolled in Easy Breathing. Of these 9339 children, 8324 (89%) were Medicaid or S-CHIP participants and were matched with claims data; these children constitute the study population (Table I).

Compared with all children in Hartford, children enrolled in Easy Breathing were younger ($P < .001$) and more often Hispanic ($P < .001$) (Table I). In all other respects the children who were enrolled were representative of Hartford’s children.

**Asthma in the Study Population**

Of the 8324 Medicaid children enrolled in Easy Breathing, 3748 children had a physician-confirmed diagnosis of asthma; 1799 children (48% of the 3748 children with asthma) were diagnosed with persistent disease. In the unadjusted analysis, children with asthma were more likely to be Hispanic, ≤5 years of age, and male compared with children without asthma. Children with asthma also were more likely to report a family history of asthma, exposure to environmental tobacco smoke, cockroaches, rodents, and dust but not to cat or dog (Table II). In the multivariate analysis that controlled for family history of asthma, gender, ethnicity, age of ≤5 years, a family history and exposure to cockroaches and dust (Table II).

**Prescription Drug Use**

After enrollment in Easy Breathing, there was a 25% overall increase in inhaled corticosteroid use. Children with persistent asthma filled more prescriptions for inhaled corticosteroids and fewer prescriptions for bronchodilators and oral corticosteroids (Table III). Inhaled corticosteroid use also increased in children with intermittent asthma. This increase usually occurred within 6 months of enrollment in the program and was associated with an increase in asthma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hartford children: 2000 census (n = 36,568)</th>
<th>Medicaid children enrolled (%) (n = 8324)</th>
<th>Medicaid children with asthma enrolled (%) (n = 3748)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gender</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>18,754 (51%)</td>
<td>4266 (51%)</td>
<td>2110 (56%)</td>
</tr>
<tr>
<td>Female</td>
<td>17,814 (49%)</td>
<td>4058 (49%)</td>
<td>1638 (44%)</td>
</tr>
<tr>
<td>Ethnicity</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>18,850 (51%)</td>
<td>4830 (58%)</td>
<td>2436 (65%)</td>
</tr>
<tr>
<td>African American</td>
<td>13,785 (38%)</td>
<td>2228 (27%)</td>
<td>825 (22%)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>2,172 (6%)</td>
<td>226 (3%)</td>
<td>50 (1%)</td>
</tr>
<tr>
<td>(Mixed, Other, Unknown)</td>
<td>1761 (5%)</td>
<td>1040 (12%)</td>
<td>437 (12%)</td>
</tr>
<tr>
<td>Age</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.5 - 4 y</td>
<td>10,116 (28%)</td>
<td>3105 (37%)</td>
<td>1308 (35%)</td>
</tr>
<tr>
<td>5 - 9 y</td>
<td>10,746 (29%)</td>
<td>2442 (29%)</td>
<td>1169 (31%)</td>
</tr>
<tr>
<td>10 - 14 y</td>
<td>9,959 (27%)</td>
<td>2,058 (25%)</td>
<td>952 (25%)</td>
</tr>
<tr>
<td>15 - 18 y</td>
<td>5,747 (16%)</td>
<td>719 (9%)</td>
<td>319 (9%)</td>
</tr>
</tbody>
</table>
severity from intermittent to persistent disease as a result of a follow-up visit. The ratio of inhaled corticosteroid to bronchodilator prescriptions almost doubled in Easy Breathing participants (0.20 to 0.35). Before Easy Breathing, 38% of the 1799 children with persistent disease were treated with anti-inflammatory therapy and half of these children were treated with cromolyn. After enrollment in Easy Breathing, 724 of the 1799 children with persistent disease were prescribed an appropriate anti-inflammatory drug resulting in a 96% adherence to the NAEP guidelines for the treatment of persistent asthma. Eighty-five percent of these children were treated with inhaled corticosteroids. Paid claims for prescriptions of inhaled corticosteroids in children never enrolled in Easy Breathing but cared for by the same primary care clinicians in the same clinics increased 10% between 1998 and 1999, and the increase was temporally related to program training. This suggests a “spill-over” effect of the training on the provider’s prescribing behavior, but changes in secular trends also could be occurring and cannot be ruled out.

### Table II. Risk factors for asthma prevalence and increased asthma severity

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted odds ratio</th>
<th>95% CI</th>
<th>Adjusted odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Asthma prevalence</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic ethnicity</td>
<td>1.79 (1.62, 1.96)</td>
<td></td>
<td>1.50 (1.36, 1.67)</td>
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</tr>
<tr>
<td>≥5 years of age</td>
<td>1.21 (1.10, 1.32)</td>
<td></td>
<td>1.18 (1.07, 1.31)</td>
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</tr>
<tr>
<td>Male gender</td>
<td>1.45 (1.33, 1.58)</td>
<td></td>
<td>1.50 (1.36, 1.65)</td>
<td></td>
</tr>
<tr>
<td>Family history</td>
<td>3.95 (3.54, 4.41)</td>
<td></td>
<td>3.35 (2.97, 3.77)</td>
<td></td>
</tr>
<tr>
<td>Asthma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tobacco smoke</td>
<td>1.56 (1.41, 1.72)</td>
<td>1.47</td>
<td>(1.32, 1.65)</td>
<td></td>
</tr>
<tr>
<td>Cockroaches</td>
<td>1.97 (1.76, 2.21)</td>
<td>1.35</td>
<td>(1.18, 1.55)</td>
<td></td>
</tr>
<tr>
<td>Rodents</td>
<td>2.08 (1.77, 2.44)</td>
<td>1.34</td>
<td>(1.11, 1.61)</td>
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</tr>
<tr>
<td>Cat</td>
<td>1.03 (0.89, 1.20)</td>
<td>N/A‡</td>
<td>N/A</td>
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<tr>
<td>Dog</td>
<td>1.1 (0.98, 1.24)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Dust</td>
<td>1.8 (1.63, 1.99)</td>
<td>1.47</td>
<td>(1.32, 1.65)</td>
<td></td>
</tr>
</tbody>
</table>

**Increased asthma severity**

<table>
<thead>
<tr>
<th></th>
<th>Unadjusted odds ratio</th>
<th>95% CI</th>
<th>Adjusted odds ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hispanic ethnicity</td>
<td>1.26 (1.09, 1.44)</td>
<td>1.22</td>
<td>(1.05, 1.40)</td>
<td></td>
</tr>
<tr>
<td>≥5 years of age</td>
<td>1.16 (1.02, 1.32)</td>
<td></td>
<td>1.16 (1.02, 1.33)</td>
<td></td>
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<tr>
<td>Male gender</td>
<td>1.05 (0.93, 1.18)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Family asthma</td>
<td>1.34 (1.12, 1.61)</td>
<td>1.30</td>
<td>(1.08, 1.57)</td>
<td></td>
</tr>
<tr>
<td>Tobacco smoke</td>
<td>1.09 (0.95, 1.25)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Cockroaches</td>
<td>1.48 (1.28, 1.71)</td>
<td>1.40</td>
<td>(1.19, 1.65)</td>
<td></td>
</tr>
<tr>
<td>Rodents</td>
<td>1.32 (1.09, 1.60)</td>
<td>1.02</td>
<td>(0.82, 1.26)</td>
<td></td>
</tr>
<tr>
<td>Cat</td>
<td>1.00 (0.81, 1.23)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Dog</td>
<td>1.06 (0.90, 1.24)</td>
<td>N/A</td>
<td>N/A</td>
<td></td>
</tr>
<tr>
<td>Dust</td>
<td>1.37 (1.20, 1.57)</td>
<td>1.25</td>
<td>(1.09, 1.45)</td>
<td></td>
</tr>
</tbody>
</table>

*CI: 95% Confidence interval.
‡N/A: not included in multivariate analysis.

DISCUSSION

This study demonstrates the effectiveness of a disease management program based on use of the NAEP guidelines in reducing asthma morbidity in a large group of low-income, urban, minority children whose asthma is managed entirely by PCPs. Asthma diagnosis and treatment using the NAEP’s asthma severity categories and recommended therapies including the development of a written Asthma Treatment Plan increased inhaled corticosteroid use and decreased overall hospitalizations and asthma-specific ED visits and outpatient visits.

Inhaled anti-inflammatory therapy has been shown to decrease asthma exacerbations leading to hospitalizations but continues to be under-prescribed by primary care physicians.
In this study, adherence to the NAEPP recommendations for treatment of persistent asthma by PCPs increased from 38% before Easy Breathing to 96% and has remained high for the past 3 years. In 1997, before beginning Easy Breathing, 18% of all of the Medicaid children with asthma in Hartford filled a prescription for an anti-inflammatory drug, but only half of these prescriptions were for an inhaled corticosteroid. After enrollment in the program, 46% (1724/3748) of all of the children with asthma in Easy Breathing and 96% of all the children with persistent asthma filled at least one prescription for an anti-inflammatory drug, and 85% of these prescriptions were for an inhaled corticosteroid. Prescribing an inhaled corticosteroid alone without the program, however, was insufficient in decreasing medical services utilization, suggesting that other program elements such as the written treatment plan or the standardized approach to therapy within the practice may be important contributors to the success of this disease management program. Use of a written Asthma Treatment Plan has been implicated in reducing asthma hospitalization and ED visits.

Almost all (85%) of the children cared for in these urban clinics were either Hispanic or African American, and all were from low-income families. Racial disparities in asthma care, particularly medical prescription, have been found in children and adults in both managed care and urban clinic settings. Easy Breathing decreased medical services utilization and increased inhaled corticosteroid therapy in children with a full range of asthma severities and appeared to be effective in both younger and older children, and in Hispanic and African American children. Importantly, the benefits of the program have been sustained for 3 years.

We believe that Easy Breathing has been successful in changing provider behavior because it focused almost exclusively on asthma diagnosis and therapy, areas that were important to the providers rather than taking a multi-domain, all-encompassing, comprehensive approach. This prioritization of the components of care might have helped clinicians better allocate their limited time. Furthermore, some clinicians have argued that not all components of asthma care are necessary, and some have even eliminated parts of the asthma guidelines that they consider “inapplicable” and “nonpractical.” In this project, clinicians conceptually agreed that proper diagnosis and treatment of asthma were important, and the program focused on this outcome.

The study has a number of limitations. First, study participants were not randomized and although all children in Hartford were eligible and more than one third of the children identified with asthma (using ICD-9 codes and Medicaid

Table III. Rate of prescription drug use for children with asthma before and after enrollment in Easy Breathing

<table>
<thead>
<tr>
<th>Drug</th>
<th>Before Easy Breathing</th>
<th>After Easy Breathing</th>
<th>Adjusted relative rate (CI)§</th>
<th>P value</th>
<th>Before Easy Breathing</th>
<th>After Easy Breathing</th>
<th>Adjusted relative rate (CI)§</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Inhaled corticosteroid</td>
<td>0.064</td>
<td>0.238</td>
<td>2.539 (1.784, 3.614)</td>
<td>&lt;.0001</td>
<td>0.797</td>
<td>1.283</td>
<td>1.155 (1.031, 1.295)</td>
<td>.01</td>
</tr>
<tr>
<td>Bronchodilator</td>
<td>1.309</td>
<td>1.388</td>
<td>0.806 (0.728, 0.893)</td>
<td>&lt;.0001</td>
<td>2.947</td>
<td>2.868</td>
<td>0.839 (0.769, 0.914)</td>
<td>&lt;.0001</td>
</tr>
<tr>
<td>Nonsteroidal anti-inflammatory††</td>
<td>0.093</td>
<td>0.06</td>
<td>N/A</td>
<td>N/A</td>
<td>0.484</td>
<td>0.332</td>
<td>0.741 (0.584, 0.939)</td>
<td>.01</td>
</tr>
<tr>
<td>Oral corticosteroid</td>
<td>0.151</td>
<td>0.055</td>
<td>0.821 (0.513, 1.316)</td>
<td>.41</td>
<td>0.353</td>
<td>0.105</td>
<td>0.675 (0.505, 0.901)</td>
<td>.01</td>
</tr>
</tbody>
</table>

CI: 95% Confidence Interval.
N/A, Insufficient number of events.
*Crude rates.
†Asthma severity at time of enrollment.
††Includes cromolyn and leukotriene modifiers.
§Adjusted for gender, severity, age, ethnicity, clinic site, calendar time.
We did not directly measure the cost and savings associated with the Easy Breathing program. Children with asthma enrolled in Easy Breathing experienced an overall decrease in hospital days of 0.209 days/child-year. Using the average cost of a hospital day in Connecticut, this amounts to an average decrease of approximately $355/enrolled child with asthma per year. Implementation of the program required a coordinator, a physician champion, a data manager, and forms at a cost of $34/enrolled child per year. These net savings are sufficient to offset the cost associated with the increased use of prescription medication. We also observed that total hospitalizations decreased more than hospitalizations for asthma. This would seem to indicate that some of the burden of asthma is not reflected in hospital primary discharge diagnoses, and that children are hospitalized with other diagnoses that are probably aggravated by their asthma (eg, pneumonia). A similar association, although not significant, has been previously observed.30

In summary, a citywide asthma disease management program for PCPs was successful in increasing adherence to the NAEPP guidelines. In addition, adherence to the guidelines resulted in a reduction in hospitalizations, ED visits, and outpatient visits.

We thank the clinicians and office staff of Asylum Hill Family Practice Center, Burgdorf/Fleet Health Center, Community Health Services, Family Health Center, St. Francis Hospital/Pediatrics Ambulatory Care, and Connecticut Children’s Medical Center/Primary Care Center for their dedication to patient care and their willingness to participate in the Easy Breathing program. We also thank Dr Walter F. Stewart for assistance with the statistical analysis and Ms Krissy Larrow for administrative support.

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Table IV. Rates of medical services utilization for children with asthma before and after enrollment in Easy Breathing

<table>
<thead>
<tr>
<th>Medical service (#/child/year)</th>
<th>Intermittent asthma†</th>
<th>Persistent asthma†</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before Easy Breathing*</td>
<td>After Easy Breathing*</td>
</tr>
<tr>
<td></td>
<td>Adjusted Relative Rate (CI)§</td>
<td>P value</td>
</tr>
<tr>
<td>Hospitalization § 0.058</td>
<td>0.042</td>
<td>0.782</td>
</tr>
<tr>
<td>ED visit 0.631</td>
<td>0.568</td>
<td>0.915</td>
</tr>
<tr>
<td>Outpatient visit 3.76</td>
<td>3.182</td>
<td>0.782</td>
</tr>
<tr>
<td>Hospitalization (493.xx) 0.012</td>
<td>0.012</td>
<td>N/A</td>
</tr>
<tr>
<td>ED visit (493.xx) 0.07</td>
<td>0.067</td>
<td>0.689</td>
</tr>
<tr>
<td>Outpatient visit (493.xx) 0.526</td>
<td>0.445</td>
<td>0.607</td>
</tr>
</tbody>
</table>

CI: 95% Confidence Interval.
*Crude rates.
†Asthma severity at time of enrollment.
‡‡N/A: insufficient number of events.
§Adjusted for gender, severity, age, ethnicity, clinic site, calendar time.

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claims data, Department of Public Health, State of Connecticut) were surveyed, this study sample was not random. Providers appear to have targeted children with asthma. Many other eligible children with asthma were not enrolled in the program. Reasons for not enrolling children in Easy Breathing included time constraints during office visits and the need to deal with other urgent issues at the time of the visit including acute asthma management. Children with asthma continue to be enrolled in the program, and it is hoped that eventually all children will be enrolled. Children with asthma who used more medical services were more likely sampled. These children also experienced high rates of hospitalization and ED visits. Nevertheless, these children represent the burden of asthma on the medical community. Over-sampling of persons who utilize medical services may be appropriate for programs like Easy Breathing that are designed to decrease medical services utilization. Despite this over-sampling, the risk factors for asthma prevalence and increased asthma severity are similar to what have been reported in other urban, minority communities with similar ethnic groups.27-29

We also used paid claims data that underestimate the number of prescriptions written by PCPs (eg, dispensed samples). This underestimation should be the same for children in Easy Breathing and for children with asthma not enrolled in the program. We also have no measure of patient adherence to therapy. Although these factors are undoubtedly significant, they do not change the results of improved asthma management in the enrolled children. Finally, all of the study sites were urban clinics, and thus, our results may not be generalized to private practices.
REFERENCES


PRELIMINARY DIAGNOSTIC GUIDELINES FOR MACROPHAGE ACTIVATION SYNDROME COMPLICATING SYSTEMIC JUVENILE IDIOPATHIC ARTHRITIS

ANGELO RAVELLI, MD, SILVIA MAGNI-MANZONI, MD, ANGELA PISTORIO, MD, PhD, CRISTINA BESANA, MD, TIZIANA FOTI, MD, NICOLINO RUPERTO, MD, MPH, STEFANIA VIOLA, MD, AND ALBERTO MARTINI, MD

Objective To develop diagnostic guidelines for macrophage activation syndrome (MAS) complicating systemic juvenile idiopathic arthritis (S-JIA).

Study design We followed the classification criteria approach that is based on the comparison of patients with the index disease with patients with a “confusable” disease. The former group included 74 patients with S-JIA-associated MAS reported in the literature or seen by the authors; the latter group included 37 patients with S-JIA who had 51 instances of “high disease activity” seen by the authors. The relative power of clinical, laboratory, and histopathologic variables in discriminating patients with MAS from patients with high disease activity was evaluated by calculating the sensitivity rate, specificity rate, area under the receiver operating characteristic curve, and diagnostic odds ratio (DOR). The combinations of variables that led to best separation between patients and control subjects were identified through “the number of criteria present” method.

Results The strongest clinical discriminators were hemorrhages (DOR = 67) and central nervous system dysfunction (DOR = 63); the strongest laboratory discriminators were decreased platelet count (DOR = 1092), increased aspartate aminotransferase (DOR = 247), leukopenia (DOR = 70), and hypofibrinogenemia (DOR = 165). The best separation between patients and control subjects occurred when any 2 or more laboratory criteria (DOR = 1309) were simultaneously present; the second best performance was provided by the presence of any 2, 3, or more clinical and/or laboratory criteria (DOR = 765 and 743, respectively).

Conclusion We identified preliminary diagnostic guidelines for MAS complicating S-JIA. These guidelines deserve prospective validation. (J Pediatr 2005;146:598-604)

Macrophage activation syndrome (MAS) is a life-threatening complication of chronic rheumatic diseases in childhood, which is seen most commonly in systemic juvenile idiopathic arthritis (S-JIA).1,2 It is characterized by fever, hepatosplenomegaly, lymphoadenopathy, profound depression of all 3 blood cell lines, deranged liver function, intravascular coagulation, and central nervous system dysfunction, and is thought to be caused by the activation and uncontrolled proliferation of T lymphocytes and macrophages, resulting in an unrestricted release of inflammatory cytokines. It is still unclear whether MAS is a discrete event or the severe end of the spectrum of very active systemic disease. The diagnostic hallmark of MAS is found in bone marrow aspiration, by which numerous well-differentiated macrophages actively phagocytosing haematopoietic cells are revealed.3 There are no true estimates of the incidence of MAS in S-JIA. Although it has been considered a rare complication, it is probably more common than previously thought. Furthermore, MAS accounts for a significant proportion of the morbidity and mortality seen with S-JIA. Two recent case series reported a mortality rate of 8% to 22%.4,5

Because MAS is a serious condition that can follow a rapidly fatal course, prompt recognition of its clinical and laboratory features and immediate therapeutic intervention are critical. However, because it lacks a single distinguishing manifestation

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MAS  Macrophage activation system
S-JIA Systemic juvenile idiopathic arthritis
DOR Diagnostic odds ratio
AUC-ROC area under receiver operating characteristic curve
HLH hemophagocytic lymphohistiocytosis

See related article, p 591.

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Submitted for publication May 8, 2004; last revision received Nov 8, 2004; accepted Dec 8, 2004.

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and is clinically heterogeneous, early diagnosis can be difficult. The diagnostic challenges posed by MAS in S-JIA are compounded because it may mimic a flare of the underlying disease. Other important differential diagnoses would include intercurrent infections and adverse effects of medications. The difficulty in diagnosing MAS in S-JIA and the recent therapeutic advances, such as the demonstration of the distinctive efficacy of cyclosporin A and etanercept, emphasize the need for diagnostic tools and well-established diagnostic guidelines.

The purpose of this study was to evaluate the diagnostic accuracy of the clinical, laboratory, and histopathological features of MAS, with the aim of defining diagnostic guidelines for MAS complicating S-JIA.

### METHODS

#### Study Design

To identify the features that were suitable candidates as diagnostic criteria for MAS, we followed the classification criteria approach. The purpose of this approach is to separate patients with a disease from patients without the disease. Classification criteria, ideally, have a high sensitivity

<table>
<thead>
<tr>
<th>Table I. Comparison of the frequency of clinical, laboratory, and histopathological features in the instances of systemic juvenile idiopathic arthritis-associated macrophage activation system in patients reported in the literature and seen at the authors' units and in patients with systemic juvenile idiopathic arthritis who have active disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Instances of MAS reported in the literature (N = 57)</strong></td>
</tr>
<tr>
<td>Positive/ % Positive</td>
</tr>
<tr>
<td>tested</td>
</tr>
<tr>
<td><strong>Clinical features</strong></td>
</tr>
<tr>
<td>Fever (&gt;38°C)</td>
</tr>
<tr>
<td>Cutaneous rash</td>
</tr>
<tr>
<td>Hepatomegaly</td>
</tr>
<tr>
<td>Splenomegaly</td>
</tr>
<tr>
<td>Lymphadenopathy</td>
</tr>
<tr>
<td>Central nervous system dysfunction</td>
</tr>
<tr>
<td>Hemorrhages</td>
</tr>
<tr>
<td><strong>Laboratory features</strong></td>
</tr>
<tr>
<td>White blood cells ≤4.0 × 10^9/L</td>
</tr>
<tr>
<td>Hemoglobin ≤90 g/L</td>
</tr>
<tr>
<td>Platelets ≤150 × 10^9/L</td>
</tr>
<tr>
<td>Erythrocyte sedimentation rate ≤50 mm/hr</td>
</tr>
<tr>
<td>Aspartate aminotransferase ≥40 U/L</td>
</tr>
<tr>
<td>Alanine aminotransferase ≥40 U/L</td>
</tr>
<tr>
<td>Bilirubin ≥1.2 mg/dL (20.5 μmol/L)</td>
</tr>
<tr>
<td>Lactate dehydrogenase ≥900 U/L</td>
</tr>
<tr>
<td>Albumin ≤2.5 g/dL</td>
</tr>
<tr>
<td>Fibrinogen ≥2.5 g/L</td>
</tr>
<tr>
<td>Triglycerides ≥160 mg/dL (2.5 nmol/L)</td>
</tr>
<tr>
<td>Serum sodium ≤130 mmol/L</td>
</tr>
<tr>
<td>Ferritin ≥10,000 μg/L</td>
</tr>
<tr>
<td><strong>Histopathological feature</strong></td>
</tr>
<tr>
<td>Bone marrow hemophagocytosis</td>
</tr>
</tbody>
</table>

*Comparison between instances of MAS in patients reported in the literature versus those seen by the authors.*
rate for the disease in question and a high specificity rate against other diseases (that is, a high proportion of cases of the disease are found to be positive and a high proportion of cases without disease are found to be negative). These criteria are generally created by comparing patient groups with the index disease with control patients who have a "confusable disease." In our study, the index disease was represented by MAS complicating S-JIA, and the confusable condition was represented by active S-JIA.

Patients

The group with the index disease included 74 patients with S-JIA (17 observed at the authors' units and 57 reported in the literature) and MAS (in case of repeat episodes, only the first episode was examined). Patients seen at the authors' units were identified through a database search, whereas patients reported in the literature were identified through a Medline search in the years 1971 to 2003. Because the term MAS was coined in 1993, case reports published before this year were included only when they displayed a clinical and laboratory picture consistent with the syndrome that is currently named MAS. Only reports that were sufficiently detailed were analyzed. The control group with a confusable condition included 37 patients with S-JIA observed at the authors' units who had 51 instances of "high disease activity." An instance of high disease activity was defined as any of the following: the time of diagnosis, before the start of a disease-specific treatment; or the first flare with active systemic manifestations, including as a high-spiking intermittent fever, a cutaneous rash, or both. All patients observed at the authors' units were consecutive patients seen between November 1983 and May 2001 who were diagnosed as having S-JIA by the International League of Associations for Rheumatology criteria. Of the 74 patients with MAS, 37 were boys and 37 were girls; their mean age at onset of JIA was 6.3 years (range, 4 months–17 years); the mean interval between JIA and MAS onset was 3.0 years (range, 0 months–14 years). Of the 37 patients with active systemic JIA, 14 were boys and 23 were girls; their mean age at disease onset was 4.3 years (range, 5 months–10.3 years). In all study patients, the clinical, laboratory, and histopathological features that could be regarded as potential diagnostic criteria for MAS were recorded through standardized forms. Three investigators (S.M.M., C.B., and T.F.) performed the chart and literature review. Clinical criteria were considered positive when documented as present either in clinical charts or literature reports and negative when documented as such or when not documented as present. The presence/absence of each laboratory criterion was evaluated only in patients who had the correspondent test performed. When the charts or literature reports were ambiguous, reviewers met to reach consensus. Among the potentially eligible literature cases, 8 were disqualified because they did not have systemic JIA, and 11 (all in a single paper) were disqualified because of insufficient data.

Statistics

The frequency of clinical, laboratory, and histopathological features in the instances of S-JIA-associated MAS seen by the authors and in those reported in the literature was compared with the chi-square or the Fisher exact test in case of expected frequencies <5. The ability of each feature to discriminate the episodes of MAS from those of active systemic disease was evaluated by calculating the sensitivity rate, specificity rate, area under receiver operating characteristic (AUC-ROC) curve, and diagnostic odds ratio (DOR). The DOR of a test is the ratio of the odds of positivity in subjects with the disease to the odds of positivity in subjects without the disease; the value of DOR ranges from 0 to infinity, with higher values indicating better performance of a discriminatory test. For laboratory tests, the AUC-ROC and relative 95% CI were calculated. The discriminative ability of laboratory tests was assessed by using either the "standard" threshold (ie, the threshold reported in the literature or judged as clinically meaningful) or the threshold obtained through the ROC curve analysis that produced the most appropriate trade off between sensitivity and specificity (defined as "best" threshold).

The selection of a classification/diagnostic rule requires making some specific trade off between sensitivity and specificity rates, through changing the definition of "a positive." To reach this goal, we used the "number of criteria
present’ approach, which is done by varying \( l \), which is the minimum number of criteria required to be present for a patient to be classified as positive.\(^9\) In other words, if any \( l \) or more of a list of criteria are present in a patient, then the patient can be classified as positive. Notably, all criteria must be able to be judged as being either present or absent (that is, they must be dichotomous) to allow using this method. The smaller the decision threshold, the larger the number of patients who will be judged positive, resulting in high sensitivity and low specificity rates. Conversely, when the threshold chosen is large, more patients will be judged negative, resulting in low sensitivity and high specificity rates. By varying the threshold, a table can be produced, which allows the selection of the combination of variables that shows the “best” diagnostic accuracy. For each combination of variables tested, the sensitivity rate, specificity rate, and DOR were calculated.

### RESULTS

Table I shows the frequency of clinical, laboratory, and histopathological features in patients with S-JIA, and MAS who were reported in the literature and were seen at the authors’ units and in patients with S-JIA who had active systemic disease. The frequency of features observed in our patients with MAS and in patients reported in the literature was comparable, with the exceptions of bilirubin increase and erythrocyte sedimentation rate decrease, which were more common in patients reported in the literature, and of hepatomegaly, which was more frequent in our patients.

The sensitivity rate, specificity rate, AUC, and DOR obtained for each clinical, laboratory, and histopathological feature are presented in Tables II and III. For laboratory parameters, the results obtained using both standard and best threshold are shown. In general, laboratory variables and histopathological features revealed superior discriminating.
Table IV. Results of the “number of criteria approach” to the selection of diagnostic criteria: sensitivity, specificity, diagnostic odds ratio, and 95% CI for combination of clinical and laboratory criteria

<table>
<thead>
<tr>
<th>Number of clinical criteria present</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Diagnostic Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or more</td>
<td>0.82</td>
<td>0.80</td>
<td>19.2 (7.7-48.0)</td>
</tr>
<tr>
<td>2 or more</td>
<td>0.42</td>
<td>1.00</td>
<td>74.6 (4.4-1255)</td>
</tr>
<tr>
<td>3 criteria</td>
<td>0.14</td>
<td>1.00</td>
<td>16.8 (1.0-293)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of laboratory criteria present</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Diagnostic Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or more</td>
<td>1.00</td>
<td>0.87</td>
<td>319.9 (16.9-6051)</td>
</tr>
<tr>
<td>2 or more</td>
<td>1.00</td>
<td>0.97</td>
<td>1309 (51.3-33409)</td>
</tr>
<tr>
<td>3 or more</td>
<td>0.76</td>
<td>1.00</td>
<td>237.0 (12.7-4426)</td>
</tr>
<tr>
<td>4 criteria</td>
<td>0.32</td>
<td>1.00</td>
<td>38.4 (2.1-702.4)</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Number of clinical and laboratory criteria present</th>
<th>Sensitivity</th>
<th>Specificity</th>
<th>Diagnostic Odds Ratio (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 or more</td>
<td>1.00</td>
<td>0.69</td>
<td>112.2 (6.3-1994)</td>
</tr>
<tr>
<td>2 or more</td>
<td>1.00</td>
<td>0.95</td>
<td>765.0 (35.2-16609)</td>
</tr>
<tr>
<td>3 or more</td>
<td>0.92</td>
<td>1.00</td>
<td>742.6 (34.2-16142)</td>
</tr>
<tr>
<td>4 or more</td>
<td>0.84</td>
<td>1.00</td>
<td>377.4 (19.4-7347)</td>
</tr>
<tr>
<td>5 or more</td>
<td>0.60</td>
<td>1.00</td>
<td>116.6 (6.4-2114)</td>
</tr>
<tr>
<td>6 or more</td>
<td>0.24</td>
<td>1.00</td>
<td>26.3 (1.4-491.8)</td>
</tr>
<tr>
<td>7 criteria</td>
<td>0.16</td>
<td>1.00</td>
<td>16.5 (0.8-321.9)</td>
</tr>
</tbody>
</table>

*Clinical criteria: hepatomegaly, central nervous system dysfunction, hemorrhages.
†Laboratory criteria: aspartate aminotransferase >59 U/L; platelets ≥ 262 × 10^8/L; white blood cells ≤ 4.0 × 10^9/L; fibrinogen ≥ 2.5 g/L.

Table V. Preliminary diagnostic guidelines for macrophage activation system complicating systemic juvenile idiopathic arthritis

<table>
<thead>
<tr>
<th>Laboratory criteria</th>
<th>Clinical criteria</th>
<th>Histopathological criterion</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decreased platelet count (≤ 262 × 10^9/L)</td>
<td>Central nervous system dysfunction (irritability, disorientation, lethargy, headache, seizures, coma)</td>
<td>Evidence of macrophage hemophagocytosis in the bone marrow aspirate</td>
</tr>
<tr>
<td>Elevated levels of aspartate aminotransferase (&gt;59 U/L)</td>
<td>Hemorrhages (purpura, easy bruising, mucosal bleeding)</td>
<td>Diagnostic rule</td>
</tr>
<tr>
<td>Decreased white blood cell count (≤ 4.0 × 10^9/L)</td>
<td>Hypofibrinogenemia (≤ 2.5 g/L)</td>
<td>The diagnosis of MAS requires the presence of any 2 or more laboratory criteria or of any 2 or 3 or more clinical and/or laboratory criteria. A bone marrow aspirate for the demonstration of hemophagocytosis may be required only in doubtful cases.</td>
</tr>
<tr>
<td>Hypofibrinogenemia (≤ 2.5 g/L)</td>
<td></td>
<td>Recommendations</td>
</tr>
</tbody>
</table>

The aforementioned criteria are of value only in patients with active S-JIA. The thresholds of laboratory criteria are provided by way of example only.

Comments
1. The clinical criteria are probably more useful as classification criteria rather than as diagnostic criteria because they often occur late in the course of MAS and may be, therefore, of limited value for the early suspicion of the syndrome.
2. Other abnormal clinical features in S-JIA-associated MAS, not aforementioned, may include: nonremitting high fever, splenomegaly, generalized lymphoadenopathy, and paradoxical improvement of signs and symptoms of arthritis.
3. Other abnormal laboratory findings in S-JIA-associated MAS, not aforementioned, may include: anemia, erythrocyte sedimentation rate fall, elevated levels of alanine aminotransferase, increased bilirubin, presence of fibrin degradation products, elevated lactate dehydrogenase, hypertriglyceridemia, low sodium levels, decreased albumin, and hypofibrinogenemia.

ability, as compared with clinical features. All clinical features revealed better performances for specificity rate than sensitivity rate, with the sole exception of fever, which was highly sensitive but had very poor specificity. The presence of hemorrhages or central nervous system abnormalities reached the maximum level of specificity, although they showed relatively low sensitivity. The strongest laboratory discriminators were decreased platelet count, abnormal liver function test results, hypofibrinogenemia hyponatremia, hyperferritinemina, hypertriglyceridemia, and decreased white blood cell count. Although quite impressive, the results provided by hyperferritinemia should be regarded with caution, because this parameter was tested only in 8 patients with MAS and in 11 patients with active S-JIA.

Table IV illustrates the results obtained through the “number of criteria present” approach. Only variables that provided strong discriminating properties, were not duplicative, and were available for a sufficient number of patients were examined. The clinical variables included were: hemorrhages, central nervous system dysfunction, and hepatomegaly; the laboratory variables included were decreased platelet count, aspartate aminotransferase increase, reduced white blood cell count, and hypofibrinogenemia. For each laboratory variable, the most discriminating threshold, either standard or best, was used. The bone marrow evidence of hemophagocytosis was not included because it was regarded as a confirmatory criterion rather than a first-line diagnostic criterion. The study variables were combined in 3 different ways: clinical variables only; laboratory variables only; and clinical and laboratory variables. For each combination of variables, the sensitivity rate, specificity rate, and DOR were calculated. The best performance was provided by the presence any of 2 or more
laboratory criteria (DOR = 1309), followed by the presence of any 2, 3, or more clinical and/or laboratory criteria (DOR = 765 and 743, respectively). On the basis of these results, we set up the diagnostic guidelines for MAS complicating S-JIA, which are presented in Table V.

**DISCUSSION**

There are no formal and universally accepted criteria for the diagnosis of MAS in S-JIA. The recognition that this syndrome bears close resemblance to the clinical and laboratory picture of hemophagocytic lymphohistiocytosis (HLH) has led many clinicians to use in practice the diagnostic guidelines for this disease.\(^3\)\(^5\) There are, however, several problems with the use of HLH criteria in patients with MAS, the first of which is the need for tissue confirmation. As noted in patients with HLH\(^3\)\(^6\) and among patients with MAS reviewed in our study, the bone marrow aspirate does not always show hemophagocytosis, and furthermore, hemophagocytosis is not always demonstrable at onset. Although in HLH hemophagocytosis may be seen more frequently in liver, lymph node, or splenic biopsies than in the bone marrow biopsies, these biopsies would be difficult in children with MAS because of the frequent presence of intravascular coagulopathy. The failure to demonstrate hemophagocytosis does not negate the diagnosis of HLH. These problems emphasize the need to evaluate the sensitivity and specificity rates of clinical features and laboratory markers to try to obviate the need for tissue diagnosis. Another shortcoming of HLH criteria in MAS is caused by certain criteria not applying to patients with S-JIA. Because of the prominent inflammatory nature of the latter disease, the occurrence of a relative decrease in white blood cell count, platelets, or fibrinogen rather than the absolute decrease required by the HLH criteria may be more relevant in making an early diagnosis.

The results of our study show that MAS can be reliably identified from active S-JIA with simple clinical and laboratory criteria. Overall, clinical features were less strong discriminators as compared with laboratory features. The low specificity rate of fever, however, should be regarded with caution because the literature reports of MAS only rarely allowed a precise identification of its pattern. Because the pattern of fever in MAS is known to be non-remitting\(^5\) and is, thus, different from the remitting high spiking fever seen in active S-JIA, future investigations should focus on the definition of the change in the fever pattern during MAS. Hepatomegaly was more discriminating than splenomegaly and lymphoadenopathy, reflecting the distinctive vulnerability of the liver to the pathogenic process of MAS. Central nervous system dysfunction or hemorrhages turned out to have the maximum specificity (ie, they were not seen in any instance of active S-JIA), although their sensitivity rate was not as good because they both occurred in only one third of MAS episodes.

Most laboratory features showed excellent discriminating properties, with the use of standard threshold being more advantageous in some cases, and the use of best threshold more advantageous in other cases. That the best threshold for the platelet count (262 \(\times 10^9/L\)) would fall in the reference range in a healthy individual is not surprising, because patients with active S-JIA commonly have considerable thrombocytosis (occasionally higher than 1000 \(\times 10^9/L\)), which has been related to markedly increased production of interleukin-6,\(^3\)\(^7\) and, thus, a relative decrease of platelet count, rather than an absolute decrease, is diagnostically meaningful.

Very high ferritin levels are commonly encountered in disorders characterized by histiocyte proliferation and active phagocytosis of erythrocytes, such as malignant histiocytosis and virus-associated hemophagocytic syndrome.\(^3\)\(^8\) In recent years, hyperferritinemia has been recognized as an important laboratory hallmark of MAS.\(^8\)\(^,\)\(^3\)\(^0\)\(^,\)\(^3\)\(^9\) Although serum ferritin was assessed in only 8 of the 74 episodes of MAS reviewed in our study, its sensitivity and specificity rates were very high. Altogether, these observations suggest that measurement of serum ferritin level may have strong diagnostic value for MAS. Besides the role of ferritin, future studies should investigate the diagnostic validity of fibrin D-dimer.\(^4\)\(^0\)

On the basis of the results of statistical analyses and considering the clinical importance of the different features, we selected 3 clinical criteria and 4 laboratory criteria to be included in the final set of guidelines for MAS (Table V). Using the “number of criteria present approach”, we found that the best separation between patients and control subjects occurred when any 2 or more laboratory criteria of 4 candidates were simultaneously present. This is in keeping with the clinical experience indicating that early suspicion of MAS is most commonly raised by the detection of subtle laboratory abnormalities, whereas clinical symptoms are often delayed. The strong discriminating power shown by the aforementioned definitions led us to suggest that demonstration of macrophage hemophagocytosis in the bone marrow aspirate is reserved for diagnostic confirmation only in doubtful cases.

Our study should be viewed in the light of certain limitations, which include the retrospective collection of the clinical features, some of which may have been overlooked or not recorded in some patients, and the unavailability of several laboratory measurements in a number of patients reported in the literature. Furthermore, although most of the laboratory tests examined are widely standardized routine procedures, their execution in different laboratories and in diverse periods may have affected the reliability of comparisons. Thus, as recommended in the criteria, the thresholds for laboratory parameters are provided for example only.

In summary, we present preliminary diagnostic guidelines for MAS complicating S-JIA to enable timely diagnosis and correct classification of patients, assist in the evaluation of different therapeutic approaches, and facilitate communication among specialists in different fields who might be interested in reactive hemophagocytic syndromes. Prospective validation of these guidelines is now necessary to further examine the relative strength of all potential diagnostic criteria.
REFERENCES


EVALUATION OF AN ASSOCIATION BETWEEN GASTROINTESTINAL SYMPTOMS AND CYTOKINE PRODUCTION AGAINST COMMON DIETARY PROTEINS IN CHILDREN WITH AUTISM SPECTRUM DISORDERS

HARUMI JYONOUCHI, MD, LEE GENG, PHD, AGNES RUBY, BS, CHITRA REDDY, MD, AND BARBIE ZIMMERMAN-BER, MD

Objective To evaluate an association between cytokine production with common dietary proteins as a marker of non-allergic food hypersensitivity (NFH) and gastrointestinal (GI) symptoms in young children with autism spectrum disorders (ASD).

Study design Peripheral blood mononuclear cells (PBMCs) were obtained from 109 ASD children with or without GI symptoms (GI [+]) ASD, N = 75 and GI (−) ASD, N = 34], from children with NFH (N = 15), and control subjects (N = 19). Diarrhea and constipation were the major GI symptoms. We measured production of type 1 T-helper cells (Th1), type 2 T-helper cells (Th2), and regulatory cytokines by PBMCs stimulated with whole cow’s milk protein (CMP), its major components (casein, β-lactoglobulin, and α-lactoalbumin), gliadin, and soy.

Results PBMCs obtained from GI (+) ASD children produced more tumor necrosis factor-α (TNF-α)/interleukin-12 (IL-12) than those obtained from control subjects with CMP, β-lactoglobulin, and α-lactoalbumin, irrespective of objective GI symptoms. They also produced more TNF-α with gliadin, which was more frequently observed in the group with loose stools. PBMCs obtained from GI (−) ASD children produced more TNF-α/IL-12 with CMP than those from control subjects, but not with β-lactoglobulin, α-lactoalbumin, or gliadin. Cytokine production with casein and soy were unremarkable.

Conclusion A high prevalence of elevated TNF-α/IL-12 production by GI (+) ASD PBMCs with CMP and its major components indicates a role of NFH in GI symptoms observed in children with ASD. (J Pediatr 2005;146:605-10)

Autism spectrum disorders (ASDs) are complex developmental disorders with unknown etiology and no known curative measures. Many parents thus turn to therapeutic measures of complementary and alternative medicine. However, these measures often lack rigorous scientific validation for their efficacy/safety and could be potentially hazardous. Among such measures, a casein-free, gluten-free diet has been very popular, partly because of the high prevalence of gastrointestinal (GI) symptoms (cramping, diarrhea, constipation alternating with diarrhea, gastroesophageal reflux, bloating, and loose/undigested stool) in children with ASD. Moreover, parents/therapists/caretakers frequently report resolution of GI symptoms with the casein-free, gluten-free diet in children with ASD.

Immunoglobulin E (IgE)-mediated food allergy accounts for only a small portion of adverse reaction to dietary proteins (DPs; 2%-3%). Instead, cell-mediated immunity plays a vital role in non-allergic food hypersensitivity (NFH), with major causative DPs being cow’s milk protein (CMP), soy, and wheat.1,2 Tumor necrosis factor-α (TNF-α) production in response to CMP appears closely associated with GI inflammation and clinical features of NFH, and the elimination diet leads to a decline in TNF-α production.

See editorial, p 582.

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Supported by the Jonty Foundation (St. Paul, Minnesota).

Submitted for publication May 21, 2004; last revision received Sep 27, 2004; accepted Jan 12, 2005.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2005.01.027
with CMP.3-5 Cell-mediated immune reactions take place several hours and even 1 to 2 days after the intake of reactive DPs. In the absence of commercially available diagnostic laboratory measures, the gold standard of NFH diagnosis is a resolution of GI symptoms with the elimination diet and their recurrence with the challenge of causative DPs. Such clinical features of NFH may make diagnosis more challenging, especially in children who are developmentally delayed.6

Our previous study revealed that peripheral blood mononuclear cells (PBMCs) from a number of children with ASD produced elevated levels of TNF-α and interferon-γ (IFN-γ), with CMP as observed in NFH.1-2,5,7 In that study, there was no increase in atopic disorders or IgE-mediated food allergy.7 These findings indicate that GI symptoms observed in children with ASD are partly associated with NFH to common DPs, and this may partly explain the apparent favorable effects of the casein-free, gluten-free diet in children with ASD.

However, it is unknown how frequently children with ASD exhibit such cellular reactivity to DPs commonly associated with NFH in comparison with GI symptoms.

**METHODS**

**Study Subjects**

The study subjects included children (aged 1-10 years) in Tanner stage 1. Children with ASD were recruited from those referred to the Autism Center at the New Jersey Medical School, University of Medicine and Dentistry of NJ, Newark, NJ. ASD diagnosis was made or ascertained by means of the Diagnostic and Statistical Manual of Mental Disorders-IV, the International Classification of Diseases-10 criteria, or both, the Autism Diagnostic Interview-Revised, and Autism Diagnostic Observational Schedules. Children with NFH and typically developing control children were recruited from those seen in the Allergy/Immunology Clinic and General Pediatrics Clinic at the New Jersey Medical School. We excluded subjects taking neuropsychiatric medications and those with known immunodeficiency, metabolic disorders, genetic diseases, and illnesses involving major organs. Blood samples were collected after institutional review board-approved signed consent forms were obtained. At the time of venipuncture, all study subjects were on an unrestricted diet, not febrile, and had no evidence of acute microbial illnesses.

Children with ASD were subdivided into 2 groups by the presence or absence of GI symptoms, GI (+) or GI (−) (Table I). We defined GI symptoms as vomiting, diarrhea, chronic loose stool, colic and GI cramping, and constipation (often alternating with diarrhea) reported by parents/caretakers/physicians. Of the GI (+) ASD children, autism was diagnosed in 36 and pervasive developmental disorder NOS (PDD-NOS) was diagnosed in 27. Of the GI (−) ASD children, autism was diagnosed in 14 and PDD-NOS was diagnosed in 1. ASD not falling into clear diagnostic criteria of autism or PDD-NOS because of young age was diagnosed in the remaining children. Diagnosis of atopic asthma, allergic rhinitis, and allergic dermatitis was made by the presence of clinical features of these disorders with positive skin test reactivity, the presence of allergen-specific IgE against common airborne and food allergens. The prevalence of atopic disorders in children with ASD was similar to that reported in general population (Table I).8

**Table I. Demographics of the study subjects**

<table>
<thead>
<tr>
<th>Study group</th>
<th>Age (years)</th>
<th>Subject number (Male:Female)</th>
<th>Atopic disorders</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI (+) ASD*</td>
<td>4.7 (1.8–10.6)*</td>
<td>75 (61:14)</td>
<td>19/75 (25.3%)</td>
</tr>
<tr>
<td>GI (−) ASD*</td>
<td>5.4 (2.1–10.2)</td>
<td>34 (32:2)</td>
<td>12/34 (35.3%)</td>
</tr>
<tr>
<td>NFH</td>
<td>2.8 (1.3–7.8)</td>
<td>15 (10:6)</td>
<td>2/15 (13.3%)</td>
</tr>
<tr>
<td>Controls</td>
<td>3.8 (1.0–9.0)</td>
<td>19 (11:8)</td>
<td>unknown</td>
</tr>
</tbody>
</table>

*The results are expressed as the median (range).

Assessment of Immune Reactivity Against Dietary Proteins

PBMCs (10^6 cells/mL) were cultured in the presence of common DPs for 4 to 5 days, and levels of IFN-γ, TNF-α, Interleukin-5 (IL-5), IL-10, and IL-12p40 in the culture supernatant were determined as a measure of assessing cellular immune reactivity. IFN-γ and IL-5 were selected as representative type 1 and type 2 T-helper (Th1 and Th2) cytokines, respectively. A significant increase in TNF-α production by PBMCs with CMP has been reported in patients with non-allergic CMP hypersensitivity.3,5 IL-10 was measured as a representative regulatory cytokine produced by T cells and other lineage cells; dysregulated IL-10 production can be associated with various inflammatory and autoimmune conditions.8 IL-12p40 is a degraded product of biologically functional IL-12p70 that promotes Th1 responses. We also measured IL-4, transforming growth factor-β, and IL-2 in a few study and control subjects. However, we observed little production of these cytokines with DPs, as reported before, and thus these cytokines were not measured in the rest of the study subjects.7

Among DPs, we tested reactivity to crude cow’s milk and soy protein extracts (provided by Ross Products Division/Abbott Laboratories, Columbus, Ohio), gliadin (a major wheat protein; Sigma, St. Louis, Mo), and major components of
CMP, bovine casein, α-lactoalbumin, and β-lactoglobulin (Sigma). These DPs were major causes of NFH in children.\(^7\) Concentrations of these DPs used were 100 \(\mu g/mL\) for CMP and soy and 10 \(\mu g/mL\) for gliadin and major components of CMP. We detected <1 ng/mL of endotoxin in these DPs (1 mg/mL solution; Endotoxin kit, Sigma). We used recall Ags (tetanus toxoid and dust mite extract) and T cell mitogens (phytohemagglutinin [10 \(\mu g/mL\)] and concanavalin A [1\(\mu g/mL\)]) as positive control stimuli to ensure normal immune reactivity to control stimuli in the study subjects. Cytokine levels in the culture supernatants were measured by an enzyme-linked immunosorbent assay, using OptEIA Reagent Sets (BD Pharmingen, San Diego, Calif). Intra- and inter-variations of cytokine levels were less than 5\%.\(^7,10\)

**Statistical Analysis**

Equality of 2 sets of data values was evaluated with the Mann-Whitney test (independent samples) or Wilcoxon ranked ranks test (related samples). Multiple sets of values were evaluated with the Kruskal-Wallis test. Correlation was assessed with the 2-tailed Kendall’s \(\tau\) test. Differences with a \(P\) value <.05 were considered to be significant.

**RESULTS**

**Cytokine Production in Response to Common DPs**

Our findings in cytokine production with a stimulus of each DP tested were:

1) CMP: PBMCs from both GI (+) and GI (-) ASD children produced more TNF-α and IL-12 than those from control subjects. NFH PBMCs produced more IFN-γ, TNF-α, IL-10, and IL-12 than those from control subjects (Figure 1A).

2) Casein: Cytokine production by PBMCs was minimal and did not differ among the study groups (data not shown).

3) β-Lactoglobulin: PBMCs from GI (+) ASD children, but not from GI (-) children, produced more TNF-α and IL-12 than those from control subjects (Figure 1B). These levels are also higher than those produced by GI (-) ASD PBMCs (\(P<.05\)). NFH PBMCs produced the highest levels of IFN-γ, TNF-α, and IL-12 among the study groups (Figure 1B).

4) α-Lactoalbumin: GI (+) but not GI (-) ASD PBMCs produced more TNF-α and IL-12 than those from control subjects (Figure 1C). IL-12 production by GI (+) ASD PBMCs was also higher than that by GI (-) ASD PBMCs (\(P<.02\)). NFH PBMCs also produced more TNF-α and IL-12 than those from control subjects (Figure 1C), and their TNF-α production was the highest among the study groups (\(P<.005\)).

5) Gliadin: GI (+) but not GI (-) ASD PBMCs produced more TNF-α than those from control subjects (Figure 1D), and TNF-α levels produced were also higher than those produced by GI (-) ASD PBMCs (\(P<.05\)). NFH PBMCs also produced more TNF-α than those from control subjects.

**Figure.** Production of IFN-γ, TNF-α, IL-10, and IL-12 by PBMCs from GI (+) ASD, G (-) ASD, NFH and control children with stimuli of CMP (A), β-lactoglobulin (B), α-lactoalbumin (C), and gliadin (D). The results are expressed as control mean values ± 1SD. Marked values are higher than controls by means of the Mann-Whitney test. *\(P<.005\); **\(P<.02\); + + \(P<.05\).
Cytokine Production in ASD Children

Relationship of GI Symptoms and DP-Induced Cytokine Production in ASD Children

We also analyzed the relationship between elevated TNF-α and IL-12 production (>CM + 1SD) with DPs (CMP, β-lactoglobulin, and gliadin) and objective GI symptoms in ASD and NFH children, because subjective GI symptoms were difficult to assess in children with ASD because of their poor expressive languages. PBMCs that produced >CM + 1SD TNF-α, IL-12, or IFN-γ with gliadin also produced >CM + 1SD of these cytokines with CMP, β-lactoglobulin, or both without exception. The results with α-lactoalbumin were similar to those obtained with β-lactoglobulin.

1) GI (+) ASD group: The most common GI symptoms reported were loose stool and constipation (Table II). GI (+) ASD PBMCs produced >CM + 1SD TNF-α and IL-12 with CMP and β-lactoglobulin at high frequency irrespective of objective GI symptoms. In contrast, >CM + 1SD TNF-α production with gliadin was more frequently found in the loose stool group (Table II). Elevated IL-12 production was less frequently found with a stimulus of gliadin, irrespective of GI symptoms (Table III). A positive correlation was observed between TNF-α and IL-12 levels produced with β-lactoglobulin, α-lactoalbumin, and gliadin (P < .005), but not with CMP. In summary, with stimuli of CMP and or β-lactoglobulin, >CM + 1SD TNF-α production, IL-12 production, or both is frequently seen in PBMCs obtained from GI (+) ASD children (55/75; 73.3%).

Among 20 GI (+) ASD children without elevated TNF-α production, IL-12 production, or both with DPs, 7 of 20 had moderate to heavy growth of Candida albicans in their stool, 1 in the diarrhea group, 2 in the loose stool group, and 4 in the constipation group. Another 2 subjects had atopic dermatitis with elevated IgE antibodies against common food allergens. Another 5 subjects had a history of chronic or recurrent otitis media and sinusitis requiring frequent antibiotic treatment, 3 in the loose stool group and 2 in the constipation group.

2) GI (−) ASD Group: PBMCs from GI (−) ASD children produced >CM + 1SD TNF-α and IL-12 with high frequency, but less frequently with β-lactoglobulin or gliadin (Tables II and III). TNF-α and IL-12 levels with CMP, but not with β-LG or gliadin, were positively correlated (P < .005). In summary, with stimuli of CMP, β-lactoglobulin, or both, GI (−) ASD PBMCs produced

### Table II. Relationship between gastrointestinal symptoms and tumor necrosis factor-α production by peripheral blood mononuclear cells with cow’s milk protein, β-lactoglobulin, and gliadin

<table>
<thead>
<tr>
<th>Stimulants (&gt;CM + 1SD) (N = 75)</th>
<th>CMP</th>
<th>β-lactoglobulin</th>
<th>Gliadin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI (−) ASD</td>
<td>17 (50%)</td>
<td>13 (38.2%)</td>
<td>8 (23.5%)</td>
</tr>
<tr>
<td>(N = 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI (+) ASD</td>
<td>39 (52.0%)</td>
<td>44 (58.7%)</td>
<td>31 (41.3%)</td>
</tr>
<tr>
<td>(N = 75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea (N = 8)</td>
<td>4 (50.0%)</td>
<td>4 (50.0%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Loose stool (N = 39)</td>
<td>21 (53.8%)</td>
<td>25 (64.1%)</td>
<td>23 (59.0%)</td>
</tr>
<tr>
<td>Constipation (N = 28)</td>
<td>15 (53.4%)</td>
<td>15 (53.4%)</td>
<td>8 (28.6%)</td>
</tr>
<tr>
<td>NFH (N = 15)</td>
<td>13 (86.7%)</td>
<td>13 (86.7%)</td>
<td>10 (66.7%)</td>
</tr>
<tr>
<td>Diarrhea (N = 5)</td>
<td>4 (80.0%)</td>
<td>5 (100%)</td>
<td>3 (100%)</td>
</tr>
<tr>
<td>Loose stool (N = 10)</td>
<td>9 (90.0%)</td>
<td>8 (80.0%)</td>
<td>7 (70.0%)</td>
</tr>
</tbody>
</table>

### Table III. Relationship between gastrointestinal symptoms and interleukin-12 production by peripheral blood mononuclear cells with cow’s milk protein, β-lactoglobulin, and gliadin

<table>
<thead>
<tr>
<th>Stimulants (&gt;CM + 1SD) (N = 75)</th>
<th>CMP</th>
<th>β-lactoglobulin</th>
<th>Gliadin</th>
</tr>
</thead>
<tbody>
<tr>
<td>GI (−) ASD</td>
<td>15 (44.1%)</td>
<td>10 (29.4%)</td>
<td>4 (11.8%)</td>
</tr>
<tr>
<td>(N = 34)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI (+) ASD</td>
<td>42 (56.0%)</td>
<td>40 (53.3%)</td>
<td>12 (16.0%)</td>
</tr>
<tr>
<td>(N = 75)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Diarrhea (N = 8)</td>
<td>4 (50.0%)</td>
<td>4 (50.0%)</td>
<td>1 (12.5%)</td>
</tr>
<tr>
<td>Loose stool (N = 39)</td>
<td>25 (64.1%)</td>
<td>24 (61.5%)</td>
<td>7 (17.9%)</td>
</tr>
<tr>
<td>Constipation (N = 28)</td>
<td>12 (42.9%)</td>
<td>13 (46.4%)</td>
<td>4 (14.3%)</td>
</tr>
<tr>
<td>NFH (N = 15)</td>
<td>9 (60.0%)</td>
<td>13 (86.7%)</td>
<td>4 (26.7%)</td>
</tr>
<tr>
<td>Diarrhea (N = 3)</td>
<td>4 (80.0%)</td>
<td>5 (100%)</td>
<td>0 (0.0%)</td>
</tr>
<tr>
<td>Loose stool (N = 10)</td>
<td>5 (50.0%)</td>
<td>8 (80.0%)</td>
<td>4 (40.0%)</td>
</tr>
</tbody>
</table>
>CM + 1SD TNF-α more frequently (17/34; 50%) than IL-12 (8/34; 23.5%).

3) NFH group: The most common GI symptoms were loose stool in NFH children (Table III). Most NFH PBMCs produced >CM + 1SD TNF-α and IL-12 with CMP, β-lactoglobulin, or both, irrespective of GI symptoms. With gliadin, >CM + 1SD IL-12 production was only seen in NFH children with loose stool (Table III). TNF-α and IL-12 production were positively correlated in NFH PBMCs with stimuli of β-lactoglobulin and gliadin (P < .05).

DISCUSSION

Our results revealed a high prevalence (>70%) of cellular immune reactivity to CMP and its major components in GI (+) ASD children when positive reactivity is defined as >CM + 1SD production of TNF-α, IL-12, or both with CMP, β-lactoglobulin (a major component of CMP), or both. Such cellular reactivity was less remarkable with gliadin. Our findings indicate a possible role of NFH against CMPs in GI symptoms observed in children with ASD.

GI symptoms are frequently observed in children with ASD, with evidence of GI inflammation by means of imaging and endoscopic examinations. Other authors also reported non-specific colitis with ileal-lymphoid nodular hyperplasia accompanied by colonic CD8 and TCRγδ T cell infiltration and prominent epithelial cell damage in regressive autism. In 24 children with regression autism, pathological findings included epithelial IgG and complement deposition with infiltration of enterocytes and lymphocytes in epithelium and lamina propria of duodenum. Other authors also reported an increase in T cells in intestinal epithelium and increased T and B cells in lamina propria in children with ASD who had a resolution of GI symptoms with the implementation of the casein-free, gluten-free diet. These findings indicate a role of T cell mediated, cellular immune responses in the GI inflammation observed in children with ASD.

As noted in the introductory section, cellular immune reactivity to DPs plays a vital role in NFH. Increase in TNF-α production by PBMCs with CMP appear closely associated with clinical features of NFH in children who are reactive to CMP. In this study, we observed marked elevation of IFN-γ, TNF-α, and IL-12, but not IL-5 or IL-10, in most NFH children when stimulated with CMP and its major components (β-lactoglobulin and α-lactoalbumin). We obtained a similar but less remarkable result in GI (+) ASD PBMCs. Namely, 55 of 75 GI (+) ASD PBMCs (73.3%) produced >CM + 1SD TNF-α/IL-12 with CMP, β-lactoglobulin, or both. Prevalence of AD with or without food allergy and other atopic disorders was equivalent among the study groups and similar to that in the general population. We also obtained similar results with α-lactoalbumin in ASD PBMCs. Taken together, our results indicate that GI symptoms found in children with ASD are partly attributed to NFH to CMP and its 2 major components, α-lactoalbumin and β-lactoglobulin. All the GI (+) ASD children with elevated (>CM + 1SD) TNF-α/IL-12 production responded favorably to the elimination diet per parental report, and we are in the process of conducting a prospective study in children with ASD who have positive or negative cellular immune reactivity to CMP, β-lactoglobulin, or both, as defined as aforementioned.

Our study also revealed that β-lactoglobulin and α-lactoalbumin, but not casein, are major CMP components inducing significant TNF-α, IL-12, and IFN-γ production in GI (+) ASD and NFH subjects. However, in children with ASD who have atopy, especially atopic dermatitis (AD), we observed an increase in IL-5 production with casein (unpublished observation). Our results provide practical information that children with ASD who have NFH to CMP but do not have atopic disorders may have a good probability of tolerating casein-containing processed food, making the implementation of a diary-free diet easier. An increase in TNF-α/IL-12 production to gliadin, a major wheat protein, was less frequently found in GI (+) ASD children, especially those with diarrhea or constipation. Our finding indicates that in GI (+) ASD children, especially those with diarrhea or constipation, a gluten-free diet may not be required for resolution of GI symptoms. These questions need to be addressed further in a prospective study of children with ASD enrolled in a trial of the elimination diet on the basis of defined cellular immune reactivity.

Another notable finding in this study is that 17 of 34 GI (-) ASD PBMCs produced >CM + 1SD TNF-α/IL-12 with CMP, β-lactoglobulin, or both. Among them, 8 subjects also produced >CM + 1SD TNF-α/IL-12 with gliadin. Eleven of these 17 children underwent a trial of the casein-free diet or casein-free, gluten-free diet on the basis of cellular immune reactivity; soy products were not substituted as dairy products in these children, because children with NFH to CMP are at a high risk of developing NFH to soy when it is substituted. Parents of all 11 of these children reported more regular bowel movements with less hard stool and an improvement of behavioral symptoms (less irritability, less hyperactivity, less stimulatory behaviors; unpublished observation). These preliminary findings suggest that certain GI symptoms, such as GI cramping because of NFH, may be under-appreciated in children with ASD, most likely because of their poor expressive language. Delayed implementation of intervention measures for NFH could aggravate behavioral symptoms in these children with ASD. Even in children with normal cognitive activity, non-IgE mediated NFH can be frequently under-diagnosed. Further addressing this possibility will require a prospective study examining changes in GI and behavioral symptoms in children with ASD enrolled in a trial of elimination diets on the basis of defined cellular immune reactivity to DPs.

Another notable finding in this study is that 20 of the 75 of GI (+) ASD children did not have elevated immune reactivity against DPs. As described in the Results section, 2 of these 20 children had IgE-mediated food allergy. In the remaining 18 subjects, GI symptoms appeared not to be associated with the NFH to common DPs tested in this study. Five of these 18 subjects had a history of frequent
antibiosis caused by recurrent otitis media and sinusitis, and 7 of them had evidence of candida overgrowth on stool cultures. These findings might be associated with their GI symptoms, but further evaluation will be required in these children, and we cannot completely rule out NFH to other DPs in these children.

In addition to IFN-γ and TNF-α that are implicated with the pathogenesis of NFH, we also assessed regulatory cytokine production (IL-10 and IL-12) by PBMCs when stimulated with common DPs. IL-12 produced by innate immune cells promotes Th1 responses that are characterized by Th1 cytokine (IFN-γ and TNF-α) production and potent cellular immune reactivity. PBMCs from GI (+) ASD and NFH children produced more IL-12 with CMP/β-lactoglobulin/α-lactalbumin than those from control subjects. Even GI (−) ASD PBMCs produced more IL-12 with CMP than those from control subjects. Moreover, IL-12 production was positively correlated with TNF-α production with these stimuli in PBMCs from both ASD and NFH children. Thus, IL-12 production in our assay system likely related to activation of DP-specific T cells, because T cell cytokines promote the production of IL-12 by monocytes.

In contrast to IL-12 production, IL-10 production did not differ among the study groups. IL-10 is one of the major cytokines produced by regulatory T cells and exerts inhibitory actions on monocytes and T cells, partly suppressing production of IFN-γ and TNF-α. Our results revealed that only NFH PBMCs produced more IL-10 with CMP, pointing out a possibility that the production of regulatory cytokines such as IL-10 might be less in children with ASD who have NFH to CMP. A prospective study evaluating the effects of the elimination diet in ASD children with or without positive cellular immune reactivity to CMP and other common DPs is needed to address this possibility.

REFERENCES

PHYSICIANS’ WILLINGNESS TO GRANT REQUESTS FOR ASSISTANCE IN DYING FOR CHILDREN: A STUDY OF HYPOTHETICAL CASES

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Objective  To study the willingness of Dutch physicians to use potentially life-shortening or lethal drugs for severely ill children.

Study design  We asked 63 pediatricians about their approach to 10 hypothetical cases of children with cancer. The age of the child (15, 11, or 6 years), the child’s (explicit) request, and the opinion of the parents varied. Two hypothetical cases were also presented to 125 general practitioners and 208 clinical specialists.

Results  Most pediatricians were willing to increase morphine in all cases. A total of 48% to 60% of pediatricians were willing to use lethal drugs in children at the child’s request, when the parents agreed; when parents requested ending of life of their unconscious child, 37% to 42% of pediatricians were willing; 13% to 28% of pediatricians were willing when parents did not agree with their child’s request. General practitioners and clinical specialists were as willing as pediatricians to use lethal drugs at the child’s request, but less willing to grant a request of parents for their unconscious child.

Conclusions  Many Dutch pediatricians are willing to use potentially life-shortening or lethal drugs for children. The legal limit of 12 years, as the age under which voluntary euthanasia is forbidden, is not fully supported by Dutch physicians. (J Pediatr 2005;146:611-7)

End-of-life decisions, that is, decisions that may intentionally or unintentionally hasten death, include decisions to use drugs with possible life-shortening effects and lethal drugs. In the Netherlands, the use of lethal drugs with the explicit intention to hasten death is defined as euthanasia when someone other than the patient administers the drugs at the explicit request of the patient and as physician-assisted suicide when the patient takes these drugs himself or herself. Before April 2002, Dutch law prohibited euthanasia and physician-assisted suicide. However, physicians who performed euthanasia or physician-assisted suicide were not prosecuted when they applied the established rules for careful decision-making. In recent years, whether euthanasia or physician-assisted suicide should be allowed for children has been debated. The law on euthanasia that came into effect in April 2002 allows physicians to grant requests for euthanasia or physician-assisted suicide to adults aged 18 years or older. Euthanasia, or physician-assisted suicide, for minors aged 16 or 17 years is allowed when parents are informed, and for minors aged 12 to 16 years when parents agree with the request. For children younger than 12 years of age, euthanasia or physician-assisted suicide is not allowed, and the use of lethal drugs without the request of a patient is still legally prohibited for all age groups.

In the Netherlands in 2001, 20% of all deaths were preceded by the use of a drug with a possible life-shortening effect to alleviate pain or other symptoms, whereas approximately 3.5% of deaths were preceded by the use of lethal drugs, mostly at the request of the patient. In 1995, 23% of all deaths of neonates and infants were preceded by the use of a drug with a possible life-shortening effect to alleviate pain or other symptoms, and 9% of deaths were preceded by the use of lethal drugs. No data have been published about end-of-life decision-making in children after the neonatal period.

Dutch physicians are more willing to perform euthanasia in a cancer patient who is in excruciating pain than American physicians from Oregon (59% versus 24%). However, the attitudes of pediatricians or other physicians toward using lethal drugs, or drugs with
a possible life-shortening effect (such as morphine) in severely ill children have rarely been studied.4-6

End-of-life decision-making in children is complex, because it almost always involves 3 parties: physicians, the child, and the parents.7,8 Questions arise, such as “who should have the most important vote in the decision?” and “at what age should children be involved in the decision-making?” It is often difficult to decide whether and when it is possible or desirable to discuss end-of-life decisions with the child and how to address, for example, children’s requests to receive assistance in dying.9-14 Parents are often assigned an important role in the decision-making process. However, there are different opinions about whether parents should make decisions themselves, should be consulted before the physician makes a decision, or should be protected from participating in such emotionally charged issues.9,12-20

Therefore, this study was designed to gain insight about the willingness of Dutch pediatricians, other clinical specialists, and general practitioners to use lethal or potentially life-shortening drugs in children and about the characteristics of cases and physicians that determine such willingness.

METHODS

Data are presented from 2 interview studies, one among pediatricians and one among general practitioners and clinical specialists. For both studies, physicians who had worked at least 2 years and for more than 50% of their time in their current practice were sampled. Data were collected between March and December 2002.

Pediatricians

The sample consisted of specialists who attend the majority of all deaths in children in the Netherlands: pediatrician-oncologists and hematologists, pediatrician-intensivists, and pediatric neurologists. Pediatrician-oncologists/hematologists and pediatrician-intensivists work exclusively at departments within the 8 university hospitals in the Netherlands. From each department, half the physicians were randomly selected, or all were selected when only 1 or 2 physicians worked in the department. The sample of pediatric neurologists, who also work in other than university hospitals, was drawn from their professional registry. For each hospital, half the pediatric neurologists were randomly selected, or all were selected when 1 or 2 physicians worked in the department. The sample of pediatric neurologists, who also work in other than university hospitals, was drawn from their professional registry. For each hospital, half the pediatric neurologists who were randomly selected, or all were selected when only 1 or 2 pediatric neurologists worked in the hospital. In the Netherlands, pediatric neurologists have often been trained as neurologists. For readability, when we use the term “pediatrician,” we include these pediatric neurologists. Of 98 eligible pediatricians, 69 were asked for an interview; 63 agreed (27 pediatrician-oncologists/hematologists, 18 pediatrician-intensivists, and 18 pediatric neurologists; response rate, 91%).

General Practitioners and Clinical Specialists

We also interviewed random samples of general practitioners and clinical specialists (cardiologists, surgeons, and specialists in internal medicine, pulmonology, and neurology) who may also treat children. We selected addresses from the professional registries. Of 403 physicians who were asked for an interview, 333 agreed (125 general practitioners and 208 clinical specialists; response rate 83%).

Hypothetical Cases

Pediatricians were presented 10 hypothetical cases of children with cancer and metastases who had pain that could not be controlled with morphine (Table I). The age of the child (15, 11, or 6 years), whether the child (explicitly) requested ending of life, and the opinion of the parents varied. An inexplicit request was described as “the child would like to quietly fall asleep.” Eighteen combinations could be made. The age of 6 was not combined with an explicit request of the child, because this combination seemed unrealistic. Further, combinations in which the child and parents did not request ending of life were excluded. The case of a 15-year-old child who explicitly requests ending of life, with parents’ agreement, was the only one for which the use of lethal drugs would be allowed according to the law. General practitioners and clinical specialists were presented a selection of 2 hypothetical cases: a 15-year-old child who explicitly requests ending of life, with parents’ agreement, and a 15-year-old unconscious child for whom the parents requested ending of life. All physicians were asked 2 questions about these hypothetical cases. First, we asked about the willingness to use potentially life-shortening drugs (“Are you willing to increase morphine, taking into account that this may hasten death?”), and second, we asked about the willingness to use lethal drugs (“Are you willing to administer a drug with the explicit intention to hasten death?”). They could answer both questions on a 5-point Likert scale (yes; probably; maybe/maybe not; probably not; no).

Statements

Pediatricians, general practitioners and clinical specialists were asked to indicate whether they agreed with 4 statements on the use of lethal drugs in children on a 5-point Likert scale (totally agree; agree; neither agree nor disagree; disagree; totally disagree).

Statistical Analyses

All answers were dichotomized; for the hypothetical cases, the answers “yes” and “probably” were considered to be “willing to increase morphine or use lethal drugs”; for the statements, the answers “totally agree” and “agree” were considered to be “agree.” Multivariate logistic regression analysis was used to assess the influence of case characteristics on the pediatricians’ willingness. Respondent number was included in this model to correct for repeated measures per pediatrician. In subsequent models, we added factors representing possible interaction between the age and request of the child and between the child’s age and the parents’ opinion. Because the sample of pediatricians contained 64% of all eligible pediatricians (63 of 98), we decided to treat this sample as random and did not choose for multilevel analysis. Multivariate logistic regression analyses were used to analyze
the influence of physician characteristics (sex, age, specialty, years of experience, and religion) on the statements and 2 hypothetical cases that were presented to all physicians. All percentages were weighted for non-response and sampling fraction of the physicians.

RESULTS

Physician Characteristics

Table II shows that of all physicians \((n = 396)\), 22% were women and 40% considered themselves as belonging to a religious group or adhered to a certain philosophy of life. Most of the physicians were older than 40 years (88%) and had more than 10 years of experience (76%). Pediatricians were more often women and had fewer years of experience than other physicians. General practitioners considered themselves as belonging to a religious group or adhered to a certain philosophy of life less often than pediatricians and other specialists.

Hypothetical Cases for Pediatricians

In total, 13% to 60% of all pediatricians were willing to use lethal drugs in children (Table III); 6% to 35% answered “yes,” and 7% to 33% answered “probably” (not in table). About half the pediatricians were willing to use lethal drugs for children of different ages when the child (explicitly) requested for the ending of life and the parents agreed (48%-60%). When parents requested ending the life of their unconscious child, 37% to 42% of the pediatricians were willing to grant this request. Pediatricians were least often willing to use lethal drugs when parents did not agree with the explicit request of their child (13% for an 11-year-old child; 28% for a 15-year-old child). In all cases, pediatricians were more often willing to increase morphine (70%-90%) than to use lethal drugs; 49% to 79% answered “yes,” and 8% to 21% answered “probably” (not in table).

Table IV shows that pediatricians tended to be less willing to use lethal drugs for younger children and that they were less willing for children who had made an inexplicit or no request. The opinion of the parents was the strongest determinant of the decision-making process: when parents did not agree with the child's request, pediatricians were least often willing to use lethal drugs. We found a significant interaction between the child’s age and the parents’ opinion, reflecting a larger decrease in the willingness of pediatricians to use lethal drugs when parents did not agree with the explicit request of an 11-year-old child as compared with a 15-year-old child. The extremely low odds ratio (OR) for parents’ opinion reflects that pediatricians considered this factor for all ages and situations consistently, except pediatricians who were in all cases willing to use lethal drugs.

Table I. Description of hypothetical cases

<table>
<thead>
<tr>
<th>Case description</th>
<th>Case characteristics</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Patient of a certain age has cancer with extensive metastases. The pain is severe and cannot be controlled with morphine.</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Case characteristics</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
</tr>
</thead>
<tbody>
<tr>
<td>A Age (y)</td>
<td>15</td>
<td>11</td>
<td>6</td>
<td>The child is disordered, has reduced consciousness and is not responsive since last week; the child had earlier said that he/she would like to quietly fall asleep.</td>
</tr>
<tr>
<td>B Request child</td>
<td>Child makes an explicit and, to your impression, well-considered request for ending of life</td>
<td>The child is disordered, has reduced consciousness and is not responsive since last week; the child had earlier said that he/she would like to quietly fall asleep.</td>
<td>Child says that he/she would like to quietly fall asleep.</td>
<td>The child is disordered, has reduced consciousness and is not responsive since last week; the subject of physician-assisted death is never discussed with the child.</td>
</tr>
<tr>
<td>C Opinion parents</td>
<td>Parents agree with this request</td>
<td>Parents make a well-considered and explicit request for ending the life of their child</td>
<td>Parents cannot accept the hopeless situation and ask for the continuation of treatment</td>
<td></td>
</tr>
</tbody>
</table>

Composition case characteristics

Pediatricians A1B1C1*, A1B1C3, A1B2C1, A1B4C2; A2B1C1; A2B1C3; A2B2C1; A2B4C2; A3B3C1; A3B4C2

General practitioners and clinical specialists A1B1C1*, A1B4C2

Questions

1. Are you willing to increase morphine taking into account that this may hasten death?
2. Are you willing to administer a drug with the explicit intention to hasten death?

*This hypothetical case is allowed according to the Dutch law on euthanasia.
When the child had made an inexplicit request, pediatricians were more often willing to increase morphine than when the child had made an explicit request for ending of life.

Differences Between Physicians

For the hypothetical case of a 15-year-old child who requested ending of life, with which the parents agreed, pediatricians were willing as often as general practitioners and clinical specialists to use lethal drugs (54%-60%; Table V). When parents requested ending of life of their 15-year-old unconscious child, pediatricians were somewhat more often (37%) willing to use lethal drugs than general practitioners (23%) and clinical specialists (27%). There was no difference between pediatricians, general practitioners, and clinical specialists in the willingness to increase morphine (81%-87%).

Most pediatricians, general practitioners, and clinical specialists thought that euthanasia is acceptable for children who are able to assess their interests (67%; Table VI). A minority felt that euthanasia is never acceptable for children younger than 12 years (15%). Pediatricians more often (40%) agreed with the statement that children’s requests for euthanasia can be granted without permission of the parents than general practitioners (28%) or clinical specialists (28%). Further, pediatricians more often (68%) agreed with the statement that active life-ending can be acceptable when parents think their child suffers unbearably than general practitioners (45%) or clinical specialists (43%).

Female physicians were less often willing than male physicians to use lethal drugs at the request of either the child (OR, 0.43; 95% CI, 0.26-0.73) or the parents (OR, 0.58; 95%
CI, 0.31-1.10); female physicians also more often agreed with the statement that euthanasia is never acceptable for children aged younger than 12 years (OR, 2.10; 95% CI, 1.10-4.00; not in table). Religious physicians were also less often willing to grant a request for euthanasia of a child than non-religious physicians (OR, 0.46; 95% CI, 0.30-0.70). Further, religious physicians less often agreed with the first and third statement of Table V on acceptability and allowance of euthanasia in children (OR, 0.47; 95% CI, 0.31-0.72; OR, 0.42; 95% CI, 0.26-0.67, respectively) and more often agreed with the second statement on unacceptability of euthanasia for children younger than 12 years (OR, 2.83; 95% CI, 1.61-4.99).

**DISCUSSION**

A substantial proportion of Dutch pediatricians is willing to use lethal or potentially life-shortening drugs in children, including when legal conditions are not met. Whether parents agree is a more important factor in the decision-making process of pediatricians than whether the child requests ending of life or the age of the child. Pediatricians are more willing than general practitioners and clinical specialists to grant a request from parents for ending of life of their unconscious child. Female pediatricians and religious physicians are less willing to use lethal or potentially life-shortening drugs, as found in other studies.3,4,21,22

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Table IV. Influence of case characteristics of 10 hypothetical cases on willingness of pediatricians (n = 60) to use lethal drugs or increase morphine

<table>
<thead>
<tr>
<th>Case characteristics †</th>
<th>OR</th>
<th>95% CI</th>
<th>OR</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>15</td>
<td>1</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>0.49</td>
<td>0.24-1.01</td>
<td>0.84</td>
<td>0.32-2.20</td>
</tr>
<tr>
<td>6</td>
<td>0.57</td>
<td>0.23-1.43</td>
<td>0.16</td>
<td>0.04-0.60</td>
</tr>
<tr>
<td>Child’s request</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Explicit request</td>
<td>238</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Inexplicit request</td>
<td>178</td>
<td>0.23</td>
<td>0.08-0.67</td>
<td>5.92</td>
</tr>
<tr>
<td>No request</td>
<td>178</td>
<td>0.05</td>
<td>0.02-0.16</td>
<td>0.85</td>
</tr>
<tr>
<td>Parents’ opinion</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Agreement/request</td>
<td>475</td>
<td></td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>No agreement/no request</td>
<td>119</td>
<td>0.004</td>
<td>0.00-0.02</td>
<td>0.04</td>
</tr>
</tbody>
</table>

*Total of all hypothetical cases; 3 pediatricians did not answer the questions about the hypothetical cases; 1 pediatrician answered questions about only 4 of the 10 hypothetical cases. †All answers were dichotomized; the answers “yes” and “probably” were considered to be “willing to use lethal drugs or increase morphine”. ‡Multivariate logistic regression analysis; respondent number is included in the model to control for repeated measures of the pediatrician; first category of each variable is the reference category. For example, the OR for willingness to use lethal drugs in case of an inexplicit request of a child is 0.23 (95% CI, 0.08-0.67). This means that physicians were significantly less willing to use lethal drugs when the child made an inexplicit request as compared with a case in which the child made an explicit request.

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Table V. Percentage of pediatricians and other physicians who would use lethal drugs or increase morphine for a 15-year-old child

<table>
<thead>
<tr>
<th>Use lethal drugs</th>
<th>Pediatricians (N = 60*)</th>
<th>General practitioners (N = 120*)</th>
<th>Clinical specialists (N = 198*)</th>
<th>Total (N = 378)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% † (95% CI)</td>
<td>% † (95% CI)</td>
<td>% † (95% CI)</td>
<td>% † (95% CI)</td>
</tr>
<tr>
<td>Request of child, parents agree</td>
<td>60 (47-71)</td>
<td>58 (49-67)</td>
<td>54 (47-61)</td>
<td>57 (52-62)</td>
</tr>
<tr>
<td>No request of child, request of parents</td>
<td>37 (25-49)</td>
<td>23 (16-31)</td>
<td>27 (21-33)</td>
<td>26 (22-30)</td>
</tr>
<tr>
<td>Increasing morphine</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Request of child, parents agree</td>
<td>84 (72-91)</td>
<td>84 (76-90)</td>
<td>81 (75-86)</td>
<td>83 (79-87)</td>
</tr>
<tr>
<td>No request of child, request of parents</td>
<td>83 (71-90)</td>
<td>85 (77-90)</td>
<td>87 (82-91)</td>
<td>85 (81-89)</td>
</tr>
</tbody>
</table>

*Three pediatricians, 5 general practitioners, and 10 clinical specialists did not answer the questions about hypothetical cases. †Percentages are weighted for non-response and sampling fraction of the physicians. ‡Pearson chi-square test.
Some limitations of our study should be kept in mind. Concise information about the hypothetical cases can lead to differences in interpretation of the respondents. End-of-life practice is often more complex than is presented in the hypothetical cases, and there might be barriers to performing intended behavior in practice. Therefore, physicians may act differently in practice. Possible differences in interpretation were minimized by instructing the interviewers not to give additional information and enabling respondents to explain their answers.

In general, there are no indications that the practice of end-of-life decision-making has significantly altered in the last 10 years, during which a regulatory system was developed.1,3 For children, we do not know whether the new Dutch law on euthanasia is a regulation of existing practice or whether it will expand the practice of euthanasia.

The rules on euthanasia for children 12 years or older imply a legal recognition of the competence of children to form an opinion and make a well-considered request, albeit with the parents’ agreement. Our study shows that pediatricians did not distinguish between the explicit request of a 15-year-old child and that of an 11-year-old child, provided that parents agree with the decision. In both cases, more than half the pediatricians were willing to use lethal drugs. However, when parents do not agree with the explicit request of the child, the willingness to use lethal drugs substantially decreases, especially for 11-year-old children. Further, most physicians find euthanasia acceptable for children with decision-making capacity and think that euthanasia can be acceptable for a child younger than 12 years. Thus, although the age of 12 years does not seem to be regarded as a clear cutoff for the capacity of a child to be involved in end-of-life decision-making, physicians tend to weigh the opinion of parents more heavily in children younger than this age. The opinion of the parents in decision-making has also been found to be important elsewhere, but opinions about age cutoffs for capacity of children to participate in the decision-making have not been clearly studied.7,9,15,19,23

### Table VI. Percentage of pediatricians and other physicians who agreed with statements on the use of lethal drugs in children

<table>
<thead>
<tr>
<th>Statement</th>
<th>Pediatricians (N = 63)</th>
<th>General practitioners (N = 125)</th>
<th>Clinical specialists (N = 207)</th>
<th>Total (N = 395)</th>
<th>P value‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Euthanasia is acceptable for children who are able to assess their interests</td>
<td>75 (63-84)</td>
<td>67 (59-75)</td>
<td>63 (56-69)</td>
<td>63 (62-71)</td>
<td>.24</td>
</tr>
<tr>
<td>2. For a child younger than 12 years, euthanasia is never acceptable</td>
<td>13 (6-23)</td>
<td>17 (11-25)</td>
<td>14 (10-20)</td>
<td>15 (12-19)</td>
<td>.63</td>
</tr>
<tr>
<td>3. It must be allowed to grant a request for euthanasia of incurably ill children in the age group 12-16 without permission of the parents</td>
<td>28 (21-36)</td>
<td>28 (22-34)</td>
<td>30 (25-34)</td>
<td></td>
<td>.17</td>
</tr>
<tr>
<td>4. If parents of a child who is not able to value his or her interests think their child suffers unbearably and hopelessly, active ending of life is acceptable</td>
<td>68 (56-78)</td>
<td>45 (36-53)</td>
<td>43 (36-49)</td>
<td>47 (43-52)</td>
<td>.00</td>
</tr>
</tbody>
</table>

*Percentages are weighted for non-response and sampling fraction of the physicians.
†One clinical specialist did not address the statements.
‡Pearson chi-square test.
A Study Of Hypothetical Cases

justifies ending the life of children with decision-making incapacity when their suffering is unmitigated. Further, pediatricians may be more familiar than other physicians with discussing important medical decisions with parents who decide for their child. The answers of other physicians are probably based more often on a theoretical perspective or abstract principles.

Remarkably, pediatricians are more often willing to use potentially life-shortening drugs for a child who asks to be allowed to quietly fall asleep than for a child who explicitly requests ending of life. The child’s request to quietly fall asleep is apparently more often interpreted as indicating a need to be relieved of pain or other symptoms, for instance by sedation, than as a request for ending of life. Further, most pediatricians and other physicians are willing to increase morphine for all hypothetical cases. Because all case descriptions indicated that the pain was severe and could not be controlled with morphine, the goal of increasing morphine can be questioned. Although some respondents might have thought that increasing morphine (or another narcotic analgesic) could further relieve pain, it is likely that the possible effect of hastening death is appreciated by many of the physicians who are willing to increase morphine. Whether such practices can be justified is doubtful, especially when dosages are increased without taking notice of the degree of symptom relief. To what extent the use of potentially life-shortening drugs represents good end-of-life care or should be seen as an option to avoid the illegal practice of the use of lethal drugs remains to be discussed.

A substantial proportion of Dutch physicians is willing to use lethal drugs in children at different ages. The finding that general practitioners and clinical specialists are less willing than pediatricians to grant a parental request for ending of a child’s life suggests that for them the legally required patient’s request is a more important condition than it is for pediatricians. Further, the legal rule in the Dutch law on euthanasia that ending life at the request of a child 12 years or older is allowed when parents agree is more consistent with views of physicians than the rule that it is not allowed for children younger than 12 years.

The authors thank Rob Pieters, Willem–Frans Arts, and Edwin van der Voort for their contributions to the design of the study; the members of the steering committee for their continuous support throughout the study; the physicians who participated; the interviewers; the research assistants; and the chairman of the Dutch Society of Paediatric Neurology, the chairman of the Royal Dutch Medical Association and the Inspector General for Health Care for their support to the study.

REFERENCES


TWO-YEAR FOLLOW-UP RESULTS FOR HIP-HOP TO HEALTH JR.: A RANDOMIZED CONTROLLED TRIAL FOR OVERWEIGHT PREVENTION IN PRESCHOOL MINORITY CHILDREN

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Objectives To assess the impact of a culturally proficient dietary/physical activity intervention on changes in body mass index (BMI) (kg/m²).

Study design Randomized controlled trial (Hip-Hop to Health Jr.) conducted between September 1999 and June 2002 in 12 Head Start preschool programs in Chicago, Illinois.

Results Intervention children had significantly smaller increases in BMI compared with control children at 1-year follow-up, 0.06 vs 0.59 kg/m²; difference −0.53 kg/m² (95% CI −0.91 to −0.14), \( P = .01 \); and at 2-year follow-up, 0.54 vs 1.08 kg/m²; difference −0.54 kg/m² (95% CI −0.98 to −0.10), \( P = .02 \), with adjustment for baseline age and BMI. The only significant difference between intervention and control children in food intake/physical activity was the Year 1 difference in percent of calories from saturated fat, 11.6% vs 12.8% (\( P = .002 \)).

Conclusions Hip-Hop to Health Jr. was effective in reducing subsequent increases in BMI in preschool children. This represents a promising approach to prevention of overweight among minority children in the preschool years. (J Pediatr 2005;146:618-25)

Prevalence of overweight in the United States (US) is strikingly high among minority children. Interventions that can successfully alter the trajectory toward overweight among high-risk children are critical if we are to effectively address this public health crisis. The most recent national estimates of overweight for children 2 to 5 years of age indicate comparable rates across ethnic groups. However, the rates shift as children age. For example, for children 6 to 11 years of age, 13.5% of Non-Hispanic Whites and 19.8% of Non-Hispanic Blacks are overweight. Ethnic differences in obesity-related risk factors begin as early as 6 to 9 years of age. As children age, the probability of childhood overweight persisting into adulthood increases from approximately 25% at 4 years of age to approximately 80% by late adolescence.

Schools provide an ideal setting for prevention efforts because most children are enrolled in school, schools provide ongoing contact, and there are no costs to families. To date, most published school-based interventions have focused on healthy eating, exercise, and cardiovascular risk reduction, with no significant impact on body weight. Recent randomized controlled trials have specifically targeted changes in body weight with mixed results. Other trials have shown some success but were conducted with smaller samples, intervened with older children, did not target those at highest risk (underserved minorities), or were not designed to assess longer-term change. The primary aim of this efficacy trial was to alter the trajectory toward overweight among preschool minority children by reducing increases in weight following a weight control intervention. Our primary outcome was change in body mass index (BMI) at Year 1 and Year 2 post-intervention in children from schools randomized to a weight control intervention compared with children from schools randomized to a control group.

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**BMI**: Body mass index  
**CDC**: Centers for Disease Control  
**GHI**: General health intervention  
**WCI**: Weight control intervention

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See related article, p 586.

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From Feinberg School of Medicine, Northwestern University, and Children’s Memorial Hospital, University of Illinois at Chicago, Chicago, Illinois. Supported by a grant from the National Heart, Lung, and Blood Institute (Grant HL58871).

Preliminary findings of this study were presented at the First Virgin Island WIC Conference on Childhood Obesity, St Thomas, United States Virgin Islands, June 2003.

Submitted for publication Jul 27, 2004; last revision received Nov 8, 2004; accepted Dec 9, 2004.

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0022-3476/$ - see front matter

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METHODOLOGIES

Study Design

In September of 1999, 12 Head Start sites administered through the Archdiocese of Chicago were recruited. The schools were paired based only on class size, and one member of each pair was randomly assigned to the weight control intervention (WCI) or to the general health intervention (GHI). All children at the sites were eligible to participate. Parents provided informed consent for themselves and their children.

The primary outcome was the difference in change in BMI between children in WCI schools versus children in GHI schools from baseline (fall 1999) to Year 1 post-intervention (spring 2001), and Year 2 post-intervention (spring 2002). Secondary outcomes included percent of total calorie intake from fat and saturated fat and grams per 1000 kcal of fiber from a single dietary recall by parents for their children, physical activity (frequency per week and intensity), and television viewing behavior (hours per day). The study was approved by the Institutional Review Board on Human Subjects at the Northwestern University Feinberg School of Medicine.

Interventions

The rationale and development of the intervention are described elsewhere. Briefly, the intervention was developed with input from early childhood educators, pediatricians, nutritionists, exercise physiologists, community health advocates, experts in minority health, and focus groups. Children in the six WCI schools participated in a 14-week (40 minutes, three times weekly) healthy eating and exercise intervention. Each week of the intervention covered a particular topic such as “Go and Grow” foods, fruits, and reducing TV viewing. Based on these topics, the thrice weekly lesson plans incorporated two major components: (1) a 20-minute lesson that introduced a healthy eating or exercise concept with an activity and (2) 20 minutes of ongoing physical activity. The 20-minute lessons and activities often involved the use of colorful, friendly, handheld puppets that represented the seven food groups of the food pyramid. Throughout the intervention, the puppets led the children in various activities and adventures. For the physical activity component, the teacher led the children in a 5-minute warm-up, 10 minutes of aerobic activity, and a 5-minute cooldown. The teachers used multiple games and approaches, such as aerobic “trips to the zoo” where children pretended to be different animals.

The WCI intervention did not target overweight children specifically. We deliberately chose an inclusive approach that was aimed at altering the distribution of weight among the intervention children by reducing further development of overweight in already overweight children and preventing overweight in normal-weight children. Intensive treatment interventions with already overweight children have shown both short- and long-term success, but may be less applicable in school settings because being identified as overweight and in need of treatment could embarrass young children and cause them to be further ostracized.

In addition to the child curriculum, parents in the WCI schools received weekly newsletters with information that mirrored the children’s curriculum. Each newsletter had a section on healthy eating and a section on healthy exercise. The newsletters also included a homework assignment. The weekly homework assignments required about 5 to 10 minutes daily or 15 minutes in one sitting (depending on nature of assignment) to complete items that reinforced concepts presented in the weekly newsletters. For example, in “Five a Day” week, parents were asked to keep track (on an enclosed form) of their and their children’s fruit and vegetable intake for 1 week. Parents also were asked to write down specific ways they might increase their family’s fruit and vegetable intake. Parents received a $5.00 grocery store coupon for each homework assignment they completed and turned in.

Children in the six GHI schools received a 14-week (20 minutes, 1 time weekly) class in which they learned about a variety of general health concepts such as dental health, immunization, seat belt safety, and 911 procedures. Parents received weekly newsletters that mirrored the GHI. No information on diet or physical activity was presented.

Measurements

Measures used in the study are described in detail elsewhere.

DEMOGRAPHICS. Parents reported the age, gender, and ethnicity of their child and their own date of birth, education, and marital status.

BODY MASS INDEX (BMI). Weight was measured using a Seca (Hanover, Md) digital scale, and height was measured using a portable stadiometer. BMI was calculated as weight in kilograms divided by the square of height in meters. BMI Z scores were calculated for each child based on the 2000 Centers for Disease Control (CDC) growth charts, using the NutStat module of Epi Info 2000.

DIETARY INTAKE. Dietary intake data were obtained from the parent of the child for a 24-hour period by a trained and certified registered dietitian, blinded to treatment group. This method has been used in other studies with very young children. Depending on the amount of time the parent had spent with the child during the 24-hour period before the recall, a partial (including only those meals observed) or full 24-hour recall was obtained. At baseline (99%) and post-intervention (95%), most recalls were collected in person using three-dimensional visual aids. However, because many of the children were no longer at the Head Start sites at follow-up, 48% of the dietary recalls at Year 1 and 100% at Year 2 were collected by phone. We collected a single recall in order to ease participant burden and because our aim was to document group means, not individual-level dietary intake. The recalls were documented by hand and then were entered into the
most recent version of the Nutrition Data System for Research.26 Dietary recalls were tape recorded. Probing techniques were used to verify portion sizes and preparation techniques and to account for plate waste. Duplicate entry was performed on a randomly selected 10% sample of recalls with a different dietitian performing the analysis. All discrepancies were adjudicated by the same dietitian.

**PHYSICAL ACTIVITY.** Parents were asked to assess the frequency and intensity of their child’s current activities and the hours per day that their child watched television.

All baseline, post-intervention (14 weeks), and follow-up (1 and 2 years post-intervention) assessments were conducted by trained data collectors who were unaware of group assignment at follow-up, though not at baseline. Baseline and post-intervention health screenings were conducted at the schools. At subsequent follow-ups, because many children were no longer in preschool, follow-up assessments were conducted in their homes or in local community facilities such as libraries. School personnel, parents, and children were unaware that the primary outcome was change in BMI.

**Statistical Analyses**

Baseline comparability of intervention and control schools was assessed using two-sample t tests for continuous variables and χ² tests for categorical variables. To test the primary hypothesis, SAS (SAS Institute Inc., Cary, NC) Proc Mixed was used with the individual school as the unit of randomization, yielding a test statistic with 10 degrees of freedom for 12 schools.27 Change in BMI and change in BMI Z score at follow-up were the dependent variables. Analyses were conducted with and without adjustment for baseline age quartile and baseline BMI or BMI Z score. We adjusted for age quartile rather than age as a continuous variable because of nonlinear associations between baseline age and change in BMI at Year 1 and Year 2. For dietary, physical activity, and television viewing measures, we used SAS Proc Mixed and the absolute values at follow-up, adjusted for the baseline value, rather than change scores. For BMI and BMI Z score, differences between groups were considered significant if the two-tailed P value was <.05. For secondary outcome measures and height and weight, differences between groups are reported along with their corresponding 95% CIs.

To assess consistency of the effects of the intervention, we also report change in BMI for children in the WCI and GHI schools separately for boys and girls, children above and below the median age at baseline, Black children, and those above and below the 85th percentile of BMI at baseline, as determined using the NutStat module of Epi Info 2000.22 P values are not reported for these comparisons because the study was not designed to have adequate power for comparisons within subgroups.

To assess possible bias in results because of children leaving school or missing anthropometric data at a specific follow-up, we also conducted two additional analyses in which we imputed BMI 1 and 2 years post-intervention from prior (baseline, post-intervention, or Year 1) or subsequent (Year 2) values of BMI. In the more conservative of the two approaches, for the 43 children missing BMI at Year 1 but not Year 2, we estimated BMI at Year 1 from a regression of Year 1 BMI on Year 2 BMI plus baseline age and gender for the 130 control group children with data at both Year 1 and Year 2. For the 32 children with Year 1 BMI but no Year 2 BMI, we estimated Year 2 BMI from a regression of Year 2 BMI on Year 1 BMI plus the same two variables in the 130 control children. For the 56 children with post-intervention BMI but no Year 1 or Year 2 BMI, we estimated Year 1 and Year 2 BMI from regressions of Year 1 or Year 2 BMI on post-intervention BMI plus baseline age and gender in control children. For the 21 children with no post-baseline BMI, we estimated Year 1 and Year 2 BMI from regressions of Year 1 or Year 2 BMI on baseline BMI, age, and gender in control children.

In the less conservative approach, we used the above estimates for intervention and control children if they had no data at Year 1 or Year 2 follow-up. However, if the children had other data at Year 1 or Year 2 but no BMI, we replaced the above estimates with estimates based on similar regressions that included both intervention and control children and a variable for treatment group in addition to the prior or subsequent BMI and age and gender.

**Sample Size**

The original study design called for the recruitment of 35 children per Head Start site (420 total). This number, along with the number of Head Start sites, ie, 12, was selected to provide at least 80% power to detect a difference between groups of 0.35 standard deviations (within-site) of the change in BMI, assuming one-sided tests at the 5% level, an intra-school correlation no greater than .015, and a retention rate of ≥80% at each follow-up assessment, ie, that at least 336 children would complete the final follow-up assessment. Four hundred and nine children were recruited.

Because fewer than 336 children provided anthropometric data at Year 1 (289 children) and Year 2 (300 children), the number of children varied by school, and the results reported here are based on two-sided tests, we re-calculated power to detect a difference of 0.35 standard deviations. In these calculations, we used the standard error of the difference in BMI from SAS Proc Mixed as well as the estimate of the within-school component of variance from Proc Mixed to obtain estimates of power of 80% at Year 1 and 82% at Year 2. The higher power in these calculations reflects lower than expected or negative values for the intra-school correlation at both Year 1 and Year 2, ie, −0.0027 at Year 1 and −0.0033 at Year 2.

**RESULTS**

No adverse events were reported for any participants as a function of the intervention. Because of the nature of the intervention, neither the interventionists nor the participants could be blinded to the content of the intervention. Quality control for the interventions was ensured by intensive training for the early childhood educators who served as
interventionists, weekly supervision, and unscheduled live observations of intervention delivery conducted by study investigators. Approximately 61% of the parents in the WCI group returned at least one homework assignment, and 88% reported reading the newsletters.

### Baseline Comparability

WCI and GHI participants were comparable at baseline for gender, BMI, BMI Z score, percentage of children at or above the 85th percentile of BMI for age and sex, hours of television viewing, exercise intensity, energy from total fat and saturated fatty acids, and dietary fiber per 1000 kcal (Table I). GHI children were, however, older than WCI children by 2.2 months ($P = .001$). Because of their slightly older age, GHI children also were taller and heavier than WCI children ($P < .01$ and $P = .014$, respectively; no longer significant when adjusted for age). In addition, more GHI children exercised seven or more times per week (54% vs 44% $P = .043$), and there were more non-Black children in GHI ($P < .001$). Female parents of WCI and GHI children were comparable in BMI, age, years of education, and marital status.

### Primary Outcome

Post-intervention changes in BMI and BMI Z score did not differ significantly between intervention and control children (0.05 kg/m$^2$ vs 0.14 kg/m$^2$, $P = .234$ for BMI; and 0.06 vs 0.08, $P = .655$ for BMI Z score). At Year 1 post-intervention, the increase in mean BMI in WCI children was 0.02 kg/m$^2$, whereas mean BMI increased by 0.64 kg/m$^2$ in GHI children ($P = .002$) (Table II), even though mean weight was 3.79 and 4.65 kg higher in the two groups, respectively. Mean change in height was similar for WCI and GHI children, 10.37 and 10.10 cm, respectively. Change in BMI Z score also differed significantly between groups ($–0.06$ vs 0.13, $P = .024$). Adjustment for age and BMI at baseline reduced the difference between WCI and GHI.

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**Table I. Child and parent measures at baseline, by treatment group; Hip-Hop to Health Jr.**

<table>
<thead>
<tr>
<th></th>
<th>Intervention</th>
<th>Controls</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Children</strong></td>
<td>N Mean (SD)</td>
<td>N Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Age (mo)</td>
<td>197 48.6 (7.6)</td>
<td>212 50.8 (6.4)</td>
<td></td>
</tr>
<tr>
<td>Gender (% female)</td>
<td>197 49.7</td>
<td>212 50.5</td>
<td></td>
</tr>
<tr>
<td>Race (%)</td>
<td>197</td>
<td>212</td>
<td></td>
</tr>
<tr>
<td>Black</td>
<td>99.0</td>
<td>80.7</td>
<td></td>
</tr>
<tr>
<td>Latino</td>
<td>0.0</td>
<td>12.7</td>
<td></td>
</tr>
<tr>
<td>Multiracial/Other</td>
<td>1.0</td>
<td>6.6</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>197 16.5 (1.5)</td>
<td>212 16.7 (2.0)</td>
<td></td>
</tr>
<tr>
<td>BMI &lt;85th percentile</td>
<td>135 15.7 (0.7)</td>
<td>135 15.6 (0.9)</td>
<td></td>
</tr>
<tr>
<td>BMI ≥85th percentile</td>
<td>62 18.2 (1.3)</td>
<td>77 18.5 (2.0)</td>
<td></td>
</tr>
<tr>
<td>BMI Z score for age and sex</td>
<td>197 0.62 (0.90)</td>
<td>212 0.67 (1.11)</td>
<td></td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>197 17.6 (2.9)</td>
<td>212 18.3 (3.4)</td>
<td></td>
</tr>
<tr>
<td>Height (cm)</td>
<td>197 102.8 (6.4)</td>
<td>212 104.6 (5.9)</td>
<td></td>
</tr>
<tr>
<td>BMI ≥85th percentile (%)</td>
<td>197 31.5</td>
<td>212 36.3</td>
<td></td>
</tr>
<tr>
<td>Total fat (% kcal)</td>
<td>175 33.6 (7.6)</td>
<td>183 33.4 (8.3)</td>
<td></td>
</tr>
<tr>
<td>SFA (% kcal)</td>
<td>175 12.1 (3.9)</td>
<td>183 11.9 (3.8)</td>
<td></td>
</tr>
<tr>
<td>Dietary fiber (g/1000 kcal)</td>
<td>175 6.4 (2.9)</td>
<td>183 6.6 (3.6)</td>
<td></td>
</tr>
<tr>
<td>TV viewing (h/d)</td>
<td>174 3.4 (1.9)</td>
<td>182 3.1 (1.6)</td>
<td></td>
</tr>
<tr>
<td>Exercise frequency (% ≥7 × /wk)</td>
<td>174 43.7</td>
<td>182 54.4</td>
<td></td>
</tr>
<tr>
<td>Exercise intensity (Borg scale)</td>
<td>174 5.3 (2.2)</td>
<td>182 5.2 (2.1)</td>
<td></td>
</tr>
<tr>
<td><strong>Parents</strong></td>
<td>N Mean (SD)</td>
<td>N Mean (SD)</td>
<td></td>
</tr>
<tr>
<td>Age (y)</td>
<td>137 30.0 (9.7)</td>
<td>152 30.8 (9.5)</td>
<td></td>
</tr>
<tr>
<td>BMI (kg/m$^2$)</td>
<td>122 30.6 (8.6)</td>
<td>148 31.5 (8.5)</td>
<td></td>
</tr>
<tr>
<td>Education (y)</td>
<td>136 12.4 (1.8)</td>
<td>152 12.7 (1.7)</td>
<td></td>
</tr>
<tr>
<td>Married/living as married (%)</td>
<td>136 19.9</td>
<td>152 21.7</td>
<td></td>
</tr>
</tbody>
</table>

SFA, Saturated fatty acids.

*a* Overweight or at risk for overweight is defined as BMI ≥85th percentile for age and sex.

†Deviation from the mean BMI for age and sex for the reference population divided by the age- and sex-specific standard deviation for the reference population.

‡If the child was in school the previous day, the parent completed a recall for the child’s time at home; otherwise, the parent completed a 24-hour recall for the child.

§Female parents with some baseline data only.
children in change in BMI from 0.62 to 0.53 kg/m² ($P = .012$), whereas adjustment for baseline age and BMI Z score increased the difference in BMI Z score from $-0.19$ to $-0.23$ ($P = .006$). When BMI was imputed at Year 1 for those without Year 1 BMI, the unadjusted estimate of the difference was 0.57 kg/m² using the more conservative approach and 0.60 kg/m² using the less conservative approach. These estimates became 0.51 and 0.54 kg/m², respectively, with adjustment for baseline age and BMI.

At Year 2 post-intervention, the mean increase in BMI was 0.65 kg/m² higher in GHI children than WCI children, or 1.14 and 0.48 kg/m², respectively (Table II) ($P = .008$), whereas the difference in change in BMI Z score was $-0.14$, or 0.02 and 0.16, respectively ($P = .021$). The mean increases in weight over this period were 7.95 kg for GHI children and 6.84 kg for WCI children. As at Year 1 follow-up, change in height was similar in WCI and GHI children, 16.36 and 16.08 cm, respectively. Adjustment for age and BMI at baseline reduced the difference between WCI and GHI children in change in BMI at Year 2 follow-up to 0.54 kg/m² ($P = .022$), whereas baseline adjustment increased the difference in BMI Z score to $-0.18$ ($P = .015$). When BMI was imputed at Year 2 for those without Year 2 BMI, the unadjusted estimate of the difference was 0.65 kg/m² with the more conservative approach and 0.67 kg/m² with the less conservative approach. These estimates became 0.54 and 0.57 kg/m², respectively, with adjustment for baseline age and BMI.

Relative effects of the intervention were similar for boys and girls at both Year 1 and Year 2 follow-ups (0.67 and 0.58 kg/m² at Year 1 and 0.79 and 0.53 kg/m² at Year 2), for children below and above the median age at baseline (0.58 and 0.64 kg/m² at Year 1 and 0.50 and 0.70 kg/m² at Year 2), and for children below and above the 85th percentile of BMI at baseline (0.47 and 0.80 kg/m² at Year 1 and 0.57 and
The differences in Black children also were similar to those for all children, ie, 0.67 kg/m² at Year 1 and 0.62 kg/m² at Year 2.

Secondary Outcomes

Intakes of total fat and dietary fiber per 1000 kcal, exercise frequency and intensity, and hours of TV viewing per day were similar among treatment and control children post-intervention and at both Year 1 and Year 2 follow-up (Table III), whereas saturated fat intake was significantly lower in WCI children at Year 1 (P = .002) but not post-intervention or at Year 2 follow-up.

DISCUSSION

Hip-Hop to Health Jr., a randomized controlled efficacy trial in minority preschool-age children, demonstrated success in reducing increases in BMI (and BMI Z score) as children age, not only at Year 1 follow-up but also through Year 2 follow-up. Other intervention studies that targeted dietary fat and physical activity have not demonstrated a reduction in BMI increases.

Other promising prevention trials have been conducted with older children but have not targeted high-risk minority children exclusively. For example, in an 18-lesson television viewing reduction trial with two schools of third to fourth graders, the intervention school had significant relative decreases in BMI, television viewing, and meals eaten in front of the television. However, >70% of the sample was White and there was no follow-up to assess whether the BMI change was maintained. In Planet Health, Gortmaker et al focused on reduction of television viewing and consumption of high-fat foods, as well as increasing fruit and vegetable intake and physical activity over 2 school years among 10 schools of sixth to seventh grade students. Post-intervention results showed a decrease in overweight among girls, with the largest effect among Black girls, but no change among boys. The Pathways program, which did target underserved American Indian third-grade school children, produced a reduction of dietary fat. Additionally, although there were positive changes in knowledge and attitudes, there were no significant differences in physical activity measured by motion sensors or percentage body fat between treatment and control schools.

There are several limitations to this study. First, this was an intervention designed to meet the developmental, cultural, and financial needs of low-income minority preschool children. Therefore, we do not know if this could be generalized to nonminority and higher-income populations. For example, we incorporated the use of foods in our intervention that are

### Table III. Adjusted* child diet† and physical activity at 1-Year and 2-Year follow-up; Hip-Hop to Health Jr.

<table>
<thead>
<tr>
<th>Follow-up</th>
<th>Intervention</th>
<th>Control</th>
<th>Difference (WCI-GHI) (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N  Mean (SE)</td>
<td>N  Mean (SE)</td>
<td></td>
</tr>
<tr>
<td><strong>Post-Intervention</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat (% kcal)</td>
<td>133 32.9 (0.6)</td>
<td>147 34.1 (0.6)</td>
<td>-1.16 (-3.02 to 0.71)</td>
</tr>
<tr>
<td>SFA (% kcal)</td>
<td>133 11.4 (0.3)</td>
<td>147 12.1 (0.2)</td>
<td>-0.65 (-1.45 to 0.14)</td>
</tr>
<tr>
<td>Fiber (g/1000 kcal)</td>
<td>133 7.0 (0.4)</td>
<td>147 6.7 (0.4)</td>
<td>0.31 (-0.87 to 1.49)</td>
</tr>
<tr>
<td>TV viewing (h/d)</td>
<td>143 2.9 (0.2)</td>
<td>154 3.1 (0.1)</td>
<td>-0.17 (-0.64 to 0.30)</td>
</tr>
<tr>
<td>Exercise frequency (% ≥7 × /wk)</td>
<td>143 43.8 (4.3)</td>
<td>154 43.2 (4.1)</td>
<td>0.59 (-12.60 to 13.79)</td>
</tr>
<tr>
<td>Exercise intensity (Borg scale)</td>
<td>143 5.7 (0.1)</td>
<td>154 5.5 (0.1)</td>
<td>0.14 (-0.26 to 0.54)</td>
</tr>
<tr>
<td><strong>Year 1</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat (% kcal)</td>
<td>141 33.3 (0.7)</td>
<td>147 34.7 (0.7)</td>
<td>-1.46 (-3.60 to 0.67)</td>
</tr>
<tr>
<td>SFA (% kcal)</td>
<td>141 11.6 (0.2)</td>
<td>147 12.8 (0.2)</td>
<td>-1.15 (-1.74 to -0.56)</td>
</tr>
<tr>
<td>Fiber (g/1000 kcal)</td>
<td>141 6.6 (0.3)</td>
<td>147 6.6 (0.3)</td>
<td>0.04 (-0.96 to 1.04)</td>
</tr>
<tr>
<td>TV viewing (h/d)</td>
<td>132 3.2 (0.2)</td>
<td>142 3.4 (0.2)</td>
<td>-0.17 (-0.75 to 0.42)</td>
</tr>
<tr>
<td>Exercise frequency (% ≥7 × /wk)</td>
<td>142 55.5 (5.1)</td>
<td>142 55.5 (5.1)</td>
<td>-10.55 (-27.05 to 5.95)</td>
</tr>
<tr>
<td>Exercise intensity (Borg scale)</td>
<td>132 5.3 (0.3)</td>
<td>142 5.6 (0.3)</td>
<td>-0.29 (-1.32 to 0.75)</td>
</tr>
<tr>
<td><strong>Year 2</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total fat (% kcal)</td>
<td>126 34.1 (0.8)</td>
<td>130 33.6 (0.8)</td>
<td>0.58 (-2.00 to 3.16)</td>
</tr>
<tr>
<td>SFA (% kcal)</td>
<td>126 11.9 (0.4)</td>
<td>130 11.6 (0.4)</td>
<td>0.27 (-0.89 to 1.43)</td>
</tr>
<tr>
<td>Fiber (g/1000 kcal)</td>
<td>126 6.4 (0.3)</td>
<td>130 6.9 (0.3)</td>
<td>-0.58 (-1.61 to 0.44)</td>
</tr>
<tr>
<td>TV viewing (h/d)</td>
<td>129 2.9 (0.2)</td>
<td>131 3.1 (0.2)</td>
<td>-0.11 (-0.60 to 0.38)</td>
</tr>
<tr>
<td>Exercise frequency (% ≥7 × /wk)</td>
<td>129 39.2 (5.4)</td>
<td>132 38.4 (5.2)</td>
<td>0.79 (-15.97 to 17.55)</td>
</tr>
<tr>
<td>Exercise intensity (Borg scale)</td>
<td>129 4.7 (0.4)</td>
<td>132 5.4 (0.4)</td>
<td>-0.62 (-1.77 to 0.53)</td>
</tr>
</tbody>
</table>

*SFA, Saturated fatty acids.
*Adjusted for baseline value and Head Start site using SAS Proc Mixed.
†If the child was in school the previous day, the parent completed a recall for the child's time at home; otherwise, the parent completed a 24-hour recall for the child.
approved by the Special Supplemental Nutrition Program for Women, Infants, and Children to facilitate the purchasing of these foods by parents. This tailoring would not be necessary for a higher-income population. Second, a notable limitation was the lack of significant differences between WCI and GHI for any of the dietary measures (percentage of calories from fat and saturated fat, g/1000 kcal of fiber) or activity (frequency or intensity of physical activity or weekly hours of television viewing) at Year 2 follow-up or post-intervention, although saturated fat intake was significantly lower in WCI children at Year 1 follow-up. This could reflect true absence of differences in diet and physical activity between WCI and GHI children or lack of sensitivity in the measures employed. The relationships between the increased prevalence of overweight and changes in diet and physical activity are not clear from previous studies, and questions regarding potential mechanisms remain debatable. The dietary habits of young children who are preliterate are very difficult to study, and researchers have noted the potential need for ethnic group–specific dietary assessment tools. We deliberately chose to collect a single recall at baseline and follow-up visits simply to compare group differences because of potential participant burden. However, use of a single dietary recall that did not include all meals eaten in the previous 24 hours for a majority of the children and an unvalidated physical activity measure may have reduced our ability to detect differences between groups and thus to explain observed differences in BMI via changes in energy intake or energy expenditure. Nonetheless, the documented change in the rate of weight gain in the intervention children makes it clear that research on mechanisms and use of potentially more sensitive measures for assessment of energy intake and expenditure will be an important next step in understanding how children do or do not become overweight.

Additional limitations of this research include the use of specialty trained early childhood educators rather than classroom teachers to deliver the intervention, thereby raising questions of generalizability. The enthusiasm and consistency of the intervention may be less replicable if delivered by busy classroom teachers. Attention to high-quality training and other quality control efforts can help to address this issue. Additionally, because both the WCI and GHI interventions were conducted for 14 weeks, but WCI was delivered three times weekly and GHI was delivered once weekly, a dose–response difference cannot be completely ruled out.

Another limitation is the number of children who were unavailable or did not provide anthropometric data at Year 1 or Year 2. However, because children received no additional intervention after the 14 weeks, and children who did not have anthropometric data at one follow–up assessment often returned for subsequent visits with results similar to those who did not miss an assessment, it is likely that the missing anthropometric data can be viewed as being missing completely at random and may therefore be regarded as ignorable. Analyses based on imputing the missing data based on regressions in controls only (more conservative) or in addition from regressions in intervention and control children for those children with other data at Year 1 or Year 2 (less conservative) suggested that there is no bias in the observed Year 1 and Year 2 results, given the similarity of the imputed and observed results.

These limitations notwithstanding, our results document the feasibility and efficacy of a school-based overweight prevention intervention with preschool minority children. It was effective with both genders and across the weight distribution. School-based interventions initiated during the preschool years and disseminated on a population-wide basis could play a role in changing the distribution of obesity in the population and taking young children off the trajectory toward obesity.

We gratefully acknowledge the administrators, staff, teachers, food service personnel, parents, and children who participated in this project. We would like to thank Drs. Ken Resnicow and John Himes for their insightful comments on a previous draft of the manuscript. We also would like to thank the Hip–Hop to Health Jr. staff who worked with the utmost professionalism to conduct this study in this underserved population and Jamie Gayle and Allison Thompson for their technical assistance.

REFERENCES

IMPROVED OUTCOMES OF OUTBORN PRETERM INFANTS IF ADMITTED TO PERINATAL CENTERS VERSUS FREESTANDING PEDIATRIC HOSPITALS


Objectives To examine whether admission hospital type (13 perinatal centers vs 4 freestanding pediatric hospitals) was associated with differences in risk and illness severity adjusted mortality and morbidity among outborn preterm infants.

Study design Records of singleton outborn infants ≤32 weeks' gestational age (n = 605) admitted to 17 tertiary level neonatal intensive care units participating in the Canadian Neonatal Network for the period 1996 to 1997 were examined.

Results Outborn infants admitted to freestanding pediatric hospitals were at higher risk of death (adjusted odds ratio [AOR], 2.25; 95% confidence interval [CI], 1.20, 4.20), nosocomial infection (AOR, 2.48; 95% CI, 1.64, 3.73), and oxygen dependency at 28 days of age (AOR, 1.77; 95% CI, 1.14, 2.75) when compared with outborn infants admitted to perinatal centers.

Conclusions After adjustment for perinatal risks and admission illness severity, outborn infants had better outcomes if they were admitted to perinatal centers compared with freestanding pediatric hospitals. (J Pediatr 2005;146:626-31)

Outcomes for preterm infants born in tertiary care centers (inborn) are better when compared with preterm infants born in a level II or level I hospital and then transferred to a tertiary care center for further treatment (outborn).1-4 Kitchen et al5 reported serious functional impairment in 72% of outborn infants compared with 23% of inborn infants who weighed 500 to 999 g at birth. Factors accounting for these differences may include a lack of adequate facilities at the outborn centers, ineffective or inappropriate resuscitation and postnatal stabilization, and delay in therapies such as surfactant or artificial ventilation.1,5 Outborn infants tend to be sicker than infants born at the tertiary care centers.1-4

In most countries with regionalized perinatal care systems, outborn preterm infants ≤32 weeks are routinely transferred to tertiary centers for care after birth.1,6,7 These tertiary care neonatal intensive care units (NICU) are located either in perinatal centers (attached with obstetric units) or freestanding pediatric hospitals. In Canada, preterm and low birth weight infants (ie, a more homogenous population) usually comprise the bulk of the admissions to NICUs attached to perinatal centers. In contrast, freestanding pediatric hospitals tend to care predominantly for neonates with complex diagnoses such as surgical, cardiac, genetic, or metabolic disorders. A relatively small proportion of preterm and low birth weight infants without complex diagnoses are admitted to these hospitals. Therefore, the expertise and experience of both medical and paramedical personnel and the practice patterns and infrastructure may differ between these two types of hospitals. This may result in differences in the care provided to these infants and their outcomes.

Subtle differences in the practices have been shown to influence certain neonatal outcomes even among perinatal centers.8 To our knowledge, differences in outcomes of outborn preterm infants admitted to perinatal centers and to freestanding pediatric hospitals.

---

**Abbreviations**: NICU Neonatal intensive care unit, PDA Patent ductus arteriosus, SNAP Score of Neonatal Acute Physiology

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Presented at the Annual Meeting of the Pediatric Academy Societies, San Francisco, California, May 2004.

Supported by grants 40503 and 00152 from the Medical Research Council of Canada. Additional funding was provided by the B.C.'s Children's Hospital Foundation; Calgary Regional Health Authority; Dalhousie University Neonatal-Perinatal Research Fund; Division of Neonatology, Children's Hospital of Eastern Ontario; Child Health Program, Health Care Corporation of St John's; the Neonatology Program, Hospital for Sick Children; Lawson Research Institute; Midland Walwyn Capital Inc; Division of Neonatology, Hamilton Health Sciences Corporation; Mt Sinai Hospital; North York General Hospital Foundation; St Joseph's Health Centre; University of Saskatchewan Neonatal Research Fund; University of Western Ontario; Women's College Hospital.

Submitted Sep 15, 2004; revision received Dec 26, 2004; accepted Jan 13, 2005.

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10.1016/j.jpeds.2005.01.030
hospitals have not been described. The objective of this study was to examine whether admission hospital type (perinatal center vs freestanding pediatric hospitals) is associated with differences in mortality and morbidity among outborn preterm infants after adjustment of perinatal risks and admission illness severity (using the Score of Neonatal Acute Physiology, version II [SNAP II]).

METHODS

Study Population
The Canadian Neonatal Network established a national database (including 17 hospitals with 75% of tertiary NICU beds across Canada) and collected information on all admissions over a 22-month period from January 1996 to October 1997 after approval from the research ethics board at each site. Trained research assistants prospectively abstracted relevant data from the mother’s and infant’s charts at each participating center on a daily basis. Details of data collection and data management have been published elsewhere. Singleton newborn infants with gestational age between 24 and 32 completed weeks born outside a perinatal center and transferred to either perinatal centers or freestanding pediatric hospitals within the first 4 days of age were included in this study. Perinatal centers are centers where obstetric care is provided to mothers and where newborn infants are admitted to tertiary level NICUs. Freestanding pediatric hospitals are centers with tertiary level NICUs but no obstetric services, and all NICU patients are transferred after the birth from other hospitals. All the NICUs included in this study were tertiary level referral NICUs. Four NICUs (two perinatal centers and two freestanding pediatric hospitals) had extracorporeal membrane oxygenation capabilities. Infants with lethal congenital anomalies, who were born at <23 weeks’ gestational age or who were moribund (ie, a physician, in consultation with the parents, had made an explicit decision not to provide life support at the time of the NICU admission) on admission were excluded from the analysis. The admission hospitals were divided into two groups: perinatal centers (n = 13) and freestanding pediatric hospitals (n = 4).

Variable Definitions
Study variables were defined according to the Canadian NICU Network SNAP Project Abstractor Manual. Gestational age was defined as the best obstetric estimate, based on early prenatal ultrasound, obstetric examination, and obstetric history, unless the postnatal pediatric estimate of gestation differed from the obstetric estimate by more than 2 weeks. In that case, the pediatric estimate of gestational age was used instead. An infant was defined as small-for-gestational age if the birth weight was less than the 3rd percentile for gestational age according to the British Columbia provincial growth charts established by Whitfield in 1992 for the Canadian population. Data are also presented for infants <10th percentile for gestational age. Prenatal care was defined as receipt of pregnancy-related care from a physician on at least one occasion (not related to a visit for diagnosis of pregnancy) during pregnancy. SNAP-II is a neonatal illness severity score calculated from 6 empirically weighted physiologic measurements and made during the first 12 hours of admission to the NICU. Higher SNAP score is associated with increased risk of mortality and morbidity. Chronic lung disease was defined as oxygen dependency at 36 weeks’ corrected gestational age for an infant who was born at ≤32 weeks’ gestation. Intraventricular hemorrhage was defined according to the criteria of Papile from head ultrasound performed before 14 days of life. Necrotizing enterocolitis was defined according to Bell criteria (stage 2 or higher) and was classified as medical (clinical symptoms and signs plus evidence of pneumatosisis on abdominal radiography) or surgical (histologic evidence on surgical specimen of intestine). Nosocomial infection was defined by using blood and cerebrospinal fluid culture results according to Freeman criteria. Patent ductus arteriosus was defined as clinical diagnosis plus treatment with indomethacin or surgical ligation or both.

Outcomes
Death in the NICU, severe (grade 3 or greater) intraventricular hemorrhage, chronic lung disease, necrotizing enterocolitis, patent ductus arteriosus, respiratory distress syndrome, nosocomial infection, duration (days) of mechanical ventilation, duration (days) of oxygen treatment, number of transfusions, and respiratory status on day 28 (infants needing respiratory support) were compared between the two groups.

Statistical Methods
Univariate and bivariate analyses were performed to describe the characteristics of the study population and to explore the association between population characteristics and clinical outcomes. Multivariate regression analyses, including logistic, linear, and Poisson, depending on the data type (dichotomous, continuous, and counts) of each outcome, were used to compare neonatal outcomes of infants admitted to perinatal centers and freestanding pediatric hospitals after adjustment for perinatal risks, admission severity of illness (SNAP-II), and volume of admissions (number of very low birth weight infants treated at each hospital). The SPSS version 12 (SPSS Inc; Chicago, Ill) was used for data analysis.

RESULTS
A total of 3769 singleton preterm infants born at 24 to 32 weeks’ gestation were admitted to 17 tertiary NICUs in the Canadian Neonatal Network during the study period of 1996 to 1997. Of these, 3164 infants were born in perinatal centers, whereas 605 infants were born outside. Two infants with lethal congenital anomalies, 102 infants born at ≤23 weeks’ gestation, and 21 infants who were moribund on admission were excluded from the analyses. Of the 605
eligible outborn infants, 303 infants were admitted to perinatal centers and 302 infants were admitted to freestanding pediatric hospitals. There was no significant difference in the gestational age distribution of patients admitted to the two types of hospitals (percentage of babies born at ≤26 weeks, 27 to 28 weeks, 29 to 30 weeks, and 31 to 32 weeks were 26%, 20%, 22%, and 32%, respectively, for perinatal centers; and 26%, 17%, 25%, and 32%, respectively, for freestanding pediatric hospitals). The mean (±SD) number of infants ≤32 weeks’ gestation that were admitted per center (including inborn and outborn infants) were 256 (±122) patients for the 13 perinatal centers and 110 (±76) patients for the 4 freestanding pediatric hospitals during the study period.

Baseline characteristics (Table I) of infants admitted to perinatal centers and freestanding pediatric hospitals were similar, with the exception of maternal hypertension and administration of a complete course of antenatal corticosteroids, which were more prevalent among infants admitted to freestanding pediatric hospitals. Bivariate analysis (Table II) showed that the nosocomial infection and oxygen dependency at 28 days of life were higher among infants admitted to freestanding pediatric hospitals, whereas patent ductus arteriosus was more prevalent among infants admitted to perinatal centers. There was a trend toward increased mortality rates and decreased incidence of chronic lung disease among infants admitted to the freestanding pediatric hospitals; however, it did not reach statistical significance in bivariate analysis.

Multivariate regression analyses (Table III) revealed that outborn infants admitted to freestanding pediatric hospitals were at higher risk of death (adjusted odds ratio [OR], 2.25; 95% confidence interval [CI], 1.20, 4.20), nosocomial infection (adjusted OR, 2.48; 95% CI, 1.64, 3.73), and oxygen dependency at 28 days of age (adjusted OR, 1.77; 95% CI, 1.14, 2.75) and at lower risk for development of patent ductus arteriosus (PDA) (adjusted OR, 0.46; 95% CI, 0.30, 0.69), even after adjustment for perinatal risks and admission illness severity. Volume of admission was not a significant predictor. Male sex was predictive of death (adjusted OR, 2.10; 95% CI, 1.15, 3.85) but not other outcomes. Lower gestational age was predictive of all adverse outcomes. Small for gestational age and antenatal corticosteroids (partial) were only predictive of nosocomial infections. Higher admission illness severity was predictive of death but higher SNAP-II scores were not significantly predictive of PDA, nosocomial infection, or oxygen dependency at 28 days of life. The latter probably is due to the small numbers of patients with high SNAP-II scores in the cohort. Diagnostic plots showed no departure from model assumptions.

We also examined the issue of nonlinearity and categorization of SNAP scores. When SNAP-II is fitted as a linear variable, the residuals indicate a nonlinear effect. Categorizing SNAP-II into 0 to 9, 10 to 19, 20 to 29, and ≥30 groups accommodates nonlinearity; the categories also enable a sufficient number of observations in the cells. If we fit the logistic model with SNAP-II as a continuous polynomial term, the likelihood ratio statistics is equal to 203.7 on 12 degrees of freedom. Refitting the model with the categorized SNAP-II instead gives a likelihood ratio statistic equal to 202.5 on 13 degrees of freedom. This shows that

### Table I. Characteristics of infants admitted to NICUs in perinatal centers (n1 = 303) and freestanding pediatric hospitals (n2 = 302)

<table>
<thead>
<tr>
<th>Infant characteristics</th>
<th>Perinatal centers (% of n1)</th>
<th>Freestanding pediatric hospitals (% of n2)</th>
<th>χ² (df)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sex</td>
<td>Male 59.9 57.0 0.436 (1) .51</td>
<td>Female 40.1 43.0</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age (weeks)</td>
<td>20–26 25.7 26.5 1.468 (3) .69</td>
<td>27–28 20.1 16.6</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>29–30 22.2 24.5</td>
<td>31–32 32.0 32.5</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Apgar score (5 min)</td>
<td>0–3 7.7 5.0 4.321 (2) .12</td>
<td>4–6 31.4 26.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>≥7 60.9 68.8</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Small for gestational age</td>
<td>3rd percentile 1.3 2.0 0.461 (1) .52</td>
<td>10th percentile 5.0 8.9 3.775 (1) .05</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Delivery type</td>
<td>Vaginal 65.7 58.8 3.014 (1) .08</td>
<td>Cesarean 34.3 41.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Maternal 5.7 11.4 5.691 (1) .02*</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Presentation</td>
<td>Vertex 67.6 67.0 0.367 (2) .88</td>
<td>Breech 27.7 29.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Other 4.7 3.7</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antenatal steroid use</td>
<td>Partial 19.8 22.8 0.837 (1) .36</td>
<td>Complete 6.3 12.3 6.546 (1) .01*</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prenatal care</td>
<td>None 6.2 5.8 0.025 (1) .87</td>
<td>Some 93.8 94.2</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Score for Neonatal Acute Physiology (II)</td>
<td>0–9 41.7 49.0 4.834 (3) .18</td>
<td>10–19 29.1 26.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>20–29 12.9 13.3</td>
<td>≥30 16.2 11.3</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*P < .05.
categorizing SNAP-II in the given categories eliminates nonlinearity.

**DISCUSSION**

We report that outborn preterm infants have better outcomes if they are admitted to perinatal centers compared with freestanding pediatric hospitals. Previous reports documented that regionalization of perinatal care improved the outcome both for the mother and infant. McCormick et al\(^6\) reported a decline in neonatal mortality rates after regionalization of perinatal care, which emphasized delivery of high-risk and preterm infants 31 to 32 weeks' gestation at perinatal centers. Truffert et al\(^7\) showed a similar trend among infants 31 to 32 weeks' gestational age in France, with the use of an established neonatal transport team attending birth when feasible. The Victoria Infant Collaborative Study Group\(^2\) in Australia reported an increase in the number of intrauterine transfers and survival of neonates over time (1979 to 1992), accompanied by an increasing gap between survival rates of inborn and outborn preterm infants. It is speculated that regionalization results in better outcomes because high-risk pregnancies are transferred in an orderly manner to facilities with the appropriate expertise and facilities for ongoing surveillance and timely intervention. Although Canada has a highly regionalized perinatal care system, Chien et al\(^1\) reported that in 1996 to 1997, approximately 20% of preterm infants were born outside perinatal centers, mostly due to emergent circumstances that prevented in utero transfer to a tertiary center.

Previous authors reported variations in outcomes of tertiary NICUs and suggested that hospital and patient characteristics may affect outcomes. Lee et al\(^8\) reported significant variation in mortality, morbidity, and resource use among tertiary Canadian NICUs. Horbar et al\(^15\) reported that mortality rates among very low birth weight infants admitted to the NICU were affected by factors such as volume of patients and whether the NICU was an academic hospital. Phibbs et al\(^4\) in a large population-based study in California, reported significantly lower-risk adjusted neonatal mortality rates (OR, 0.62; 95% CI, 0.47, 0.82) for neonates born in a perinatal center with a level 3 NICU with an average NICU census of >15 patients per day compared with hospitals without NICUs. Risk-adjusted neonatal mortality was not different among smaller level 3 NICUs, level 2 NICUs, and hospitals without NICUs and was significantly higher than level 3 NICUs. Lee et al\(^9\) reported that NICU mortality rates were higher if the patient volume was low, the infant was admitted at night, and the in-house medical staff were inexperienced. However, outcomes of outborn infants admitted to NICUs associated with perinatal centers and freestanding pediatric hospitals have not been previously compared.

We speculate that there might be several possible reasons for our finding that perinatal centers had better NICU outcomes than freestanding pediatric hospitals. Note that only outborn preterm infants were included in this study, to ensure comparability of findings. First, the NICUs in freestanding pediatric hospitals had lower patient volumes (especially preterm infants) than the perinatal centers (mean admissions per NICU during the study period were 110 and 256). Lee et al\(^16\) Horbar et al\(^15\) and Phibbs et al\(^4\) reported a relation between lower patient volumes and poorer outcomes. Second, proportionately more patients with surgical problems, congenital heart disease, and complex congenital anomalies were admitted to freestanding pediatric hospitals than to perinatal centers because the former were more often designated for care of infants with these conditions. Infants with predominant surgical problems are at increased risk of infections and treatment with multiple antibiotics, and this may predispose to cross-infections and resistant organisms. Although adjustment for admission illness severity using SNAP-II minimizes the impact of patient differences, it is possible that SNAP-II may not completely compensate for differences in referral patterns. Third, clinical practices and hospital infrastructure and organization may vary because of

**Table II. Crude mortality and morbidity rates among the infants admitted to perinatal centers and freestanding pediatric hospitals**

<table>
<thead>
<tr>
<th>Outcome</th>
<th>Perinatal centers (%)</th>
<th>Freestanding pediatric hospitals (%)</th>
<th>Odds ratios (95% CI)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Death in the NICU</td>
<td>12.9</td>
<td>18.3</td>
<td>1.52 (0.97–2.37)</td>
</tr>
<tr>
<td>Severe intraventricular hemorrhage (grade 3)</td>
<td>15.0</td>
<td>18.5</td>
<td>1.29 (0.81–2.06)</td>
</tr>
<tr>
<td>Chronic lung disease</td>
<td>24.4</td>
<td>18.6</td>
<td>0.71 (0.46–1.08)</td>
</tr>
<tr>
<td>Necrotizing enterocolitis</td>
<td>5.6</td>
<td>3.4</td>
<td>0.59 (0.26–1.33)</td>
</tr>
<tr>
<td>Patent ductus arteriosus</td>
<td>33.7</td>
<td>29.7</td>
<td>0.51 (0.35–0.75)</td>
</tr>
<tr>
<td>Respiratory distress syndrome</td>
<td>84.3</td>
<td>78.5</td>
<td>0.68 (0.45–1.08)</td>
</tr>
<tr>
<td>Infection</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primary</td>
<td>0.3</td>
<td>2.3</td>
<td>7.17 (0.88–58.6)</td>
</tr>
<tr>
<td>Nosocomial</td>
<td>18.2</td>
<td>34.1</td>
<td>2.33 (1.60–3.40)</td>
</tr>
<tr>
<td>Number of blood transfusions (mean ± SD)</td>
<td>3.2 ± 2.6</td>
<td>3.6 ± 3.3</td>
<td>0.45 (–0.24–1.13)*</td>
</tr>
<tr>
<td>Oxygen-dependent at 28 d</td>
<td>78.5</td>
<td>86.6</td>
<td>1.77 (1.15–2.72)</td>
</tr>
<tr>
<td>Ventilator-dependent at 28 d</td>
<td>92.4</td>
<td>96.0</td>
<td>1.98 (0.97–4.05)</td>
</tr>
</tbody>
</table>

*Using Poisson regression model.

†Outcomes for which regression models were created.
the differing patient types and volumes in perinatal centers and freestanding pediatric hospitals. Increased emphasis on larger infants with complex medical conditions may be less conducive to care for the small preterm infant. Indeed, although the mortality rate was 50% higher among preterm infants \( \leq 32 \) weeks' gestation at birth when they were admitted to freestanding pediatric hospitals compared with perinatal centers, we found no evidence that this was due to higher illness severity. Practice differences (eg, fluid management) might also explain the decreased risk of PDA among infants admitted to freestanding pediatric hospitals. Since the mortality rates and morbidity risks are highest among the very preterm infants \(<26\) weeks' gestation at birth, these infants are at greatest risk of adverse outcomes if admitted to freestanding pediatric hospitals.

Our results could be confounded by the lack of information regarding socioeconomic status, reasons for transfer, timing of maternal admission, resuscitation of infants, treatment given during and after birth, and transport stabilization. However, there were no differences in the perinatal risks and admission illness severity scores among outborn infants admitted to either type of hospitals, thus making it unlikely that there were any major baseline differences among the infants. Our results were derived from regionalized NICUs serving 75% of the Canadian population and are generalizable to NICUs in Canada and potentially worldwide.

**CONCLUSIONS**

We recognize that we had only four freestanding pediatric hospitals in our study, further studies involving more units are needed before firm recommendations can be made. However, if the results hold true, this could have significant potential implications for regionalization planning and resource utilization. If our findings are replicated in other studies, it would suggest that when in utero maternal transfers are not possible, efforts should be made to transfer outborn preterm infants to perinatal centers. This is particularly true for infants \( \leq 28\) weeks' gestation, when the risk for adverse outcomes is greatest.\(^6\) Alternatively, changes in the treatment of preterm infants admitted to freestanding pediatric hospitals may be warranted. Further research is needed to determine risk factors in freestanding pediatric hospital NICUs that are associated with good or poor outcomes and that might be amenable to intervention.

**APPENDIX I**

**Members of the Canadian Neonatal Network**

Shoo K. Lee, MBBS, FRCP, PhD (Coordinator, Canadian Neonatal Network; Centre for Healthcare Innovation and Improvement, Vancouver, BC); Wayne Andrews, MD, FRCP (Charles A. Janeway Child Health Centre, St John's, NF); Ranjit Baboolal, MD, FRCP (North York Hospital, N York, ON); Jill Boulton, MD, FRCP (St Joseph's Health Centre, London, ON; previously at Mt Sinai Hospital, Toronto, ON); David Brabyn, MBChB, FRACP, FRCP (Royal Columbia Hospital, New Westminster, BC); David S. C. Lee, MBBS, FRCP (St Joseph's Health Centre; London, ON); Derek Matthew, MRCS, FRCP, SM (Victoria General Hospital, Victoria, BC); Douglas D. McMillan, MD, FRCP (IWK-Grace Health

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**Table III. Risk factors predictive of death, patent ductus arteriosus, nosocomial infection, and oxygen dependency at 28 days (odds ratio; 95% confidence interval)**

<table>
<thead>
<tr>
<th>Variables</th>
<th>Death in NICU</th>
<th>Patent ductus arteriosus</th>
<th>Nosocomial infection</th>
<th>Oxygen dependency at 28 d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Freestanding pediatric hospitals</td>
<td>2.25 (1.20–4.20)</td>
<td>0.46 (0.30–0.69)</td>
<td>2.48 (1.64–3.73)</td>
<td>1.77 (1.14–2.75)</td>
</tr>
<tr>
<td>Male</td>
<td>2.10 (1.15–3.85)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Gestational age</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( \leq 26) wk</td>
<td>8.00 (3.45–18.52)</td>
<td>7.95 (4.12–15.3)</td>
<td>5.89 (3.23–10.7)</td>
<td>NS</td>
</tr>
<tr>
<td>27–28 wk</td>
<td>1.51 (0.53–4.30)</td>
<td>10.9 (5.63–21.2)</td>
<td>5.05 (2.72–9.37)</td>
<td>NS</td>
</tr>
<tr>
<td>29–30 wk</td>
<td>0.58 (0.16–2.04)</td>
<td>2.88 (1.45–5.72)</td>
<td>2.49 (1.37–4.54)</td>
<td>NS</td>
</tr>
<tr>
<td>Apgar score (5 min)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0–3</td>
<td>3.61 (1.37–9.53)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>4–6</td>
<td>2.14 (1.11–4.13)</td>
<td>NS</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Small for gestational age (10th percentile)</td>
<td>NS</td>
<td>NS</td>
<td>2.37 (1.16–4.87)</td>
<td>NS</td>
</tr>
<tr>
<td>Antenatal steroid</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Partial</td>
<td>NS</td>
<td>NS</td>
<td>1.98 (1.24–3.15)</td>
<td>NS</td>
</tr>
<tr>
<td>Complete</td>
<td>NS</td>
<td>NS</td>
<td>1.15 (0.57–2.31)</td>
<td>NS</td>
</tr>
<tr>
<td>Admission SNAP-II</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>10–19</td>
<td>1.80 (0.73–4.44)</td>
<td>1.78 (1.07–2.94)</td>
<td>1.79 (1.12–2.87)</td>
<td>2.21 (1.24–3.92)</td>
</tr>
<tr>
<td>20–29</td>
<td>6.43 (2.58–16.02)</td>
<td>3.34 (1.81–6.15)</td>
<td>1.20 (0.65–2.24)</td>
<td>1.78 (0.86–3.70)</td>
</tr>
<tr>
<td>( \geq 30)</td>
<td>11.7 (4.67–29.28)</td>
<td>1.50 (0.78–2.87)</td>
<td>0.36 (0.17–0.75)</td>
<td>0.81 (0.45–1.44)</td>
</tr>
</tbody>
</table>

The reference group is perinatal centers, Apgar \( \geq 7\), gestational age 31 to 32 weeks, female, not small for gestational age, no antenatal steroid use, and SNAP-II 0 to 9.
Centre for Women, Children and Families, Halifax, NS; previously Foothill's Hospital, Calgary, AB; Christine Newman, MD, FRCPC (Hospital for Sick Children; Toronto, ON); Arne Ohlsson, MD, FRCPC, MSc (Mt Sinai Hospital, Toronto, ON; formerly Women's College Hospital, Toronto, ON); Abraham Peliowski, MD, FRCPC (Royal Alexandra Hospital, Edmonton, AB); Margaret Pendray, MBBS, FRCPC (Children's and Women's Health Centre of British Columbia, Vancouver, BC); Koravangattu Sankaran, MBBS, FRCPC (Royal University Hospital, Saskatoon, SK); Barbara Schmidt, MD, FRCPC, MSc (Hamilton Health Sciences Corporation, Hamilton, ON); Mary Seshia, MBChB, FRCP(Ed), FRCPC (Health Sciences Centre, Winnipeg, MB); Anne Synnes, MDCM, FRCPC, MHSc (Children's and Women's Health Centre of British Columbia, Vancouver, BC; formerly Montreal Children's Hospital, Montreal, PQ); Paul Thiessen, MD, FRCPC (Children's and Women's Health Centre of British Columbia, Vancouver, BC); Robin Walker, MD, FRCPC (Children's Hospital of Eastern Ontario and Ottawa General Hospital, Ottawa, ON); Robin Whyte, MBBS, FRCPC (IWK-Grace Health Centre for Women, Children and Families, Halifax, NS); Canadian Neonatal Network Coordinating Centre (Vancouver, BC): Holly Bavinton, MSc; Stella Karuri, MSc; Sarka Lisonkova, MD, MSc; Lauren Anderson, BA.

REFERENCES


PRETREATMENT CORTISOL VALUES MAY PREDICT RESPONSES TO HYDROCORTISONE ADMINISTRATION FOR THE PREVENTION OF BRONCHOPULMONARY DYSPLASIA IN HIGH-RISK INFANTS

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Objectives To investigate the effect of hydrocortisone treatment on survival without bronchopulmonary dysplasia (BPD) and to study whether serum cortisol concentrations predict the response.

Study design We performed a randomized, placebo-controlled trial on infants with gestation ≤30 weeks, body weight of 501 to 1250 g, and respiratory failure. Hydrocortisone was started before 36 hours of age and given for 10 days at doses from 2.0 to 0.75 mg/kg per day. Shortly before hydrocortisone treatment, basal and stimulated (ACTH, 0.1 μg/kg) serum cortisols were measured.

Results The study was discontinued early, because of gastrointestinal perforations in the hydrocortisone group (4/25 vs 0/26, P = .05); 3 of the 4 had received indomethacin/ibuprofen. The incidence of BPD (28% vs placebo 42%, P = 0.28) tended to be lower, and patent ductus arteriosus (36% vs 73%, P = .01) was lower in the hydrocortisone group. The hydrocortisone-treated infants with serum cortisol concentrations above the median had a high risk of gastrointestinal perforation. In infants with cortisol values below the median, hydrocortisone treatment increased survival without BPD.

Conclusions Serum cortisol concentrations measured shortly after birth may identify those very high-risk infants who may benefit from hydrocortisone supplementation. (J Pediatr 2005;146:632-7)

Despite advanced respiratory treatment, bronchopulmonary dysplasia (BPD) remains a major problem among very low birth weight (VLBW) infants.1 Lung inflammation and adrenal insufficiency are among the proposed risk factors of BPD.2 Sick VLBW infants have low basal serum cortisol concentrations and inadequate responses to adrenocorticotropic hormone during the early neonatal period. Apart from BPD, low cortisol levels predispose to hypotension and patency of ductus arteriosus (PDA).2-7

Dexamethasone (DX) given to very preterm infants shortens the duration of mechanical ventilation and decreases the incidence of BPD.8-11 However, pharmacologic doses of DX increase the risk of hyperglycemia, hypertension, cardiac hypertrophy, infections, gastrointestinal (GI) bleeding and perforation, suppression of somatic and brain growth, and periventricular leukomalacia.12-18 Follow-up studies have revealed an association between very early DX treatment and neurologic problems in childhood.19-21 Because of the concerns about side effects, routine use of postnatal DX is not indicated.22,23 Watterberg et al24 demonstrated a significant increase in survival without BPD in a pilot study using low-dose hydrocortisone (HC; i.e., dosage approaching cortisol levels reached in stress) given shortly after birth.

Based on the promising early results of the pilot study by others, we conducted a randomized, placebo-controlled trial of early HC treatment beginning within 36 hours after birth. The aims were to investigate whether HC treatment improves the survival without BPD in VLBW infants and whether pretreatment serum cortisol concentrations predict the therapeutic response.

BPD Bronchopulmonary dysplasia
DX Dexamethasone
GI Gastrointestinal
HC Hydrocortisone
NSAID Nonsteroid anti-inflammatory drug
PDA Patent ductus arteriosus
VLBW Very low birth weight

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Supported by grants from Foundation for Pediatric Research, The Alma and K. A. Snellman Foundation (Oulu, Finland), and the Sigrid Juselius Foundation (Finland).

Submitted for publication Aug 2, 2004; revision received Nov 18, 2004; accepted Dec 20, 2004.

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METHODS

Study Population

This randomized, blinded, placebo-controlled trial involved three University Hospitals in Finland (Oulu, Helsinki, and Kuopio). The study protocol was approved by the ethics committee of Oulu University Hospital and National Agency for Medicines. Written informed consent was obtained from the parents. The entry criteria were birth weight 501 to 1250 g, gestational age between 23 ± 0 and 30 ± 0 weeks, and need for mechanical ventilation before the age of 24 hours. The subgroup of infants with birth weight of 1000 to 1250 g had the additional requirement of supplemental oxygen and mechanical ventilation beyond 24 hours despite surfactant therapy. Infants with lethal malformations or suspected chromosomal abnormalities were excluded.

Random Assignment and Intervention

Random assignment was performed separately in each participating center. The study drug and placebo were prepared into identical syringes. The nurses and doctors as well as the study investigators were blinded. The infants were stratified into three weight groups (501 to 750 g, 750 to 999 g, and 1000 to 1250 g). They received either HC (2.0 mg/kg IV divided into 3 doses at 8-hour intervals for 2 days, 1.5 mg/kg IV divided into 3 doses at 8-hour intervals for 2 days, and 0.75 mg/kg IV divided into 2 doses at 12-hour intervals for 6 days) or isotonic saline as placebo. The first dose was given before the age of 36 hours. We aimed to use a stress dose at the beginning, reducing then to physiologic and finally to subphysiologic dose to allow recovery from adrenal suppression. The first ACTH test was performed before the study intervention (ie, before age 36 hours) and the second on the day after the end of the intervention (day 11). Serum samples for cortisol measurements were collected immediately before (basal level) and 30 minutes after the intravenous administration of ACTH (Synachten, Novartis, Basel, Switzerland) at a dose of 0.1 μg/kg. The ACTH dose was based on previous experience using slightly lower (0.5 to 1 μg/1.73 m²) ACTH doses in premature neonates.26,28 The serum samples were frozen at −20 °C before the cortisol assays. The use of open-label postnatal corticosteroid treatment was discouraged. Any use of glucocorticoid therapy during hospitalization was recorded. An intention to treat analysis was performed.

Outcomes

The primary outcome was survival without BPD, defined as survival without need for supplemental oxygen at 36 ± 0 weeks’ postmenstrual age. Secondary outcomes were intracranial hemorrhage, grade 3 to 427 and cystic periventricular leukomalacia.28 Cranial ultrasound examination was conducted on all infants between the days 4 and 8 and at 36 weeks’ postmenstrual age. Requirements of ventilation support and oxygen therapy were evaluated at the ages of 14 and 28 days and at 36 ± 0 weeks’ postmenstrual age. The number of days on assisted ventilation, the use of supplemental oxygen, the length of open-label corticosteroid treatment, and the length of hospital stay were recorded. Nosocomial sepsis was defined as a positive blood or cerebrospinal fluid culture after day 3 of life. Diagnostic data on hyperglycemia requiring insulin treatment, hypotension, or hypertension requiring therapy, PDA requiring prostaglandin inhibitor therapy or surgery, GI bleeding, and GI perforations were recorded. Necrotizing enterocolitis was defined according to the criteria of Bell et al.29 For the diagnosis of retinopathy of prematurity,30 the first ophthalmoscopic examination was performed 4 to 7 weeks after birth or no later than at 32 weeks’ postmenstrual age. Ophthalmic examination was repeated until retinas were mature, and the highest stage of retinopathy was reported.

Laboratory Analysis

Serum samples were analyzed retrospectively for cortisol by the immunochromimometric method (Bayer ADVIA Centaur Cortisol, Bayer HealthCare LLC, Bayer Corporation, Tarrytown, NY). The detection limit was 3.2 nmol/L. The method used was linear over the standard curve (0 to 839 nmol/L). The intra-assay and interassay coefficients of variation ranged within 1.6% to 2.8% and 5.3% to 12.2%, respectively. Cross-reaction with cortisone was 7.4%.

Statistical Analyses

Sample size justification was based on unpublished data from Finland. HC treatment was expected to increase survival without BPD from 55% to 70% among the very high-risk preterm infants. This would require 160 patients in each arm (α- and β-error 0.05 and 0.2, respectively). Baseline data for the infants enrolled in the study were compared by unpaired t tests for continuous variables and by chi-square tests for categoric data. In outcome analysis, we calculated the odds ratios with 95% confidence intervals and also compared the differences by χ² tests for categoric data. The correlations between the timing of antenatal steroids and cortisol values were tested by using Spearman correlation coefficient. Single continuous variables were compared by unpaired t tests between the two groups, and repeated measurements were analyzed by repeated measurements of analysis of variance. The Mann-Whitney U test was used when the data were not normally distributed. Statistical analyses were performed using SPSS 12.0.1 for Windows (SPSS Inc, Chicago, IL).

RESULTS

Fifty-one infants were enrolled between August 12, 2002, and March 4, 2004; 25 infants were randomly assigned to receive hydrocortisone and 26 placebo treatment. At the enrolment, the groups had similar demographic characteristics and respiratory status (Table I). The steering committee discontinued enrolment according to recommendation of the safety committee, after the risk of GI perforation in the HC group became evident in the current study and in another similar trial.31
Table I. Baseline characteristics of the study groups

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Hydrocortisone group (n = 25)</th>
<th>Placebo group (n = 26)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Birth weight, g</td>
<td>888 ± 204</td>
<td>903 ± 220</td>
</tr>
<tr>
<td>Gestational age, wk</td>
<td>26.7 ± 1.6</td>
<td>26.5 ± 2.8</td>
</tr>
<tr>
<td>Male sex, n (%)</td>
<td>16 (64)</td>
<td>14 (54)</td>
</tr>
<tr>
<td>Antenatal glucocorticoid</td>
<td>23 (92)</td>
<td>25 (96)</td>
</tr>
<tr>
<td>Surfactant, n (%)</td>
<td>24 (96)</td>
<td>23 (89)</td>
</tr>
<tr>
<td>Chorioamnionitis, n (%)</td>
<td>9 (36)</td>
<td>9 (35)</td>
</tr>
<tr>
<td>PROM, n (%)</td>
<td>4 (16)</td>
<td>6 (23)</td>
</tr>
<tr>
<td>Pre-eclampsia, n (%)</td>
<td>20 (80)</td>
<td>20 (77)</td>
</tr>
<tr>
<td>Multiple birth</td>
<td>5 (20)</td>
<td>7 (27)</td>
</tr>
<tr>
<td>Vaginal delivery, n (%)</td>
<td>14 (56)</td>
<td>13 (50)</td>
</tr>
<tr>
<td>Apgar scores, median</td>
<td></td>
<td></td>
</tr>
<tr>
<td>(interquartile)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>At 1 min</td>
<td>4.0 (1.5)</td>
<td>4.5 (4.5)</td>
</tr>
<tr>
<td>At 5 min</td>
<td>7.0 (5.0)</td>
<td>6.5 (6.0)</td>
</tr>
<tr>
<td>Cord blood pH, mean ± SD</td>
<td>7.30 ± 0.08</td>
<td>7.23 ± 0.18</td>
</tr>
<tr>
<td>Surfactant, n (%)</td>
<td>24 (96)</td>
<td>23 (89)</td>
</tr>
</tbody>
</table>

*PROM, premature rupture of membranes >24 hours before the birth.  
None of the mothers had a repeated course of glucocorticoid. 
†Four mothers had clinical chorioamnionitis (2 in hydrocortisone group, 2 in placebo group).

Outcomes and Side Effects

Hydrocortisone-treated infants did not have a significant increase in survival without BPD (64% vs placebo, 54%; OR, 1.53; 95% CI, 0.46 to 5.02). The lowest glucose concentrations on each day were similar between the study groups during the first week (P = .80). The levels of diastolic and systolic blood pressures were similar between the study groups during the first week, with similar requirements for supportive medication (data not shown).

Adrenocortical Function

The ACTH test was performed on 41 infants before the onset of the trial and on 46 infants after the HC treatment. At the onset, the ACTH test was incomplete in 3 patients. Although the low number of analyzed cases limits the strength of the findings, the characteristics of the infants with missing serum cortisol measurements were similar to the other participants of the trial. There was nonsignificant correlation between the timing of the antenatal corticosteroids and basal (P = .06) or stimulated (P = .11) cortisol values soon after birth. There were no significant differences in the basal and ACTH-stimulated cortisol levels between the intervention groups at the baseline or after the intervention (Table III).

Among the infants with basal cortisol below the median, the incidence of BPD or death at 36 weeks' postmenstrual age was lower in the HC-treated group (P = .02) (Table IV). Similarly, the HC-treated infants with the ACTH-stimulated cortisol below the median had lower incidence of BPD/death than the placebo-treated ones with lower than median stimulated cortisol (P = .04). There was no such difference among the patients with basal or stimulated cortisol values above the median.

We analyzed separately the infants with and without GI perforation including the HC and placebo groups. There was no difference in the basal cortisol levels at the onset of the study when the infants who had GI perforation were compared with those with no GI perforation. On the other hand, stimulated cortisol levels tended to be higher in the infants with later GI perforation compared with the ones with no GI perforation (median, 419 nmol/L vs 240 nmol/L; P = .126). Three of the 4 GI perforations occurred in the group of patients with basal cortisol above the median at the baseline. Three GI perforations occurred in the HC group with stimulated cortisol levels above the median; the fourth case of GI perforation had no stimulated cortisol value available (Table IV).

DISCUSSION

The current randomized study demonstrated evidence that hydrocortisone supplementation of VLBW infants shortly after birth promoted spontaneous closure of PDA, shortened the length of oxygen therapy, and decreased the severity of respiratory failure during the first week. Although the trial was underpowered, as it was discontinued early due to safety concerns, the results are consistent with the meta-analyses, indicating that pharmacologic dosage of DX shortly after birth facilitates weaning from assisted ventilation, decreases the risk of PDA, and lowers the incidence of BPD at the age of 36 postmenstrual weeks.8–10 In a previous study of
40 infants, supplementation of hydrocortisone appeared to reduce the development of BPD, which supports our findings. According to a preliminary report, the efficacy of HC treatment was found to be more obvious among infants with histologic chorioamnionitis. In the current study, there was no detectable association between the histologic chorioamnionitis and the efficacy of HC treatment.

Systemic DX is associated with GI complications. In the preliminary report of a larger trial, the increased risk of GI perforations in the HC group was shown, and the study was discontinued early because of this side effect. Another risk factor causing GI perforations is the use of nonsteroid anti-inflammatory drugs (NSAIDs) as treatment for PDA. According to a cohort study of adult patients, corticosteroid treatment combined with NSAIDs increased the risk of GI bleeding more than 2-fold compared with the use of corticosteroid alone. Administration of corticosteroid simultaneously with indomethacin or ibuprofen treatment to promote closure of PDA may increase the risk of GI perforation among very preterm infants.

### Table II. Outcome and treatment requirements in the study groups

<table>
<thead>
<tr>
<th></th>
<th>Hydrocortisone group (n = 25)</th>
<th>Placebo group (n = 26)</th>
<th>OR (95% CI)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Outcomes and side effects</td>
<td>n (%)</td>
<td>n (%)</td>
<td>(95% CI)</td>
<td>.28</td>
</tr>
<tr>
<td>BPD, n (%)</td>
<td>7 (28)</td>
<td>11 (42)</td>
<td>0.53 (0.17–1.71)</td>
<td>.67</td>
</tr>
<tr>
<td>Death</td>
<td>2 (8)</td>
<td>3 (12)</td>
<td>0.67 (0.10–4.37)</td>
<td>.01</td>
</tr>
<tr>
<td>Patent ductus arteriosus Indomethacin/ibuprofen treatment</td>
<td>9 (36)</td>
<td>19 (73)</td>
<td>0.21 (0.06–0.68)</td>
<td>.04</td>
</tr>
<tr>
<td>Intracranial hemorrhage</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Any grade</td>
<td>13 (52)</td>
<td>11 (42)</td>
<td>1.48 (0.49–4.46)</td>
<td>.49</td>
</tr>
<tr>
<td>Severe (gr 3+)</td>
<td>3 (12)</td>
<td>4 (15)</td>
<td>0.75 (0.15–3.75)</td>
<td>.73</td>
</tr>
<tr>
<td>Cystic PVL</td>
<td>3 (12)</td>
<td>2 (8)</td>
<td>1.64 (0.25–10.7)</td>
<td>.61</td>
</tr>
<tr>
<td>Retinopathy of prematurity</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Severe (gr 3+)</td>
<td>1 (4)</td>
<td>1 (4)</td>
<td>1.10 (0.06–18.6)</td>
<td>.95</td>
</tr>
<tr>
<td>Sepsis at 3–28 days’ age</td>
<td>8 (32)</td>
<td>4 (15)</td>
<td>2.59 (0.67–10.1)</td>
<td>.16</td>
</tr>
<tr>
<td>GI disorders</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>NEC</td>
<td>2 (8)</td>
<td>1 (4)</td>
<td>2.17 (0.19–25.6)</td>
<td>.53</td>
</tr>
<tr>
<td>GI hemorrhage</td>
<td>2 (8)</td>
<td>1 (4)</td>
<td>2.17 (0.19–25.6)</td>
<td>.53</td>
</tr>
<tr>
<td>GI perforation</td>
<td>4 (16)</td>
<td>0 (0)</td>
<td></td>
<td>.05</td>
</tr>
<tr>
<td>Treatment requirements</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Open glucocorticoid treatment</td>
<td>7 (28)</td>
<td>12 (46)</td>
<td>0.45 (0.14–1.45)</td>
<td>.18</td>
</tr>
<tr>
<td>Days of oxygen therapy</td>
<td>34 (19–59)</td>
<td>62 (30–110)</td>
<td></td>
<td>.02</td>
</tr>
<tr>
<td>Days of intubation</td>
<td>4 (2–20)</td>
<td>13 (2–40)</td>
<td></td>
<td>.25</td>
</tr>
<tr>
<td>Days of hospitalization</td>
<td>82 (72–103)</td>
<td>91 (70–110)</td>
<td></td>
<td>.73</td>
</tr>
</tbody>
</table>

BPD, bronchopulmonary dysplasia; PVL, periventricular leukomalacia; NEC, necrotizing enterocolitis; GI, gastrointestinal; md, median; iq, interquartiles.

### Table III. Basal and ACTH-stimulated serum cortisol concentrations in the study groups before and after the intervention

<table>
<thead>
<tr>
<th>Serum cortisol, nmol/L</th>
<th>Hydrocortisone group</th>
<th>Placebo group</th>
<th>P value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Basal cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before intervention</td>
<td>139 (62–236)</td>
<td>130 (53–324)</td>
<td>.52</td>
</tr>
<tr>
<td>After intervention</td>
<td>130 (90–260)</td>
<td>159 (99–340)</td>
<td></td>
</tr>
<tr>
<td>Stimulated cortisol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Before intervention</td>
<td>290 (121–419)</td>
<td>255 (118–566)</td>
<td>.64</td>
</tr>
<tr>
<td>After intervention</td>
<td>330 (251–470)</td>
<td>420 (245–470)</td>
<td></td>
</tr>
</tbody>
</table>

*Significance levels were analyzed by repeated-measures analysis of variance.
3 of the 4 infants in the HC group with GI perforation had NSAID treatment for PDA.

Low serum basal and/or stimulated cortisol levels during the first week were associated with an increased risk of BPD and with lung inflammation and high-permeability edema. Low adrenocortical activity is also associated with hypotension and an increased risk of PDA. Ng et al reported recently that in the preterm infants at high risk of BPD, serum cortisol concentrations at 7 days of age correlated negatively but at 14 days of age positively with the length of oxygen therapy. Although many very preterm infants may have higher basal serum cortisol levels than healthy more mature infants soon after birth, the premature infants in general have a lower response to ACTH compared with near-term infants. Furthermore, some premature newborn infants also have low basal serum cortisol levels despite severe early lung disease. This phenomenon could be a consequence of an inadequate response to stress after preterm birth. The current study of very preterm VLBW infants revealed no difference in basal or stimulated cortisol levels soon after birth, which can be explained by the small sample size and/or the timing of the antenatal steroids. Together, the findings indicate that regardless of a single course of antenatal glucocorticoid, very preterm infants are capable of responding to ACTH. In the current study, nearly all infants were exposed to antenatal glucocorticoid. The timing of antenatal glucocorticoid therapy in relation to delivery had no statistically significant effect on the basal or stimulated cortisol levels soon after birth, which can be explained by the small sample size and/or the timing of the antenatal steroids. Together, the findings indicate that regardless of a single course of antenatal glucocorticoid, very preterm infants are capable of responding to ACTH. In the current study, the basal cortisol levels of the HC group were similar to those in the placebo group within 1 day after the end of the intervention, and there was no significant decrease in stimulated cortisol levels. Similarly, Watterberg et al failed to find any suppression of basal or stimulated cortisol levels after a 15-day course of low-dose HC treatment.

In the current study, the basal or ACTH-stimulated serum cortisol concentration above the median shortly after birth appeared to associate with the increased risk of gastrointestinal perforations in infants treated with HC. Although the use of NSAIDs for PDA was evenly distributed between the infants with cortisol levels above and below the median, the NSAID recipients with stimulated cortisol levels above the median appeared to be at risk. In contrast, the infants with basal and/or stimulated cortisol levels below the median shortly after birth tended to have a high BPD risk and a favorable therapeutic response to HC. Our sample size was too small to allow definite conclusions about the efficacy and safety of HC supplementation for prevention of BPD in VLBW infants. Furthermore, the cutoff levels for basal and stimulated serum cortisol concentrations that influence the efficacy and safety of HC therapy remain to be determined.

Taken together with the results of another multicenter trial of early hydrocortisone therapy in VLBW infants, combination with NSAID therapy appears to increase the risk of gastrointestinal perforation. We propose that serum cortisol concentrations below the median at the onset may identify those very high-risk infants who benefit from HC supplementation. Whether early HC supplementation is efficacious and safe in very high-risk infants, who additionally have low serum cortisol levels at the onset, remains to be tested in further randomized trials.

The authors thank Jorma Kokkonen, MD, PhD, for serving as the chairman of the Safety Committee.

REFERENCES

Objectives  To review the success of pediatric trainees for neonatal intubation over a 10-year interval at a single academic center.

Study design  We reviewed a database of all neonatal intubations designed as a quality assurance process at our institution. Respiratory care practitioners recorded the number of attempts at the time of each procedure. Attempts were defined as each time a laryngoscope was placed in the baby’s mouth. Success rates were calculated as the number of successful intubations divided by the attempts.

Results  From January 1992 through September 2002, 5051 successful intubations with 9190 attempts were performed by all practitioners. Pediatric residents intubated neonates successfully on 1676 occasions requiring 3719 attempts. The median success rates were 33% for pediatric level (PL)1 residents; 40% for PL2 and PL3 residents, and 68% for neonatal fellows ($P < .001$). The success rates for residents who had more than 20 total attempts versus those who had fewer than 20 attempts were 49% versus 37% ($P < .001$).

Conclusions  Developing proficiency at intubation requires a significant amount of experience. Current pediatric residents at our institution have inadequate opportunity to achieve consistent success. (J Pediatr 2005;146:638-41)

The pediatric Residency Review Committee (RRC), of the Accreditation Council for Graduate Medical Education, has indicated that pediatric trainees must be competent at endotracheal intubation.¹ The minimal experience required to become competent at neonatal endotracheal intubation has not, to our knowledge, been determined. The anesthesia literature suggests that proficiency at intubation in controlled circumstances takes 40 or more procedures.²⁻⁴

Since 1994, changes in pediatric residency training limit the amount of time pediatric residents may spend in intensive care. The current pediatric RRC program requirements are that residents must spend a minimum of 4 months and a maximum of 6 months in intensive care training, including neonatal and pediatric intensive care and including all nighttime coverage.

We reviewed our neonatal intubation database to determine the success rates of pediatric residents throughout their training over a 10-year period. The years of the database include the time before and after the introduction of intensive care limitations.

METHODS

The University of California, San Diego (UCSD) Infant Special Care Center is a 40-bed, level III neonatal intensive care unit (NICU) with approximately 600 admissions per year. This hospital provides full perinatal care with a regional NICU and has approximately 2800 deliveries per year, of which approximately 45% are considered high risk. The NICU at UCSD has maintained a neonatal intubation database since 1991 as part of a hospital-approved quality improvement project. The neonatal intubation database contains information about all intubations of babies admitted to the UCSD Infant Special Care Center, including the date and time of intubation, the names and training levels of the operators attempting intubation, the number of intubation attempts, and the name of the operator completing the intubation.

The definitions of attempted and successful intubations have remained constant since the creation of the database. An attempt is defined as placement of the laryngoscope in the baby’s mouth. A successful intubation is defined as an attempt that leads to placement of the endotracheal tube in the baby’s trachea. Evidence of successful intubation includes
auscultation of breath sounds, chest expansion, improvement in clinical condition, mist on the endotracheal tube, and since 2001, change in color of a colorimetric CO2 detector for confirmation. A preprinted card with the information to be collected is completed by a respiratory care practitioner at the time of each intubation and entered into a computerized database. The preprinted card has remained unchanged since the initiation of the database, and the data collectors have been the respiratory care practitioners throughout the existence of the database.

The database for the time period from January 1992 through September 2002 was reviewed, evaluating the number of attempted and successful intubations performed by pediatric residents and neonatal fellows. Two of the authors independently reviewed the database to ensure a uniform interpretation. When differences were found, all authors looked at the data again to resolve the differences. Success rates were calculated as the total number of successful intubations divided by the total number of attempted intubations. Success rates were evaluated by individual and by training level. Because success cannot be confirmed during intubations for meconium, success rates were also determined for all nonmeconium indications. Pediatric level (PL)-2 and PL-3 residents were grouped together as a training level because the "senior" rotation in the NICU was done in either the PL-2 or PL-3 year. These residents all previously had experience in the NICU at the PL-1 level. Before any experience in the NICU, pediatric trainees are all instructed in the Neonatal Resuscitation Program and in Pediatric Advanced Life Support. Practice with intubation of manikins occurs in both courses. Trainees also are taught intubation in a cat lab for a half day before experience in the NICU. Throughout the NICU rotation, residents participate in our video review conference, which is a bimonthly meeting in which videotaped performances of delivery room resuscitations and NICU intubations are reviewed. Proper techniques, including handling of the laryngoscope and positioning of the infant, are discussed. Trainees are taught to maintain a controlled and safe environment during the procedure. Trainees are able to see their own performances as well as those of others and will be able to apply this education to improvement of their technique with subsequent intubation experiences. With every intubation a nurse, respiratory care practitioner, and fellow or attending are present to assist and supervise residents with the procedure. Residents are generally allowed 2 to 3 attempts at intubation, provided the baby is stable before the fellow or attending performs the procedure.

The total numbers of attempted and successful intubations performed by each resident throughout training were determined from the database. To evaluate whether the amount of experience influenced success, success rates were compared for residents with 0 to 10 intubation attempts, 10 to 20 attempts, and more than 20 attempts.

Approval to review the database was obtained from the institution's Human Subjects Research Review Board. Unique patient identifiers and names of trainees were removed from the database in compliance with the Health Insurance Portability and Accountability Act regulations before any analyses.

Statistical analyses for data were performed using SigmaStat for Windows (SPSS, Chicago, Ill) statistical software. Data were compared by using 1-way analysis of variance (ANOVA) for normally distributed data with a Tukey test post hoc analysis and by Kruskal-Wallis 1-way ANOVA on ranks with Dunn method post hoc analysis, in which data were not normally distributed. Comparisons were considered significant if the probability value was < .05.

RESULTS

From January 1992 through September 2002, a total of 5051 successful intubations were documented, with 9190 (55% success rate) attempts at intubation performed by all practitioners including pediatric trainees, respiratory care practitioners, neonatal nurse practitioners, attending neonatologists, and anesthesiologists. In addition, 24 infants had unsuccessful attempts at intubation for nonmeconium indications by any provider. The majority of those encounters (21/24) were abandoned because of improvement in the infant's status. Of the remaining three infants, attempts at intubation were abandoned because of severe lethal airway anomaly (1), the clinical diagnosis of trisomy 13 (1), and extreme prematurity (1).

In the delivery room, 2676 intubations were successfully completed by all operators, including 1877 intubations for meconium. Coinciding with the revised Neonatal Resuscitation Program guidelines in the fourth edition of the textbook (2000) recommending selective intubation for suctioning meconium stained infants, our number of intubations per year decreased from 810 in 1992 with 376 (46%) for meconium to 344 intubations in 2001 with 43 (12%) for meconium.

Pediatric residents (208) intubated neonates successfully on 1676 occasions, requiring 3719 attempts (overall 45% success rate). An additional 1412 successful intubations with 2167 attempts (65% success rate) were performed by neonatal fellows. Median success rates (25th to 75th quartiles) by training level were PL-1 residents, 33% (17 to 50); PL-2 and PL-3 residents, 40% (25 to 60); and neonatal fellows, 68% (57 to 78). Kruskal-Wallis 1-way ANOVA on ranks showed a difference among groups (P < .001. Post hoc analysis using the Dunn method showed that PL2 residents had higher success rates than PL1 residents, and fellows had higher success rates than PL1 and PL2 residents. Success rates for pediatric residents were not significantly different for delivery room nonmeconium intubations than for NICU intubations (36% vs 36.5%).

The mean (standard deviation) total number of successful intubations a graduating resident accomplished throughout the entire 3 years of training decreased from 24 (±14) in 1994 to 8 (±2) in 2002. Similarly, the mean number of intubation attempts per resident throughout training decreased from 38 (±19) in 1994 to 12 (±6) in 2002 (Figure). The success rate for residents who had more than 20 total attempts (49%) was
significant differences existed between year 1 (60% success rate) and years 6 (35.3%), 7 (36.6%), 8 (32.9%), and 9 (31.7%). There was a significant decrease in overall success rates throughout training for each graduating class of residents during the period of the study (ANOVA, P < .001). Significant differences existed between year 1 (60% success rate) and years 6 (35.3%), 7 (36.6%), 8 (32.9%), and 9 (31.7%) by post hoc analysis with the Tukey test.

**DISCUSSION**

To our knowledge, this review of our neonatal intubation database represents the largest recorded compilation of neonatal intubation experience that has been described. Although we have evaluated these data retrospectively, the information was collected in a prospective manner by using preprinted data collection forms at the time of each event and the results are remarkably similar to our prospective observations using videotaped intubations.

We have used a consistent definition of intubation attempt and success. Our definition of an attempt included any encounter during which a laryngoscope was placed in the baby’s mouth for the purpose of intubation, regardless of passage of the endotracheal tube. We have used this definition of attempt because it is the laryngoscope being in the mouth that causes many of the adverse effects associated with intubation such as bradycardia and hypertension. Our success rates appear lower than many others cited because we include every attempt in the calculation of success rate as opposed to the determination that an operator successfully intubated a patient irrespective of the numbers of attempts as has been reported by others.

Although this experience is from a single institution, our results are consistent with the data reported in a recent review of neonatal intubation skills by pediatric residents in another single institution over a 3-year period. Falck et al defined competence as success at intubation on the first or second attempt ≥80% of the time and reported that none of the trainee levels who participated in the study were deemed competent by that definition. We reviewed our data using their definition and found similar success rates: for PL-1 residents, UCSD = 49.6%, Falck = 50%; for PL-2 and PL-3 residents, UCSD = 67.4%, Falck, PL-2 = 55% and PL-3 = 62%.

Since 1996, our residents have not completed more than 20 attempts at intubation throughout training. Investigators in anesthesia have shown that 40 or more intubations are required to become proficient. de Oliveira Filho used the cumulative sum method to create learning curves for basic procedures. An “acceptable failure rate” of 20% on the first attempt was set as an appropriate level of proficiency. Only half of the participating anesthesia residents achieved this level of proficiency after an average of 43 attempts. Konrad et al trained nonanesthesia students without previous intubation experience how to intubate and recorded their subsequent progress. Using statistical modeling, it was found that trainees had a 90% probability of being successful after 47 attempts. Konrad et al created learning curves for anesthetic procedures and found that success rates for intubation were 90% after a mean of 57 procedures. After training on manikins, emergency medical technicians have been about 50% successful at intubating adult patients in the field.

We document a significant reduction in the number of intubations for meconium in the delivery room consistent with the revised Neonatal Resuscitation Program guidelines. The major reduction in numbers of intubation opportunities occurred after the first 2 years of our review, and the numbers of intubations remained relatively constant thereafter. This difference after the first 2 years is related to the changes in meconium practices. We have also had a reduction in the amount of time spent in the NICU by pediatric trainees coinciding with the change in RRC guidelines. Together, these changes have decreased the opportunities available for pediatric trainees to learn intubation and are reflected in lower success rates. The neonatal unit alone, in our experience, provides an inadequate exposure to intubation for pediatric residents to become competent. The same level of proficiency expected of anesthesiologists and neonatologists should not necessarily be applied to pediatric residents. However, if a higher level of proficiency is desired than is currently being achieved, additional training opportunities are necessary. Other areas such as the operating room, emergency room, and pediatric intensive care unit may add to a trainee’s experience.

**CONCLUSIONS**

Pediatric trainees are currently provided inadequate experience to allow development of proficiency at neonatal intubation. The determination of competency at intubation for general pediatricians requires further consideration.
The authors thank Ellen Knodel, RRT, and Sheryl Zimmerman, RRT, for their efforts in creating and maintaining the neonatal intubation database.

REFERENCES

OBJECTIVES  To examine the effect of supplemental glutamine (0.6 g kg\(^{-1}\) d\(^{-1}\)) on whole body protein/nitrogen and glutamine kinetics in low birth weight (LBW) infants receiving parenteral nutrition in the immediate neonatal period.

STUDY DESIGN  Premature infants \(\leq 32\) weeks gestation with a birth weight from 694 to 1590 g were randomly assigned to either a glutamine-supplemented group (n = 10) or to a control group (n = 10). Tracer isotope studies were performed when the infants were 6 to 7 days old and had been receiving an amino acid intake of approximately 3.0 g kg\(^{-1}\) d\(^{-1}\) for at least 3 days. Whole body glutamine and nitrogen kinetics were measured with [5-\(^{15}\)N]glutamine, [\(^{2}\)H\(_5\)]phenylalanine, [1-\(^{13}\)C,\(^{15}\)N]leucine, \([^{15}\text{N}_2]\)urea, and GC-mass spectrometry.

RESULTS  Supplemental glutamine was associated with a lower rate of appearance of glutamine (\(P = .003\)), phenylalanine (\(P = .001\)), and leucine C (\(P = .003\)). There was no significant difference in leucine N turnover, urea turnover and plasma cortisol, and C-reactive protein levels in the 2 groups.

CONCLUSION  Parenteral glutamine supplement in LBW infants was associated with lower whole-body protein breakdown. Because the decrease in whole body proteolysis is associated with protein accretion, parenteral glutamine supplement may be beneficial in selected populations of LBW infants.

The improvements in the care of respiratory disease of low birth weight babies and the resulting decrease in mortality rate has made nutritional management in these infants a major challenge in clinical practice.\(^1\) Data from large studies have shown that despite the best efforts, low birth weight (LBW) babies, particularly those weighing <1200 g, are significantly growth-retarded by the time they reach a corrected gestational age or post-conceptional age of 40 weeks.\(^2\)

Several nutritional intervention strategies have been proposed and evaluated for the clinical treatment of LBW infants. These include early aggressive administration of parenteral nutrition with increased amounts of amino acids or the early administration of enteral feeds. Although some of these studies have suggested clinical benefits, the data have been generally equivocal.\(^3,4\)

Glutamine, a non-essential amino acid, is the most abundant amino acid in the blood and in the free amino acid pool in the body. It is synthesized virtually by every tissue, although only certain tissues (skeletal muscle, brain, and lung) release it into the circulation in significant quantities.\(^5\) Glutamine plays an important role in the inter-organ shuttle of nitrogen and carbon and is a primary oxidative fuel for dividing cells such as enterocytes and lymphocytes. Because of its critical role in a number of physiological systems, and because there is a rapid depletion of whole body glutamine pools during acute illness, trauma, and burns, glutamine has been studied extensively as a nutrient supplement, particularly during acute illness.\(^6-9\) Although data from studies of adults that show improvement in clinical and physiological parameters are compelling,\(^8,9\) the few data from LBW infants have been inconclusive.\(^10-13\) In this study, we have examined the impact of supplemental glutamine on whole-body nitrogen/protein and glutamine kinetics in carefully selected LBW infants.

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**Table:**

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tr>
<td>BCAA</td>
<td>Branched chain amino acids</td>
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<tr>
<td>CRP</td>
<td>C-reactive protein</td>
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<tr>
<td>KIC</td>
<td>(\alpha)-ketoisocaproic acid</td>
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<tr>
<td>LBW</td>
<td>Low birth weight</td>
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<tr>
<td>Ra</td>
<td>Rate of appearance</td>
</tr>
<tr>
<td>TPN</td>
<td>Total parenteral nutrition</td>
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</table>
Preterm infants (n = 20) born at <32 weeks gestation and weighing between 694 and 1590 g were recruited for study. They were randomly assigned to either the control group (C; n = 10) or the glutamine group (G; n = 10). There was no difference in their severity of illness, as determined using the Score for Neonatal Acute Physiology (SNAP; C, 14.1 ± 4.7 and G, 13.7 ± 5.1).14 All decisions to adjust the clinical support of the infants were made by their primary physicians. The investigators were not responsible for the clinical care of the infants. All infants were placed on parenteral nutrition between day 1 and 2 after birth, and the tracer isotope studies were performed on day 6 or 7, when the infants had been on protein intake of approximately 3.0 g.kg\(^{-1}\).d\(^{-1}\) for approximately 3 days (Table I). Their average nutrient and energy intake was: Protein, C group 2.5 ± 0.8 g.kg\(^{-1}\).d\(^{-1}\), G group 3.2 ± 1.1 g.kg\(^{-1}\).d\(^{-1}\) (2 infants in the group C received 1.7 and 1.4 g.kg\(^{-1}\).d\(^{-1}\); the average daily intake of the remaining 8 was 3.2 ± 0.3 g.kg\(^{-1}\).d\(^{-1}\)). Energy, C group 73.6 ± 24.8 kcal.kg\(^{-1}\).d\(^{-1}\), G group 70.5 ± 24.4 kcal.kg\(^{-1}\).d\(^{-1}\). Both groups received similar amounts of intravenous glucose, approximately 8 to 9 mg.kg\(^{-1}\).min\(^{-1}\) and lipids 0.5 to 0.8 g.kg\(^{-1}\).d\(^{-1}\). The variance in the parenterally administered nutrition was primarily the consequence of the need to adjust the volume of intravenous fluid for clinical needs. All infants received supplemental oxygen via nasal cannula, nasal continuous positive airway pressure, or ventilatory support. In addition, 5 infants in each group received antibiotics and aminophylline. Results of blood culture tests were negative in all infants. There was no difference in the 2 groups in clinical support. The blood gases and oxygen saturation of the infants were within acceptable ranges for our nursery. The protocol was approved by the institutional review board. Written informed consent was obtained from the mother or both parents (when available) before the study.

### Methods

Glutamine supplement (Trophamine 10%, McGaw) was used for these studies. The G group was supplemented with 0.6 g.kg\(^{-1}\).d\(^{-1}\) of intravenous glutamine. To maintain an isonitrogenous intake, the parenteral amino acid mixture was adjusted by adding a corresponding amount. The amino acid composition and the rate of delivery of individual amino acids in the parenteral mixtures are displayed in Table II. The glutamine-supplemented Trophamine was purchased from and prepared by Central Admixture Pharmacy Services (CAPS, Garden Grove, Calif) with an investigational drug authorization from the Food and Drug Administration (IND #60,909).

### Isotopic Tracer Protocol

After 3 to 5 days of parenteral nutrition (with or without glutamine supplement), tracer studies were performed while the infants were receiving the amino acid mixture continuously. L-[1\(^{-13}\)C\(^{15}\)N]leucine (99% \(13\)C,15N), [\(^{2}\)H\(_{5}\)]phenylalanine (98% \(^{2}\)H), L-[5-\(^{15}\)N]glutamine (99% \(^{15}\)N), and [\(^{15}\)N\(_{2}\)]urea (99% \(^{15}\)N) were purchased from Isotec (Miamisburg, Ohio). Weighed amounts of isotopic tracer were mixed in 0.45% NaCl, and sterilized with Millipore filtration as described previously.15-17 The tracer solution was infused at 3.0 mL/h for 5 hours. The tracers were administered as prime-constant rate infusions as: L-[1\(^{-13}\)C,\(^{15}\)N]leucine prime 7.5 \(\mu\)mol.kg\(^{-1}\), constant infusion 7.5 \(\mu\)mol.kg\(^{-1}\).h\(^{-1}\); L-[5-\(^{15}\)N]glutamine prime 30 \(\mu\)mol.kg\(^{-1}\), constant infusion 30 \(\mu\)mol.kg\(^{-1}\).h\(^{-1}\); [\(^{15}\)N\(_{2}\)]urea prime 33 \(\mu\)mol.kg\(^{-1}\), constant infusion 3.3 \(\mu\)mol.kg\(^{-1}\).h\(^{-1}\); and [\(^{2}\)H\(_{5}\)]phenylalanine prime 6 \(\mu\)mol.kg\(^{-1}\), constant infusion 4 \(\mu\)mol.kg\(^{-1}\).h\(^{-1}\). The tracer amino acid solution was piggy-backed to the parenteral amino acid line.

Blood samples were obtained during isotopic steady state at between 165 and 300 minutes of tracer infusion. The volume of blood drawn was adjusted to the infant’s weight and limited to a maximum of 5% of the infant’s blood volume, estimated to be 80 mL.kg body weight\(^{-1}\). Blood was mixed with cold trichloracetic acid (10% TCA), centrifuged, and the separated supernatant was stored at –70°C for later analysis. An aliquot of plasma was also separated and stored. All blood samples were drawn from an indwelling (umbilical artery) line already in place for clinical reasons.

### Analytical Procedures

All the analytical procedures used by this laboratory have been previously described.15-17 Plasma cortisol levels were measured with a commercial radioimmunoassay kit (Coat-A-Count, DPC; Los Angeles, Calif). The concentration of CRP in the plasma was measured with a high-sensitivity enzyme-linked immunosorbent assay (Diagnostic Systems Laboratory; Webster, Tex).

### Calculations

The total rates of appearance (Ra) of leucine, phenylalanine, glutamine, and urea were calculated with tracer dilution using steady state kinetics. Ra = I x ([Ei/Ep] – 1), in

<table>
<thead>
<tr>
<th>Table I. Clinical characteristics</th>
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<tr>
<td>Birth weight (grams)</td>
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<tr>
<td>------------------------</td>
</tr>
<tr>
<td>Group C (n = 10)</td>
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<tr>
<td>Group G (n = 10)</td>
</tr>
</tbody>
</table>

*P* = NS for all comparisons with the Wilcoxon rank sum test.
which I is the rate of infusion of the tracer (\(\mu\text{mol.kg}^{-1}\text{.h}^{-1}\)), \(E_i\) is the isotopic enrichment of tracer infused, and \(E_p\) is the enrichment of plasma amino acid or urea at steady state. The coefficient of variation for the tracer enrichment data in the plasma during isotopic steady-state in individual babies was between 3% and 5%, and the slope was not different from 0. Enrichment data obtained between 210 and 300 minutes were used for calculations. Leucine carbon flux was calculated with \(^{13}\text{C} \) enrichment of plasma KIC, whereas leucine N flux was calculated with \(^{15}\text{N} \) enrichment, \([^{13}\text{C},^{15}\text{N}]\), of plasma leucine.\(^{15}\) The endogenous rates of appearance of glutamine and phenylalanine were calculated by subtracting the exogenous rate of infusion of the respective amino acid from total Ra.

The contribution of glutamine N to urea nitrogen was calculated from the \(^{15}\text{N} \) enrichment of urea during isotopic steady state with a precursor-product relationship. As discussed previously,\(^{16}\) the \(^{15}\text{N} \) enrichment of urea mostly represents the incorporation of amide \(^{15}\text{N} \) of glutamine into urea, with minimal or no contribution by reincorporation of \(^{15}\text{N} \) from infused \(^{15}\text{N}_2\)urea.

### Statistical Analysis

In addition to descriptive statistics, groups were compared with both the 2-sample \(t\) test and Wilcoxon rank sum test. Data are presented as means plus or minus SD. A \(P\) value < .05 (2-tailed) was considered to be significant. Spearman Correlations were calculated for linear regression analysis. Statistical analyses were performed with commercial software (Statistix 7.0; Analytical Software, Tallahassee, Fla).

### RESULTS

Infants in the C and the G groups did not differ in mean birth weight, gestational age, or weight and age at the time of the tracer isotope study (Table I). The concentrations of cortisol and CRP in plasma, measured as an index of stress, were not different in the 2 groups.

There was no significant difference in the acid-base status of the C or G groups. The blood ammonia levels were also not different (C, 50.8 ± 13.3 mg/dL; G, 58.5 ± 4.4 mg/dL). The blood urea N of the G group (30.0 ± 4.2 mg/dL) was slightly higher (\(P\) = not significant [NS]) than that of the C group (26.2 ± 3.3 mg/dL).

Plasma amino acid concentrations are shown in Table III. Parenteral amino acid mixture that was supplemented with glutamine and administered for 3 to 5 days did not cause significant differences in any of the plasma amino acid levels nor in the concentration of total amino acid in plasma (C, 2422 ± 650 \(\mu\text{mol.L}^{-1}\); G, 2127 ± 423 \(\mu\text{mol.L}^{-1}\); \(P\) = NS).

The effects of supplemental glutamine on whole body phenylalanine, glutamine, and urea fluxes are displayed in Table IV. The total Ra of phenylalanine was significantly (\(P = .005\)) lower in the G group. The endogenous rate of appearance of phenylalanine (endogenous = Total Ra – rate of infusion of phenylalanine in the TPN) was also significantly (\(P = .001\)) lower in the G group, which suggests a lower rate of

### Table II. Amino acid composition and rate of delivery of parenteral amino acid mixtures

<table>
<thead>
<tr>
<th>Amino Acid</th>
<th>C group</th>
<th>G group</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g.100 mL</td>
<td>g.kg(^{-1}).d(^{-1})</td>
</tr>
<tr>
<td>Isoleucine</td>
<td>0.82</td>
<td>0.246</td>
</tr>
<tr>
<td>Leucine</td>
<td>1.40</td>
<td>0.421</td>
</tr>
<tr>
<td>Lysine</td>
<td>0.82</td>
<td>0.246</td>
</tr>
<tr>
<td>Methionine</td>
<td>0.34</td>
<td>0.102</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>0.48</td>
<td>0.144</td>
</tr>
<tr>
<td>Threonine</td>
<td>0.42</td>
<td>0.126</td>
</tr>
<tr>
<td>Tryptophan</td>
<td>0.20</td>
<td>0.060</td>
</tr>
<tr>
<td>Valine</td>
<td>0.78</td>
<td>0.234</td>
</tr>
<tr>
<td>Histidine</td>
<td>0.48</td>
<td>0.144</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>0.24</td>
<td>0.072</td>
</tr>
<tr>
<td>Alanine</td>
<td>0.54</td>
<td>0.162</td>
</tr>
<tr>
<td>Arginine</td>
<td>1.20</td>
<td>0.361</td>
</tr>
<tr>
<td>Proline</td>
<td>0.68</td>
<td>0.204</td>
</tr>
<tr>
<td>Serine</td>
<td>0.38</td>
<td>0.114</td>
</tr>
<tr>
<td>Glycine</td>
<td>0.36</td>
<td>0.108</td>
</tr>
<tr>
<td>Aspartic acid</td>
<td>0.32</td>
<td>0.096</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>0.50</td>
<td>0.150</td>
</tr>
<tr>
<td>Taurine</td>
<td>0.025</td>
<td>0.008</td>
</tr>
<tr>
<td>Glutamine</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>9.99</td>
<td>3.00</td>
</tr>
</tbody>
</table>

Cysteine was added as cysteine hydrochloride, 30 mg/g protein, to both control and glutamine supplemented solutions.
protein turnover and protein breakdown. The total Ra of glutamine was not significantly different in the 2 groups. However, when corrected for exogenous glutamine infusion, the endogenous Ra of glutamine was significantly lower \( (P = .003) \) in the G group.

The rate of urea synthesis was slightly higher \( (P = NS) \) in the infants who received glutamine supplementation.

Glutamine supplementation resulted in a lower rate of turnover of leucine N \( (P = .003; \text{Table V}) \). The Ra of leucine C was not different in the 2 groups. The fractional contribution of glutamine N to urea N was approximately 10% of urea N turnover and was not different in the 2 groups.

**Correlation**

There was a significant positive correlation between the Ra of endogenous glutamine and total Ra of phenylalanine \( (r = 0.755, P = .0007) \), endogenous glutamine and endogenous phenylalanine \( (r = 0.747, P = .001) \), and leucine N \( (r = 0.803, P = .0002) \), and leucine C \( (r = 0.553, P = .03) \). There was no correlation between plasma glutamine levels and the rate of appearance of total or endogenous glutamine. These correlations remained significant when the data in each group were analyzed separately.

**DISCUSSION**

Our data show that in LBW infants, parenteral glutamine supplementation results in a decrease in the Ra of phenylalanine and leucine C and therefore a lower rate of whole-body proteolysis. The rate of turnover of leucine N was also lower in the G group, suggesting a lower rate of BCAA transamination. In addition, glutamine supplementation was also associated with a lower endogenous rate of appearance of glutamine.

These data demonstrate a decrease in the whole body rate of proteolysis \( (r = 0.755, P = .0007) \), endogenous glutamine and endogenous phenylalanine \( (r = 0.747, P = .001) \), total Ra of phenylalanine \( (r = 0.803, P = .0002) \), and leucine N \( (r = 0.553, P = .03) \). These correlations remained significant when the data in each group were analyzed separately.

Parenteral glutamine in a dose of approximately 0.4 g.kg\(^{-1}\).d\(^{-1}\) for an average of 14 days did not have any significant impact on plasma glutamate or ammonia levels. The concentration of glutamine in plasma was slightly increased, whereas urea nitrogen levels were found to be higher in some infants. Similar data have been reported by other investigators in their studies of LBW infants.

The rationale for glutamine supplementation is based on the observation of a rapid depletion of glutamine pools in response to acute stress, infection, trauma, and burns.8,9 The mechanism responsible for this decrease has been attributed to a decreased rate of glutamine synthesis or to a rapid rate of transport of glutamine out of tissues.10 Rennie et al10 suggested that an increase in the intramuscular sodium concentration, an increase in blood cortisol, adrenaline, or glucagon, a decrease in plasma insulin, or exposure to endotoxin will result in a net outflow of glutamine and a fall in the intramuscular concentration. The lower concentrations of cortisol in the plasma of the G group in our study may have contributed to the observed effect by increasing the inflow of glutamine.

Experimental depletion of glutamine in humans by either phenylbutyrate20 or by inhibition of glutamine synthase with methionine sulfoximine in animal studies21-23 did not have any effect on the whole body rate of appearance of leucine, glutamine, or phenylalanine, which suggests a lack of any effect on skeletal muscle protein turnover. In contrast, a significant decrease in the hepatic release of proteins and the oxidation of KIC was observed when isolated rat livers were
perfused with a glutamine deficient medium. 22 A similar decrease in whole body oxidation of leucine was observed as a result of a phenylbutyrate-induced decline in plasma glutamine in healthy adults. 21 In contrast to these in vivo studies, a positive relationship between intramuscular concentration of glutamine and protein synthesis was shown by MacLennan et al 24,25 using isolated perfused rat gastrocnemius muscle. In addition, glutamine significantly inhibited protein breakdown and net protein loss in skeletal muscle. The addition of insulin enhanced protein synthesis, but it did not appear to have an additional effect on the glutamine-induced decrease of protein breakdown. 25 Similar correlations between the levels of tissue glutamine and protein synthesis in skeletal muscle in protein deficient, starved, and endotoxemic rats have been reported. 26 Other groups did not find a significant relationship between intramuscular glutamine levels and protein turnover. These differences are probably related to the methods used to reduce glutamine levels (eg, use of a glutamine synthase inhibitor), the type of perfusion system used, or the method used to quantify protein synthesis. Nonetheless, the strong correlation among glutamine levels and rate of protein turnover in skeletal muscle and alterations in hepatic protein metabolism with glutamine depletion in humans have provided a rationale for the clinical studies aimed at improving the physiological and clinical parameters in acutely sick patients.

Glutamine administration in LBW infants resulted in a lower rate of appearance of phenylalanine, which suggests a lower rate of whole-body and specifically skeletal muscle protein breakdown. There was no significant effect on the rate of turnover of leucine C, which is similar to that observed by Des Robert et al, 27 who also showed a minimal impact of acute supplementation of glutamine (0.5 g.kg 1.d -1 for one day) in a small group of LBW infants. The lack of any effect on leucine C kinetics may be caused by the lack of significant impact on leucine metabolism in splanchnic compartments, the major site of perturbation of protein metabolism in experimentally induced glutamine depletion. 21-23

The translation of these data into clinical practice remains controversial. Previous studies in LBW infants have examined only the clinical outcome in these populations. Lacey et al 10 demonstrated that, in very sick infants, parenteral glutamine resulted in a shorter length of hospital stay, shorter duration of ventilation, and rapid transition to enteral feeds. Thompson et al 11 also showed early transition to enteral feedings and a shorter length of hospital stay in infants who received glutamine supplementation. In contrast, a large multicenter study of LBW infants failed to show any impact of glutamine supplementation on the risk of late-onset sepsis, death, or necrotizing enterocolitis. 12,28 In this study, however, the infants did not receive the targeted protein and glutamine intake (3 g.kg 1.d -1 ) until 10 days of age, by which time acute perturbations in glutamine metabolism may have ameliorated. Thus, there may be an optimal period during acute illness when the requirements of glutamine are high and supplemental glutamine may be of benefit.

It is important to emphasize that data from studies with adults and with newborn babies have provided compelling evidence for the compartmental metabolism of glutamine administered via the enteral or parenteral route. 17,29 We
recently reported that in growing LBW infants glutamine administered via the enteral route is entirely metabolized in the splanchnic compartment. Thus, the clinical and physiological data of supplemental glutamine administered via the enteral route cannot be compared with that given by the parenteral route. Finally, as shown here and by Hankard et al in healthy adults, glutamine administration resulted in a lower rate of appearance of glutamine in the blood as a result of a lower rate of de novo synthesis of glutamine. The mechanism of this decrease is unclear and may be the consequence of down-regulation of glutamine synthesis by glutamine.

In summary, in a selected population of clinically stable LBW infants, parenteral amino acid nutrition supplemented with glutamine resulted in a decrease in the whole-body rate of protein breakdown and suppressed endogenous rate of glutamine synthesis. These data suggest that supplemental parenteral glutamine may be of physiological/clinical benefit in these LBW infants.

The authors thank the staff of the General Clinical Research Center at MetroHealth Medical Center, Cleveland, Ohio, for assistance in the conduct of these studies, and Mrs Joyce Nolan for secretarial assistance.

REFERENCES


INITIAL PRESENTATION OF CHILDHOOD-ONSET SYSTEMIC LUPUS ERYTHEMATOSUS: A FRENCH MULTICENTER STUDY

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Objective To describe the clinical and laboratory manifestations of childhood-onset systemic lupus erythematosus (SLE) at presentation.

Study design This retrospective French multicenter study involved 155 patients in whom SLE developed before the age of 16 years. Mean patient age at onset was 11.5 ± 2.5 years (range, 1.5-16 years). The female to male ratio was 4.5.

Results The most common initial manifestations were hematologic (72%), cutaneous (70%), musculoskeletal (64%), renal (50%), and fever (58%). Thirty-two percent of children had atypical symptoms, mainly including abdominal involvement in 26 patients, which lead to negative laparotomy results for presumed appendicitis. Severe renal, neurologic, hematologic, abdominal, cardiac, pulmonary, thrombotic, and/or cutaneous manifestations occurred within the first month after the diagnosis in 40% of patients. The mean erythrocyte sedimentation rate was 72 ± 29 mm/h, and the mean C-reactive protein value 22 ± 21 mg/L. Antinuclear antibodies an, anti-double stranded DNA antibodies, and low C3 or C4 level were retrieved in 97%, 93%, and 78% of patients, respectively.

Conclusion Initial manifestations of childhood-onset SLE are diverse and often severe. The diagnosis of SLE should be promptly considered in any febrile adolescent with unexplained organ involvement, especially when associated with an increased erythrocyte sedimentation rate. (J Pediatr 2005;146:648-53)
questionnaire was sent to 89 pediatric centers of French university hospitals (28 general pediatrics, 23 nephrology, 13 rheumatology, and 25 hematology). It included questions on the family history, age at onset and at diagnosis of SLE, sex, ethnicity, and an exhaustive list of clinical and laboratory examinations performed at disease onset. The initial presentation of the disease included events recorded within the first month after diagnosis. The first laboratory and imaging testing available at presentation were reported, including complete blood cell count, erythrocyte sedimentation rate (ESR), urinalysis, serum creatinine level, complement level (CH50, C3, C4), antiphospholipid antibodies, specific auto-antibodies comprising antinuclear antibodies (ANA), anti-double-stranded DNA (anti-dsDNA), anti-SSA, anti-SSB, anti-Sm, and anti-RNP antibodies. Other requested information included results of renal biopsy, chest radiography, echocardiography, and ophthalmoscopic examination. The criteria for renal involvement were proteinuria (>0.5 g/d) or cellular casts in urinalysis. Renal biopsies were classified according to the classification proposed by the World Health Organization (WHO): type I, normal; type II, mesangial glomerulonephritis; type III, focal proliferative glomerulonephritis; type IV, diffuse proliferative glomerulonephritis; and type V, membranous nephropathy. The criteria for hematologic involvement were hemolytic anemia, thrombocytopenia (<100 × 10⁹/L), leukopenia (<4 × 10⁹/L), and/or lymphopenia (<1.5 × 10⁹/L). Musculoskeletal manifestations included arthritis, arthralgia, and/or myalgia. ANA titer equivalent or greater than 1:80 was considered significant.

We used the events recorded within the first month after diagnosis to define initial presentation of the disease. Children whose disease required >0.5 mg/kg/d of prednisone within 6 months of initial diagnosis, who experienced a life-threatening organ dysfunction at onset, or both were regarded as having severe-onset disease.

Statistical analysis was performed with the chi-square test. A statistical difference was considered to be significant when the P value was < .05.

RESULTS

Patients

The pediatric centers’ response rate to our SLE questionnaire was 73% (65/89). Thirty-three centers included at least 1 patient and are listed at the end of this paper. Responses originated from nephrology units (56%, 13/23), rheumatology units (77%, 10/13), hematology units (8%, 2/25), and general pediatrics units (28%, 8/28). The remaining 32 centers included hematology and general pediatrics units that answered that they did not deal with patients with SLE. A total of 155 patients were included, 70 of whom (45.2 %) were initially referred to a pediatric nephrologist, 43 (28%) to a pediatric rheumatologist, 35 (22.6%) to a general pediatrician, 5 (3.2%) to a pediatric hematologist, and 2 to other specialists. Eighteen patients experienced incomplete SLE (ie, less than 4 revised ACR criteria at onset) and had autoimmune cytopenia, rheumatic complaints, or nephropathy associated with ANA and/or anti-dsDNA antibodies. Four of them still had only 3 ACR criteria by the end of the study, but were included because of the presence of lupus nephritis. In the remaining 14 patients, including 7 children with autoimmune thrombocytopenic purpura, hemolytic anemia associated with ANA and anti-dsDNA antibodies, or both, complete SLE developed during the course of the study: skin involvement, joint involvement, or both developed in 8; type III or IV nephropathy developed in 4; pericarditis developed in 2; and pleuritis developed in 1. The mean interval for completion of disease was 3.5 years (range, 3 months-8 years).

The median age at onset was 12 years (mean, 11.5 ± 2.5; range, 1.5-16.0), with SLE developing in 83% after the age of 8 years (Figure 1). The female-to-male ratio was 4.5:1 and was not influenced by the age of onset (age <12 years, 5.1:1; older children, 4.1:1). In children from multiplex SLE families, the median age at onset was 12.5 years, and the female-to-male ratio was 4:1, which was not significantly different from the age and sex ratio in sporadic cases. Forty-six percent (71/155) of patients were white, 31 % were black (48/155), 20% (31/155) were North African, and 3% (5/155) were Asian. The mean time interval between onset and diagnosis was 2.8 months (range, 0-24 months).

Comorbidities in 11 patients included sickle cell disease (2), Williams–Beuren syndrome (1), celiac disease (1), and Immunoglobulin A deficiency (1). Seven of 30 patients tested (20%) had inherited heterozygous C4 deficiency. Prothrombotic genetic factors were identified in 2 children who had thrombophlebitis and were related to prothrombin gene mutation and methyltetrahydrofolate reductase gene mutation.

A history of autoimmune disease was present in 40 unrelated families (26%) and included SLE in 13 non-consanguineous families (8.4%). The relationship between affected members of SLE multiplex families was parent-offspring pairs (47% of all the pedigrees), second degree (47%), and siblings (6%). Three families had more than 2 affected members. No inherited complement deficiency was ascertained in these familial cases of SLE. Other familial autoimmune diseases included rheumatoid arthritis (8 families), autoimmune thyroiditis (5 families), type 1 diabetes

Figure 1. Age at onset in 155 children with SLE.
mellitus (7 families), chronic autoimmune thrombocytopenia (2 families), and Crohn’s disease, autoimmune chronic hepatitis, Raynaud’s phenomenon, myasthenia gravis, and multiple sclerosis (1 family each); they mainly occurred in the maternal family (63%) and affected second-degree relatives (55%), parents (44%), and siblings (1%).

**Initial Manifestations**

The overall incidence rate of clinical features is presented in Figure 2. Hematologic, cutaneous, and musculoskeletal signs were the most common. The cutaneous manifestations typically included malar rash and vasculitis, although a variety of other lesions were also observed (Table I). Arthritis and non-erosive arthritis mostly affected the knees (48/95 children), fingers (34/95 children), and wrists and ankles (28/95 children each), whereas the shoulders (9/95 children), toes (5/95 children), cervical spine (4/95 children), and sacroiliac joint (1 child) were rarely involved. Abnormal hematologic findings included autoimmune cytopenia and coagulation factor deficiency (Table II). Renal disease was present at onset in 81 patients (50%), including proteinuria (>0.5 g/d; 73 patients), cellular cast cells (38 patients), and renal failure (26 patients). Forty of the 67 children (60%) who underwent renal biopsy had WHO class III-IV histology, 15 had class I-II (22%), 8 had class V (11%), and 4 (6%) could not be classified according to the WHO classification. Renal disease was present at onset in 81 patients (50%), including proteinuria (>0.5 g/d; 73 patients), cellular cast cells (38 patients), and renal failure (26 patients). Forty of the 67 children (60%) who underwent renal biopsy had WHO class III-IV histology, 15 had class I-II (22%), 8 had class V (11%), and 4 (6%) could not be classified according to the WHO classification. Two children had hemolytic uremic syndrome (HUS). The incidence of severe type III or IV nephropathy did not significantly differ among white (17.5%), black (27%), or North African (16%) children, but was significantly higher in Asian children (75%; P < .01) when compared with other groups. Neurologic symptoms developed in 27 patients (17%), including headache (16/27), mood disorder (8/27), seizures (6/27), cerebrovascular disease (3/27), and chorea (1/27). The clinical presentation was not different in patients younger than 12 years compared with older patients (Figure 2).

A variety of atypical features were observed in 49 patients (32%; Table III) and were associated only with fever in 7 of them. They mainly consist of abdominal involvement in 26 patients (17%), ocular involvement, and parotitis. Twenty-one of these patients experienced abdominal pain, which led in 3 cases to an appendectomy before the diagnosis of SLE could be established; abdominal pain was related to pancreatitis (6/21 children), intestinal pseudo-obstruction (2 children), cholecystitis (1 child), and lupus peritonitis (1 child); no cause was demonstrated in the remaining children.

The median value of ESR was 71 (mean, 72 ± 29; range, 2-150) and was <40 in only 17% of patients. The median value of C-reactive protein (CRP) was 10 mg/L (mean, 22 ± 21; range, <6-200); an increase in CRP level to >100 mg/L was found in only 2 children, one with gangrene of fingers and one with severe polyseritis. The ANA were positive in 97% of the patients; the mean ANA titer was 1:1280 and was >1:640 in all patients except 15. All 4 children who were ANA-negative had at least a malar rash associated with several mild manifestations. All 4 children who were ANA-negative harbored anti-ds DNA antibodies; 2 of them had complete SLE, whereas the other 2 had only malar rash associated with several mild manifestations. Different methods of detection were used for Anti-dsDNA antibodies (enzyme-linked immunosorbent assay, 80%; Crithidia lucilae assay, 21%; Farr assay, 14%), which accounted for 93% of positivity. The other main immunologic findings are presented in Table II.

**DISCUSSION**

In this French retrospective cross-sectional multicenter study of childhood-onset SLE, we found that malar rash, arthritis, and fever were the most common manifestations at presentation. In addition, we underlined the high frequency of renal manifestations in the pediatric population, although it is underestimated in this series because only 56% of
nephrology units included patients. The respective frequency of other manifestations varied among these previously published series, because of marked selection biases, depending on their size, the patients' ethnicity, and the nature of patient recruitment. This retrospective study does not provide a true estimate of these prevalences. However, we emphasized the high frequency of atypical manifestations, whereas other studies focused on classical manifestations only.

One third of our patients had non-classical manifestations at presentation, especially abdominal involvement. SLE can involve any part of the gastrointestinal tract, with oral lesions, esophageal dysmotility, mesenteric vasculitis, protein-losing enteropathy, and pancreatitis the most frequent manifestation in adults. Abdominal symptoms have been reported in some pediatric case-reports. In this series, acute abdominal SLE was related to pancreatitis in approximately one third of cases. Pancreatitis has been reported in 3% to 8% of adult patients, usually in the setting of a generalized SLE flare or as a presenting manifestation, and requires urgent management. The underlying pathophysiology is unknown, but microthrombi, vasculitis, intimal thickening, and drug toxicity are felt to play a role. Intestinal pseudo-obstruction, a rare and recently recognized SLE complication, occurred in 2 patients; it is presumably caused by an intestinal smooth muscle dystmotility of neuropathic or myogenic pathology, which may or may not be caused by vasculitis. We reported a variety of other non-classical features that included mainly parotitis and ocular involvement. Thus, we suggest that SLE should be promptly considered in the differential diagnosis of an adolescent with unexplained organ involvement associated with fever and increased ESR.

Initial manifestations were severe in 40% of our patients (Table IV). We recognize that determination of disease severity on the basis of treatment required may be biased by differences in therapeutic strategies in the different centers; however, we believe that this approach has validity because patients with severe disease, such as active nephritis, would be treated with steroids. SLE onset has been reported to be more severe in children than in adults, especially because of a higher frequency of active nephritis. Our sample probably does not over-represent severe disease, because the subjects included outpatients and inpatients and because of the moderate rate of

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**Table II. Laboratory findings in 155 children with systemic SLE at presentation**

<table>
<thead>
<tr>
<th>Findings</th>
<th>Number of patients/ Number of patients tested</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hematologic</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anemia (Hb &lt;120 g/dL)</td>
<td>42/155</td>
<td>27</td>
</tr>
<tr>
<td>Coomb's test positivity</td>
<td>56/114</td>
<td>49</td>
</tr>
<tr>
<td>Lymphopenia</td>
<td>38/155</td>
<td>24</td>
</tr>
<tr>
<td>(ALC &lt;1.5 × 10⁹/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Leukopenia (WBC &lt;4 × 10⁹/L)</td>
<td>55/155</td>
<td>35</td>
</tr>
<tr>
<td>Neutropenia (ANC&lt;1.5 × 10⁹/L)</td>
<td>18/155</td>
<td>12</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>44/155</td>
<td>28</td>
</tr>
<tr>
<td>(&lt;150 × 10⁹/L)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiprothrombin antibodies</td>
<td>2/155</td>
<td>1</td>
</tr>
<tr>
<td>ESR (mean value, mm)</td>
<td>72</td>
<td></td>
</tr>
<tr>
<td>CRP (mean value, mg/L)</td>
<td>22</td>
<td></td>
</tr>
<tr>
<td>Serology</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANA (≥1:80)</td>
<td>151/155</td>
<td>97</td>
</tr>
<tr>
<td>ANA (≥1:640)</td>
<td>141/155</td>
<td>91</td>
</tr>
<tr>
<td>ANA (≥1:1280)</td>
<td>84/155</td>
<td>55</td>
</tr>
<tr>
<td>Anti-dsDNA</td>
<td>144/155</td>
<td>93</td>
</tr>
<tr>
<td>Anti-RNP</td>
<td>36/101</td>
<td>35</td>
</tr>
<tr>
<td>Anti-Sm</td>
<td>33/101</td>
<td>32</td>
</tr>
<tr>
<td>Anti-SSA</td>
<td>34/102</td>
<td>33</td>
</tr>
<tr>
<td>Anti-SSB</td>
<td>20/103</td>
<td>19</td>
</tr>
<tr>
<td>LAC</td>
<td>35/119</td>
<td>29</td>
</tr>
<tr>
<td>ACL</td>
<td>54/114</td>
<td>47</td>
</tr>
<tr>
<td>Complement</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low C3 level</td>
<td>93/120</td>
<td>77</td>
</tr>
<tr>
<td>Low C4 level</td>
<td>94/120</td>
<td>78</td>
</tr>
<tr>
<td>Low CH50 level</td>
<td>81/109</td>
<td>74</td>
</tr>
<tr>
<td>Inherited complement deficiency</td>
<td>7/35</td>
<td>20</td>
</tr>
</tbody>
</table>

ACL = Anticardiolipin antibodies; WBC = white blood cell; ALC = absolute lymphocyte count; ANC = absolute neutrophil count.

**Table III. Non-classical initial manifestations of childhood-onset systemic lupus erythematosus**

<table>
<thead>
<tr>
<th>Features</th>
<th>Number of Children</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal involvement</td>
<td>26</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>21</td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>6</td>
</tr>
<tr>
<td>Intestinal pseudo-obstruction</td>
<td>2</td>
</tr>
<tr>
<td>Cholecystsis</td>
<td>1</td>
</tr>
<tr>
<td>Lupus peritonitis</td>
<td>1</td>
</tr>
<tr>
<td>Other causes of abdominal pain</td>
<td>11</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>5</td>
</tr>
<tr>
<td>Vomiting</td>
<td>2</td>
</tr>
<tr>
<td>Digestive bleeding</td>
<td>1</td>
</tr>
<tr>
<td>Ocular involvement (retinopathy, papillitis, punctuated keratitis)</td>
<td>7</td>
</tr>
<tr>
<td>Parotitis</td>
<td>6</td>
</tr>
<tr>
<td>Lung involvement (alveolar hemorrhage)</td>
<td>1</td>
</tr>
<tr>
<td>Neurological involvement (choreo)</td>
<td>1</td>
</tr>
<tr>
<td>Muscucutaneous involvement (erythromelalgia, facial swelling, genital ulceration)</td>
<td>3</td>
</tr>
<tr>
<td>Sjica syndrome</td>
<td>2</td>
</tr>
<tr>
<td>Renal involvement (HUS)</td>
<td>2</td>
</tr>
<tr>
<td>Histiocytic necrotizing lymphadenitis associated with reactive hemophagocytic syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Hypothyroidism</td>
<td>1</td>
</tr>
</tbody>
</table>

HUS = Hemolytic uremic syndrome.
response of nephrology centers (in which the most severe SLE cases are followed). The low inclusion rate of general pediatrics units and hematology units probably does not introduce a bias, because most children in France with SLE are referred to pediatrics nephrology and rheumatology units.

This series confirms the severity of the initial renal disease in pediatric SLE patients, because WHO class III and IV nephropathy was present in a quarter of our patients. Ethnicity has been shown to influence the pattern of renal involvement, with a poorer prognosis in African-American and Hispanic patients compared with other groups. Severe nephropathy was also significantly more frequent in our Asian patients.

Venous thrombosis occurred in 4 children. Single or recurrent venous or arterial thrombotic episodes were documented in 9% to 17% of children with SLE during the course of the disease, significantly associated with the presence of antiphospholipid antibodies. In a recent review of literature, lupus anticoagulant was found to have an odds ratio for patients with thrombosis that was 5- to 16-times higher than for control subjects, independent of the presence or non-presence of SLE. LACs were stronger risk factors for thrombosis than antiphospholipid antibodies, which were significantly associated with thrombosis in only half the cases, especially for medium or high titers of Immunoglobulin G isotype. Interaction of genetic defects and environmental factors with the normal hemostatic mechanism results in venous thromboembolism.

Inherited thrombophilic defects, such as factor V Leiden and prothrombin mutation, contribute to the risk of venous thrombosis in patients with SLE and potentiate this risk when combined with LACs or antiphospholipid antibodies.

Most of our patients had a marked increase of ESR at onset, in contrast with a normal or moderately increased CRP level. Despite severe disease activity, CRP level may remain within the reference range, limiting its clinical significance as a marker for disease activity. The measurement of CRP in SLE is a valuable tool for distinguishing infection from SLE exacerbation, except in the presence of serositis and severe necrotizing vasculitis. ANA were found in all but 4 children, with titers >1:640 in 92%. Positive results on an ANA test at moderate titer is frequently found in children without rheumatic disease and has little or no value for a diagnosis; conversely, screening serum dilution of 1:160 increases the specificity of this test for the diagnosis of SLE or mixed connective tissue disease without significantly increasing false-negative test results for SLE in children. Most large series of adult and pediatric patients with SLE included a variable number of patients, usually approximately 3% to 5%, who remained ANA-negative; adults who are ANA negative more often exhibited cutaneous discoid lesions and thrombosis and less often had arthritis as a first symptom. Some of them possibly had high DNA-binding capacity, although they had no detectable ANA.

Antibodies to ds-DNA are the most useful test in establishing the diagnosis of SLE. Anti-dsDNA antibodies are elevated in 85% to 95% of patients with childhood-onset SLE, in accordance with our data. Comparison of these autoantibodies profiles between adult- and childhood-onset SLE reveals a higher frequency in children. The presence of anti-Sm antibodies is a very specific, but not sensitive test for diagnosis of adult- SLE. In our study, we found only 31% of patients with anti-Sm antibodies.

In the United States, the prevalence of SLE is 6- to 8-times higher among African American patients than among white patients. In this French study, we also included a significant percentage of black patients, who represented 31% of our pediatric SLE population, compared with the 2.5% of the black population in France. However, definite conclusions cannot be drawn because this study is not an exhaustive French register.

Evidence for a genetic susceptibility to SLE in humans is based on the concordance rate (23%-57%) observed in identical twins and on the relative high incidence of familial cases (8%-12%). Candidate genes or loci for SLE susceptibility have been located on the long-arm of chromosome 1. The prevalence rate of familial SLE (8.4%) found in our population was in keeping with that generally admitted in literature. It is noteworthy that we also found a prevalence rate of autoimmune disorders other than SLE among relatives that was as high as 17%. This suggests that both organ- and non-organ-specific autoimmune diseases may share some susceptibility genes. The genetic background did not seem to influence disease expression because age at onset, sex ratio, and clinical SLE-related manifestations did not significantly differ between multiplex and sporadic cases in this study (data not shown) and in a previous study.

With respect to presentation, 12% of our children who had incomplete SLE as defined by using ACR criteria, evolved to complete SLE. These SLE criteria, developed in adults to allow classification and comparison of data from

### Table IV. Severe organ involvement in 155 patients with childhood-onset systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Manifestations</th>
<th>Number of events</th>
<th>Total (events/children)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Renal involvement WHO type III or IV</td>
<td>40</td>
<td>87/64</td>
</tr>
<tr>
<td>HUS</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pericardial tamponade</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Pancreatitis</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Intestinal myositis with ileus</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Thrombosis</td>
<td>4</td>
<td></td>
</tr>
<tr>
<td>Fingers gangrene</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Alveolar hemorrhage</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Neurological involvement</td>
<td>15</td>
<td></td>
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<tr>
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<td></td>
</tr>
<tr>
<td>Hemolytic anemia &lt;80 g/dL</td>
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<td></td>
</tr>
<tr>
<td>Coagulation factor deficiency with hemorrhage</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Hemophagocytic syndrome</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Total (events/children)</td>
<td>87/64</td>
<td></td>
</tr>
</tbody>
</table>

Organ dysfunctions were considered to be severe when disease required ≥0.5 mg/kg/d of prednisone within 6 months of initial diagnosis, when it was life-threatening, or both.
different sources, have been validated in only 1 pediatric study that demonstrated a 96% sensitivity rate and 100% specificity rate. Some studies suggested that incomplete SLE represents a mild spectrum of lupus sharing a good prognosis in adulthood, but the occurrence of renal involvement in one third of our patients with incomplete SLE at onset underlines that careful monitoring of urinalysis is mandatory in incomplete and in complete SLE in children.

REFERENCES

IMMUNODEFICIENCY IN CHILDREN WITH ACUTE LYMPHOBLASTIC LEUKEMIA AFTER COMPLETION OF MODERN AGGRESSIVE CHEMOTHERAPEUTIC REGIMENS

DANIEL H. BRODTMAN, DO, DAVID W. ROSENTHAL, DO, ARLENE REDNER, MD, PHILIP LANZKOWSKY, MD, AND VINCENT R. BONAGURA, MD

Objective  To determine the prevalence, duration, and a potential cause of humoral defect(s) in children with acute lymphoblastic leukemia (ALL) at least 1 year after completion of chemotherapy.

Study design  Antibody titers for mumps, rubeola, rubella, tetanus and diphtheria toxoid, poliovirus serotypes 1, 2, and 3, Haemophilus influenzae type b, varicella, and hepatitis B were obtained from 100 children with ALL. Children with non-protective titers to these microbial antigens were re-vaccinated and re-studied after anamnestic vaccine challenge.

Results  The percent of children with ALL who had protective titers was markedly less than that anticipated for immunized control subjects. Longitudinally, many titers fluctuate between protective and non-protective antibody responses after re-immunization. The chemotherapy protocol used did not affect the ability of these children to express protective antibody responses. T-, B-, and NK-cell numbers and proliferative responses to mitogens were all normal. Age correlated with titer results for certain vaccines.

Conclusions  Children in remission from ALL have a high prevalence of humoral immune defects that are not related to any specific chemotherapy regimen. This antibody deficiency may place children with ALL at risk for the development of these bacterial and viral diseases, even after completion of chemotherapy. Pediatricians, oncologists, or both should periodically monitor humoral immunity after chemotherapy and re-vaccinate these children, as needed, to ensure prolonged immunoprotection. (J Pediatr 2005;146:654-61)

Acute lymphoblastic leukemia (ALL) affects more than 2000 children in the United States each year.1 The short-term effects of chemotherapy on the immune system have been well documented,2-10 including the failure to express protective antibody responses and the development of acute bacterial and viral infections. Approximately 70% of children with ALL are in first remission 5 or more years after diagnosis.1 Infections are the most significant cause of morbidity and mortality during the treatment and convalescent phases of disease. Resolution of any immune defects in children who are disease-free occurs within 6 months11 to 1 year.12-14 After that period, the risks of infectious complications are thought to be equal to that of the general population.12-14 However, the long-term risk of serious infections is unknown.

Previous studies described immune abnormalities in children with ALL after the completion of chemotherapy, using in vitro laboratory evaluations,2,14-16 thus supporting the position that there may be a persistent immunodeficiency in children who are treated. However, other studies have provided conflicting evidence of normal3-6 or abnormal7-10 immune responses in these children, although all these studies were conducted during, or immediately after, the completion of chemotherapy.

This variability in immune responsiveness may be because of the kind of chemotherapy regimen used to treat ALL, because purine analogs can induce a marked depletion of CD4+ T-cells17-20 and glucocorticoids can cause profound lymholyis.21 We previously described 13 children with significant humoral defects after the completion of the Berlin-Frankfurt-Münster (BFM) protocol for ALL.22 One 8-year-old...
child described in this study had *Haemophilus influenzae* type b (HIB) sepsis and was unable to produce an anamnestic response after this infection or mount a protective antibody response to multiple HIB and *Streptococcus pneumoniae* vaccinations. Another child had similar laboratory findings, yet without increased clinical susceptibility to these organisms. A study of 43 children with ALL, treated with the Nordic protocol for ALL, confirmed our earlier results that found that some children with ALL fail to express protective antibodies against rubeola and rubella after vaccination. Neither of these earlier studies were longitudinal in design.

The aims of this study were to determine the prevalence of defective humoral responses to common bacterial and viral vaccines in a large cohort of children with ALL and determine whether the kind of chemotherapy used (BFM, or other modern aggressive chemotherapy regimens [MACR]) affected the ability of these children to express protective humoral responses.

**METHODS**

**Patient Selection and Study Design**

We performed a chart review to identify children with precursor T- or precursor B-cell ALL who had been treated and observed at the Schneider Children’s Hospital. Classification of ALL phenotype was made on the basis of a multiparameter flow cytometric analysis. The children had been treated with either the modified BFM or another MACR, including the risk-defined Pediatric Oncology Group (POG 9201, 9405, 9605, and 9406), and the NY I and NY II protocols. These other MACR protocols stratify treatment on the basis of the child’s age, white blood cell count, and cyogenetic results. The degree of immunosuppression associated with these protocols has not been evaluated. All children completed their BFM, or other MACR protocols, a minimum of 1 year before study enrollment and were considered to be clinically in remission. Children with known primary or secondary immunodeficiency and any children who were known not to be up-to-date with their immunizations at the time of their diagnosis were excluded. After approval of the project by the institutional review board of the North Shore-Long Island Jewish Health System, 100 children were prospectively studied.

**Clinical Evaluation**

A history of immunizations and serious infections was obtained. The dates of birth, ALL diagnosis, and the completion of chemotherapy were recorded. The genotype and phenotype of the ALL were also recorded. All immunizations that were given to each child before, during, and after the diagnosis of ALL were also documented.

**Immunologic Evaluation**

**Vaccine Titters.** Antibody titers for mumps, rubella, rubeola, tetanus toxoid, HIB, varicella, hepatitis B, and diphtheria toxoid were measured with enzyme-linked immunosorbent assay techniques in commercial reference laboratories.

Poliovirus antibody presence was determined with a neutralization assay at Focus Technologies (Cypress, Calif) for each of the 3 serotypes.

The humoral responses to each vaccine were classified as: protective, when levels of specific antibody to a given microbial agent were greater than the reference value; non-protective, when levels of specific antibody titers to a given microbial agent were less than the reference value or when the level was reported to be indeterminate.

We also reviewed the charts of 50 children selected at random from our Primary Immunology Clinic who were examined for suspected immunodeficiency and were found to have normal quantitative immunoglobulins, normal T- and B-cell numbers, normal responses to mitogens, and a history that was not consistent with an immune defect. These children were in a similar age range as our ALL study population, and they had also been immunized appropriately. This cohort had titers performed with the same protocols and at the same testing laboratories as our ALL study population.

**Complete Cellular and Humoral Responses.** Complete cellular and humoral responses were determined in a subgroup of 34 children with at least 1 non-protective antibody titer. Serum immunoglobulins (immunoglobulins G [IgG], M [IgM], and A [IgA]) were measured with nephelometry, and CH50 determinations were measured with an enzymatic, colorimetric assay in commercial laboratories. Peripheral blood immunophenotyping was performed with flow cytometric analysis as described. In brief, cellular responses to 3 different concentrations of phytohemagglutinin, concanavalin A, and pokeweed mitogen were measured with 3H-thymidine incorporation. Results were compared with those of age-matched control responses to the same mitogens.

**Anamnestic Vaccine Challenge.** Those children with non-protective titers to any of these microbial antigens were re-vaccinated with the appropriate vaccine(s) and were re-studied after at least 4 weeks. Re-vaccination was performed with diphtheria and tetanus toxoids, poliovirus serotypes 1, 2, and 3 (IPOL; all from Aventis Pasteur, Swiftwater, Pa), mumps, rubeola, rubella (M-M-R II), HIB (Liquid PedvaxHIB), varicella (Varivax), or hepatitis B (Recombivax HB; all from Merck & Co, West Point, Pa). Those children with protective titers to all the tested microbial antigens were given routine post-chemotherapy care and were not observed longitudinally in this study.

**Statistical Methods.** Exact binomial 95% CIs for the percent of children with non-protective titers on their first titer assessment were calculated for each specific titer. Two or more titers were used as inclusion criteria for our analysis, because this analysis required interpretation of antibody response with time. As such, this algorithm had limitations. First, the children were not all observed for the same length of time. Thus, a child who had fluctuating antibody titers categorized as “ending non-protective” or “always non-protective” might have had protective titers on the next
examination, although without re-immunization or natural disease this would be unlikely. Similarly, a child classified as “ending protective” or “always protective” might test “non-protective” on the next examination. This pattern was observed in some of the children. Therefore, in some instances, the classification of protective versus non-protective may reflect the length of follow-up and the intervals between follow-up visits.

Survival methods were used to compare the time until the next titer assessment, stratified by means of titer result (protective or non-protective). The time until the next titer was significantly shorter when the child’s result was non-protective. There were no baseline titers for these children at the time of diagnosis. Because not all the children were studied for all 11 bacterial and viral vaccines, smaller numbers of children were compared in some instances, thus possibly masking significant associations that might have been detected if larger numbers of children who had a given specific-titer were assayed. The association between the first titer result (protective or non-protective) and treatment protocol (BFM or other MACR) was examined with the Fisher exact test. The association between the first titer result and phenotype (T-cell or pre-B cell) and the association between the first titer result and genotype group (normal, hyperdiploidy, or other genetic abnormality) were also examined with the Fisher exact test.

The Mann-Whitney test was used to examine the association between the first titer result and the child’s age (in years) at the time the titers were drawn; the first titer result and the child’s age (in years) at the time of diagnosis; and the first titer result, and the time (in months) between the child’s age at diagnosis and the child’s age at the completion of chemotherapy. To correct for multiple comparisons, a Bonferroni-type adjustment was used; therefore, P values <.005 were considered to be significant.

RESULTS

Patient Population

All children were treated at the time of initial diagnosis of ALL; 77 children were treated with a modified BFM protocol,1,22 and 23 others were treated with MACR. Eight-nine percent of the children had precursor B-cell ALL, and 11% had precursor T-cell ALL. Of the patients (n = 65) who had genotype data available, 7 had T-cell ALL, whereas the remainder of patients had pre-B-cell ALL. We categorized the pre-B-cell genotypes into 3 groups: normal chromosome number (17/58), hyperdiploidy (27/58), or another genetic abnormality (14/58). Eleven percent of the children were younger than 2 years at the time of diagnosis, and 30% were older than 6 years at the time of diagnosis. The chemotherapy regimen lasted a mean of 2.5 years (95% CI, 2.5-2.6). The children had their first titers a mean of 2.2 years (95% CI, 1.8-2.7) after the completion of chemotherapy. Eighty-one percent of the children were observed 2 years after chemotherapy, and, 49% of those children were observed more than 5 years after chemotherapy.

Identification of Non-Protective Antibody Response by Children With ALL

Of the 11 specific vaccine titers examined, 93% of the children had at least 8 titers measured, with a mean of 10.0 titers per child. Thus, titers were not available for all vaccines on every child. Figure 1 shows the percent of children treated for ALL that expressed protective titers and the 95% CI for each vaccine. The percent of children with ALL with protective titers ranged from 41.8% to 85.7%. This was markedly less than the percent of immunized published control subjects (range, 95%-99%) that would be anticipated after complete immunization.31-33 Our healthy control cohort from our immunodeficiency clinic had titers to tetanus, diphtheria, H. influenzae b, and polio (serotypes 1, 2, and 3) that were 96% to 100% protective, consistent with immunized published controls for these vaccines. Thus, a significant number of children failed to produce or maintain protective antibody titers after complete immunization. Therefore, they may not have been adequately protected. Of the 93 children who had titers drawn to 7 or more different vaccines, many had multiple non-protective responses to vaccines, natural disease, or both, as depicted in Figure 2. Most of these children (73%) had 2 or more non-protective titers. Thirty percent showed a more profound inability to express specific antibody titers to more than 4 vaccines.

Figure 1. Percent of children with ALL at least 1 year after chemotherapy completion with protective titers to bacterial and viral vaccines. Horizontal gray bars represent the percent of children with protective antibody titers to a bacterial or viral vaccine. Black lines represent the 95% CIs for these children. The vertical crosshatched bar represents the anticipated percent of similarly immunized, historic control subjects that would express protective antibody titers after complete immunization with a given bacterial or viral vaccine (95%-99%).
Fluctuation in Protective Antibody Titers

Of the 100 children enrolled in the study, 89 had at least 1 non-protective titer on the initial examination; the other 11 children showed protective titers to all the titers obtained. Serial antibody titers were performed on 67 of the 89 children, and 22 others were lost to follow-up. Figure 3 shows the percent of these 67 children who persistently had non-protective titers to the various vaccines after vaccination or natural disease, had protective titers that became non-protective and then reverted back to protective after re-vaccination, or had non-protective titers that became protective after re-vaccination but reverted to non-protective thereafter. Thus, the number of children with ALL who at some point expressed non-protective antibody response to the various vaccines, regardless of whether they ended up protected or non-protected, may be an underestimation of the total number of children who have non-protective titers, if only those children with persistently non-protective antibody responses are considered. For example, if one considers the percent of children expressing HIB titers (Figure 3), only 18.5% of children showed a persistent, non-protective response. However, 87.0% of these children would show a non-protective response at some point, if the percent of children with fluctuating anti-HIB responses is added to the percent of children with persistent, non-protective responses.

CHEMOTHERAPY, ALL PHENOTYPE, OR GENOTYPE INFLUENCE ON THE EXPRESSION OF PROTECTIVE ANTIBODIES

Figure 4 shows the percent of children with ALL who had protective titers after modified BFM chemotherapy compared with other MACR. Among the children with ALL who received either the BFM regimen or another MACR, there were no statistically significant differences associated with the kind of chemotherapy used to treat ALL and the ability of a child’s expression of protective antibodies to viral and bacterial vaccines. There was no significant difference between the expression of protective first-titer responses and the ALL phenotype, specifically precursor T-, or precursor B-cell ALL. There were also no significant differences between the expression of protective first titer responses and the ALL genotype, specifically normal phenotypes, hyperdiploidy phenotype, and other abnormal phenotypes.

Comparison of Age at Diagnosis by First Titer Result

We compared the age of ALL onset with those children who showed protective titers to each of the 11 vaccines. At the time of diagnosis, the mean age was 5.2 years (95% CI, 4.5-5.9 years). A protective response to mumps vaccine \( P = .0011 \), tetanus \( P = .0038 \), and varicella \( P = .0046 \) was significantly associated with older age at diagnosis (Table IA). For the remaining 8 vaccines, the age of onset of ALL did not affect the ability of children with ALL to express protective antibody titers (data not shown).

Comparison of Age at First Titer With Titer Result

To determine whether the child’s age at first titer analysis was associated with a protective titer to any of the 11 vaccines given, we compared the age at first analysis with titer results at that time. At the time of their first titer assessment, the children’s mean age was 10.0 years (95% CI, 9.2-10.8 years).
years). There was a statistically significant association of the child's age at first titer analysis with the ability of a child to express a protective titer to mumps \( (P = .0025) \), tetanus \( (P = .0001) \), and varicella \( (P < .0001; \text{ Table I}) \). For all other titers, the child's age at first titer assessment did not correlate with finding a protective titer (data not shown). Thus, for mumps, tetanus, and varicella, older children with ALL were significantly more likely to express protective antibody responses than younger children.

Comparison of the Interval between Chemotherapy Completion and the First Titer With the Titer Result

We compared the number of months after the completion of chemotherapy with the first titer results for these children. The period between the completion of chemotherapy and the expression of a protective responses to a given vaccine was significant for hepatitis B \( (P = .0013) \), tetanus toxoid \( (P = .0005) \), and varicella \( (P = .0037; \text{ Table I}) \). Thus, children who were tested after longer periods after chemotherapy were more likely to express protective titers to these 2 vaccines than those children who were tested closer to the completion of chemotherapy.

Quantitative Immunoglobulins, Lymphocyte Enumeration, Serum CH50 Levels, and Lymphocyte Proliferation Studies

We determined that quantitative immunoglobulin levels, lymphocyte enumeration, serum complement (CH50) levels, and lymphocyte mitogenesis in response to phytohemagglutinin (PHA), concanavalin A (ConA), and pokeweed mitogen (PWM) in a subgroup of our children \( (n = 34) \). There were no significant differences in these children when compared with age-matched control subjects (data not shown).

Treatment With Intravenous Immunoglobulin

In our patient population, only 1 child had HIB sepsis and meningitis at age 8.5 years.\(^ {22} \) None of the other children described an increased incidence of unusual or serious infection. Three children with \( >2 \) non-protective titers, which failed repeatedly to express protective antibody titers after multiple revaccinations, were placed on intravenous immunoglobulin (IVIg), 400mg/kg monthly, for 1 year. Four months after terminating IVIg, these children were again re-vaccinated with the vaccines to which they had previously failed to make anamnestic antibody responses. Two of these 3 children were able to make protective antibody responses after 1 year of IVIg, whereas the third child was still unable to respond appropriately after re-vaccination. After an additional 1 year of IVIg (24 months total treatment) and re-vaccination, this child was able to make an anamnestic protective response. All 3 of these children maintained protective antibody responses to these vaccines 1 year after the termination of IVIg, and no further testing was performed.

DISCUSSION

A large number of these children persistently failed to make and maintain protective antibody responses to viral vaccines, bacterial vaccines, or both. Furthermore, an even larger number of children showed fluctuation in their ability to express and maintain protective antibody responses. This indicates that children, after chemotherapy, are more likely to be at risk of serious infection than would be predicted when only children who persistently fail to make protective antibodies are identified.

The correlation of the age at time of diagnosis and the age at first titer assessment with the ability of these children to mount protective antibody responses was only significant for protective responses made to mumps, tetanus toxoid, and varicella. This suggests that children who are older at the time of ALL diagnosis and at their first titer assessment are more likely to express protective antibody titers than younger children. Furthermore, a longer period between the end of chemotherapy and the first titer analysis was associated with the ability of these children to express protective antibodies for hepatitis B, tetanus toxoid, and varicella. We anticipated that a child's age would effect their ability to express protective antibodies to bacterial and viral vaccines, as shown with polysaccharide vaccines.\(^ {34,35} \) However, it remains uncertain why a child's age only correlated with certain vaccines. However, the rate of immune reconstitution after aggressive chemotherapy clearly depends on both the specific agent and the age of the child.\(^ {2,15,36,37} \) Furthermore, an inverse relationship has been shown between a child's age and total CD4+ T-cell reconstitution after aggressive chemotherapy.\(^ {11,38} \)

For specific chemotherapeutic protocols, several studies have examined the effects of individual drugs on antibody
immunodeficiency in children with acute lymphoblastic leukemia after treatment. Three investigations in which this defect was noted, all in patients with acute lymphoblastic leukemia (ALL), have suggested that the type of chemotherapy regimen used to treat pediatric ALL may be responsible in part for humoral deficits in these children. It is well recognized that aggressive chemotherapy can lead to a marked depletion of lymphocytes, thus clearly predisposing children to potentially life-threatening complications as a consequence of their treatment protocol. Furthermore, it is possible that variable amounts of time may be required to resolve defective antibody responses after chemotherapy. However, our comparison of children with ALL who received the BFM protocol versus those who received a different MACR showed no significant differences in these children’s ability to express protective antibody titers. Thus, the etiology of the humoral immune deficiency in children with ALL is not likely to be solely related to the dose, duration, or the individual drugs given in these chemotherapeutic protocols.

Table. Correlation of children’s age and first titer results

<table>
<thead>
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<th>Vaccine</th>
<th>Non-Protective Titer</th>
<th>Protective Titer</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Median ± IQR</td>
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<tr>
<td>Mumps</td>
<td>3.20 ± 3.15</td>
<td>52</td>
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<tr>
<td>Tetanus</td>
<td>3.25 ± 2.95</td>
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<td>Varicella</td>
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<td>Mumps</td>
<td>7.55 ± 3.52</td>
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</tr>
<tr>
<td>Tetanus</td>
<td>7.26 ± 3.02</td>
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<tr>
<td>Varicella</td>
<td>7.43 ± 3.33</td>
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<tr>
<td>Hepatitis B</td>
<td>13.39 ± 16.38</td>
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</tr>
<tr>
<td>Tetanus</td>
<td>12.66 ± 2.47</td>
<td>35</td>
</tr>
<tr>
<td>Varicella</td>
<td>13.17 ± 20.89</td>
<td>34</td>
</tr>
</tbody>
</table>

IQR, Interquartile range.

*All titers ≥1 year after chemotherapy completion. Only those titers with statistical significance (P < .005) are presented, all others were not significant.

There are other possible explanations for the humoral deficit that we and others have observed. Such defects may even be inherent in the disease itself, as has been suggested in Hodgkin’s disease, in which T-cell abnormalities have been observed before therapy and have been shown to persist long after completion of treatment. Furthermore, abnormalities in cell-mediated immunity can exist at the time of diagnosis in children with cancer. However, for most children with malignancy T-cell numbers and other measures of immune function are generally normal at presentation.

Considering the risk these child may have in acquiring serious infection as a consequence of this humoral defect, an increased risk of HIB and pneumococcal sepsis has been reported in pediatric oncology patients. The return of normal plasma cell numbers after the completion of chemotherapy in some children with ALL does not guarantee the expression of protective antibody responses after anamnestic vaccine challenge. It is possible that a major reason why most children in remission for ALL do not commonly experience severe or life-threatening bacterial infections is the heightened surveillance by parents, pediatricians, and hematologists/oncologists who quickly respond to any suspected infections with early intervention with antibiotics. In addition, herd immunity generated by routine vaccination of infants and children may also contribute to the reduction of serious infections in these children. Furthermore, it is possible that memory T-cells, generated in response to repeated viral vaccine challenges, are maintained in these children and are in sufficient quantities to prevent severe infections. In our study, only 1 child experienced a life-threatening infection with HIB. Two other children with multiple non-protective antibody responses were also given IVIg as prophylaxis for possible serious infection. Thus, it is possible that IVIg prophylaxis may have prevented the development of serious infections in these children.

Our study should alert pediatricians and hematologist/oncologists to examine humoral immune function 1 year after successful completion of chemotherapy in children with ALL. Children who demonstrate deficient responses to common pediatric vaccines should be re-evaluated, and re-vaccinated when indicated, to ensure that these children maintain protective antibody responses. We suggest that physicians caring for children who persistently show multiple, non-protective titers should consider a limited course of IVIg replacement, followed by re-evaluation of these children’s antibody responses.

Finally, the etiology of this humoral defect in a large percent of children with ALL needs further study to determine whether this defect simply represents a delay in the maturation of humoral immunity after chemotherapy, or whether it is the result of a pre-existing or acquired B- or T-cell defect, or a combination of B- and T-cell defects. Furthermore, children with new-onset ALL should be examined on diagnosis for this defect and should be studied periodically during and after treatment to identify children...
with ALL who are unable to produce long-lasting anamnestic responses to routine bacterial and viral vaccines.

The authors would like to thank Nina Kohn for her expert assistance with the statistical analysis, and Jane Moore, CPNP, and Diane Hart, PA, for their clinical assistance.

REFERENCES


INFLIXIMAB TREATMENT FOR REFRACTORY KAWASAKI SYNDROME

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Objective To evaluate the use of tumor necrosis factor (TNF)-α blockade for treatment of patients with Kawasaki syndrome (KS) who fail to become afebrile or who experience persistent arthritis after treatment with intravenous gamma globulin (IVIG) and high-dose aspirin.

Study design Cases were retrospectively collected from clinicians throughout the United States who had used infliximab, a chimeric murine/human immunoglobulin (Ig)G1 monoclonal antibody that binds specifically to human TNF-α, for patients with KS who had either persistent arthritis or persistent or recrudescent fever ≥48 hours following infusion of 2 g/kg of IVIG.

Results Response to therapy with cessation of fever occurred in 13 of 16 patients. C-reactive protein (CRP) level was elevated in all but one patient before infliximab infusion, and the level was lower following infusion in all 10 patients in whom it was re-measured within 48 hours of treatment. There were no infusion reactions to infliximab and no complications attributed to infliximab administration in any of the patients.

Conclusion The success of TNF-α blockade in this small series of patients suggests a central role of TNF-α in KS pathogenesis. Controlled, randomized clinical trials are warranted to determine the role of anti-TNF-α therapy in KS. (J Pediatr 2005;146:662-7)

Intravenous gamma globulin (IVIG) infusion is effective therapy for acute Kawasaki syndrome (KS), a self-limited, vasculitis that causes damage to the coronary arteries in up to 25% of untreated children. Administration of IVIG as a single 2 g/kg dose within the first 10 days after onset of fever in combination with high-dose aspirin (ASA) reduces the risk of coronary artery damage to 3% to 5%. However, IVIG therapy is expensive, is not available in all countries, and approximately 10% to 20% of patients with KS fail to become afebrile after the first dose. The risk of coronary artery aneurysms (CAA) is increased in these patients, and no controlled clinical trials have established their optimal management. For patients with KS with persistent or recrudescent fever after IVIG, current practice is to administer additional therapy, which may include one or more repeat doses of IVIG, high-dose pulse methylprednisolone, cyclophosphamide, methotrexate, ulinistatin (in Japan), cyclosporin A, or plasmapheresis.

Serum levels of the pro-inflammatory cytokine tumor necrosis factor (TNF)-α are elevated in acute patients with KS, with the highest levels observed in patients who develop CAA. We postulated that TNF-α blockade might be effective in the control of inflammation in patients with KS who fail to respond to IVIG. Two TNF-α antagonists are licensed for clinical use: etanercept (Enbrel, Immunex Corp., Seattle, Wash), a dimer of the soluble TNF receptor II in which the extracellular domain of the p75 receptor is fused to the constant region of human immunoglobulin (Ig)G1, and infliximab (Remicade, Centocor, Malvern, Pa), a chimeric murine/human IgG1 monoclonal antibody that binds specifically to human TNF-α.22 Infliximab, which is administered intravenously, is effective in a broad spectrum of immunologic disorders in which inflammation is mediated by TNF-α.33,24 A single case report described clinical improvement in a patient with Kawasaki syndrome. Other co-author participants in the Phase I clinical trial are Stanford T. Shulman and Marian E. Melish.

Submitted for publication Sep 24, 2004; last revision received Nov 16, 2004; accepted Dec 9, 2004.

Reprint requests: No reprints available.

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Jane C. Burns is the Principal Investigator of a grant from Centocor for an investigator-initiated clinical trial of infliximab in Kawasaki syndrome. Other co-author participants in the Phase I clinical trial are Stanford T. Shulman and Marian E. Melish.

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0022-3476/$ - see front matter

10.1016/j.jpeds.2004.12.022

ASA High-dose aspirin
CAA Coronary artery aneurysms
CRP C-reactive protein
eNOS Endothelial nitric oxide synthase
Ig Immunoglobulin
IVIG Intravenous gamma globulin
KS Kawasaki syndrome
TNF Tumor necrosis factor
re refractory KS treated with infliximab.25 We report here a series of patients with KS who failed to respond to repeated infusions of IVIG and other therapies and who then were treated with infliximab in an attempt to control fever as a sign of systemic inflammation associated with the vasculitis.

METHODS
Refractory KS was defined as the persistence or recurrence of fever (≥38.0°C or 100.4°F) at least 48 hours after the end of the IVIG infusion. Illness Day 1 was defined as the first day of fever. Cases treated with infliximab were collected from across the United States through a network of investigators studying KS and from clinicians who contacted one of the authors (J. C. B.) regarding the management of patients with refractory KS. Patient demographic characteristics, therapies administered for KS before and after infliximab treatment, C-reactive protein (CRP) levels, dose, patient response to infliximab, and coronary artery outcome were recorded for all patients. The potential risks and unproven benefits of infliximab therapy were discussed with all parents before the non-FDA approved, compassionate use of the drug.

Measurements of the internal diameter of the coronary arteries by transthoracic echocardiography were interpreted as follows: dilated if Z score >2 and <3, ectasia if Z score >3 with uniform dilatation of vessel, aneurysm if focally dilated segment with Z score >3.26

RESULTS
Patient Characteristics
Case reports were obtained on 17 patients with acute KS (median age 2.6 years, range 0.12-13.1 years, 11 males) who received infliximab infusion after at least two doses of IVIG (2 g/kg) and daily ASA (80-100 mg/kg/day) for the following indications: (1) persistent or recrudescent fever (14 patients) and (2) fever plus arthritis (15 patients) or persistent, severe arthritis without fever (1 patient) (Table). Of these 17 patients, 7 were non-Hispanic Caucasian, 4 were Hispanic Caucasian, 5 were Asian/Pacific Islander (3 Japanese, 2 Hawaiian), and one was mixed Caucasian/Japanese. All patients had fever and met either 4 of 5 standard criteria for KS or 3 of 5 criteria in addition to CAA by echocardiography.27

Therapy
Six patients received three or more IVIG infusions and eight patients received one to three doses of pulse methylprednisolone (30 mg/kg/dose intravenously). All failed to become persistently afebrile following these treatments. Before infliximab therapy, these patients had been either persistently or intermittently febrile for 8 to 53 days. Of the 17 patients, 15 received a single infusion of 5 mg/kg of infliximab. Two patients, one with fever and arthritis and one with only severe arthritis (Patients 14 and 5), were treated with 10 mg/kg of infliximab with dramatic and permanent resolution of their arthritis within 12 hours of the infusion. Response to therapy with cessation of fever was dramatic in 14 of 16 febrile patients.

All but one patient had an elevated CRP before infliximab infusion, and the CRP was lower following infusion in all 10 patients in whom it was re-measured within 48 hours of treatment. There were no infusion reactions to infliximab and no complications attributed to infliximab administration in any of the patients. Antibody levels to infliximab were not measured.

Response to therapy could not be evaluated in one patient (Patient 15), and fever recurred four and eight days after infliximab infusion in two patients (Patients 12 and 1). In Patient 15, fever subsided after infliximab infusion, but the patient underwent plasmapheresis 12 hours later, which precluded an evaluation of the patient’s long-term response to infliximab. The patient fully recovered with no further fevers and no coronary artery abnormalities by echocardiography. In a second patient (Patient 12), fever subsided with the infliximab infusion only to return 8 days later accompanied by conjunctival injection and tachycardia. The patient was re-admitted and responded to three pulses of methylprednisolone therapy (30 mg/kg/dose) followed by oral prednisone for 6 weeks. In a third patient (Patient 1), infliximab was administered for persistent fever after two doses of IVIG. Although the patient initially became afebrile, fever recurred 4 days after infliximab infusion, and pulse methylprednisolone therapy was administered. After the second dose of steroids, gastrointestinal bleeding was noted, and the steroid therapy was discontinued. The patient remained afebrile and was discharged home with multiple aneurysms of the left and right coronary arteries. On Illness Day 70, 53 days after infliximab infusion, the patient had a cardiopulmonary arrest at home and died, presumably of complications related to multiple CAA. No autopsy was performed.

Patient Outcome
Twelve patients had coronary artery abnormalities documented by echocardiography before infliximab therapy: four had transient dilatation that resolved post-infliximab infusion, three had aneurysms, and five had ectasia. Four had normal coronary arteries pre- and post-infliximab. Fifteen patients (excluding Patient 1, who died) were followed for 6 to 26 months (median follow-up 17 months) with no apparent complications of their infliximab therapy.

DISCUSSION
We present an anecdotal series of patients with refractory KS who failed to become afebrile with conventional therapy, most of whom responded rapidly and completely to a single infusion of the anti-TNF-α monoclonal antibody, infliximab. Importantly, there were no adverse reactions or complications of infliximab infusion in this small series of patients.

Although IVIG plus ASA therapy is effective for treatment of acute KS, numerous series in different ethnic/racial populations have documented a 10% to 20% failure rate with persistence of fever and clinical and laboratory signs of inflammation.5,8,10,11 Patients who have persistent or recrudescent fever are at increased risk of developing CAA. In
one US multicenter study, CAA developed in 5 of 50 (10%) patients who failed to become afebrile after the first IVIG treatment (2 g/kg) and in only 4 of 328 (1.2%) patients who responded to a single IVIG infusion.5 A variety of therapies have been proposed for treatment of IVIG-resistant patients but none have been studied in a prospective trial with sufficient power to allow conclusions about efficacy. Meanwhile, the clinician is faced with the difficult task of tailoring therapies for this important group of refractory patients in the absence of systematic information to guide the choice of treatment.

Several lines of evidence make TNF-α blockade an appealing option for treatment of acute KS. Circulating levels of TNF-α are markedly elevated during acute KS, and the degree of elevation correlates with coronary artery damage and the development of aneurysms.19 TNF-α may fuel the inflammatory process in KS through a number of pathways including stimulating cells to synthesize and release chemokines, metalloproteinases, and other cytokines, inducing expression of adhesion molecules and human leukocyte class II antigens on immune effector and endothelial cells, and activating neutrophils. Elevated levels of CRP mediated by IL-6 and fever resulting from cytokine release are clinically useful measures of systemic inflammation resulting from elevated circulating levels of TNF-α. The cessation of fever and lowering of CRP levels following TNF-α blockade in this series of patients suggest that TNF-α is indeed an important mediator of inflammation in this vasculitis.

### Table. Patient characteristics and outcome

<table>
<thead>
<tr>
<th>Patient #</th>
<th>Age (y)</th>
<th>Sex</th>
<th>Race (ethnicity)</th>
<th>Illness Day of 1st IVIG infusion</th>
<th>Other treatment*</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0.12</td>
<td>F</td>
<td>Caucasian (Hispanic)</td>
<td>Day 6</td>
<td>IVIG × 2, MP × 2, clopidogrel</td>
</tr>
<tr>
<td>2</td>
<td>0.25</td>
<td>M</td>
<td>Caucasian</td>
<td>Day 5</td>
<td>IVIG × 3, MP × 5, prednisone 2 mg/kg/d</td>
</tr>
<tr>
<td>3</td>
<td>0.75</td>
<td>M</td>
<td>Pacific Islander (Hawaiian)</td>
<td>Day 3</td>
<td>IVIG × 2</td>
</tr>
<tr>
<td>4</td>
<td>1.0</td>
<td>M</td>
<td>Pacific Islander (Hawaiian)</td>
<td>Day 4</td>
<td>IVIG × 2</td>
</tr>
<tr>
<td>5</td>
<td>1.5</td>
<td>M</td>
<td>Caucasian (Hispanic)</td>
<td>Day 7</td>
<td>IVIG × 3, MP × 3, naprosyn</td>
</tr>
<tr>
<td>6</td>
<td>1.75</td>
<td>F</td>
<td>Caucasian</td>
<td>Day 12</td>
<td>IVIG × 2</td>
</tr>
<tr>
<td>7</td>
<td>2.4</td>
<td>F</td>
<td>Asian (Japanese)</td>
<td>Day 4</td>
<td>IVIG × 2</td>
</tr>
<tr>
<td>8</td>
<td>2.5</td>
<td>M</td>
<td>Caucasian (Iranian)</td>
<td>Day 5</td>
<td>IVIG × 2, MP × 1</td>
</tr>
<tr>
<td>9</td>
<td>2.7</td>
<td>M</td>
<td>Asian (Japanese)</td>
<td>Day 5</td>
<td>IVIG × 2</td>
</tr>
<tr>
<td>10</td>
<td>2.8</td>
<td>M</td>
<td>Caucasian</td>
<td>Day 3</td>
<td>IVIG × 2, MP 1, mg/kg × 1</td>
</tr>
<tr>
<td>11</td>
<td>3.75</td>
<td>M</td>
<td>Caucasian (Hispanic)</td>
<td>Day 3</td>
<td>IVIG × 2, MP × 3</td>
</tr>
<tr>
<td>12</td>
<td>4.1</td>
<td>F</td>
<td>Caucasian (Hispanic)</td>
<td>Day 14</td>
<td>IVIG × 4, MP × 3 for two courses</td>
</tr>
<tr>
<td>13</td>
<td>4.4</td>
<td>M</td>
<td>Asian (Japanese)</td>
<td>Day 6</td>
<td>IVIG × 2</td>
</tr>
<tr>
<td>14</td>
<td>4.8</td>
<td>F</td>
<td>Caucasian</td>
<td>Day 4</td>
<td>IVIG × 3, naprosyn</td>
</tr>
<tr>
<td>15</td>
<td>9.4</td>
<td>M</td>
<td>Asian/Caucasian (Japanese)</td>
<td>Day 4</td>
<td>IVIG × 3, clopidogrel</td>
</tr>
<tr>
<td>16</td>
<td>12.9</td>
<td>F</td>
<td>Caucasian (Pakistani)</td>
<td>Day 8</td>
<td>IVIG × 3, MP × 3, prednisone 10 mg/d × 1 week</td>
</tr>
<tr>
<td>17</td>
<td>13.1</td>
<td>M</td>
<td>Caucasian</td>
<td>Day 5</td>
<td>IVIG × 2, MP × 3</td>
</tr>
</tbody>
</table>

CA, coronary artery; Circ, circumflex artery; CRP, C-reactive protein; LAD, left anterior descending artery; LMCA, left main coronary artery; MP, intravenous methylprednisolone 30 mg/kg/dose; ND, not done; RCA, right coronary artery.

*All patients received ASA 80-100 mg/kg/d while febrile except as noted.
†Measurements are given for the maximal internal diameter of the coronary arteries measured at any time during the disease course.
‡Pre-treatment studies obtained no more than 24 h before infliximab infusion; post-treatment studies obtained within 48 h after infliximab infusion.
§Complete response: afebrile within 12 h of infliximab infusion with complete resolution of clinical signs and symptoms.
Histologic examination of the coronary arteries in KS suggests focal destruction of the endothelium. Endothelial cell dysfunction as manifested by failure to respond to acetylcholine, also has been observed in vivo. There are several potential mechanisms by which TNF-α may affect endothelial cells in acute KS. In an in vitro study of cultured human umbilical vein endothelial cells, stimulation of cells with either IL-1 or TNF-α followed by incubation with plasma from acute patients with KS resulted in cell lysis. These results suggest that pro-inflammatory cytokines induce a neoantigen on endothelial cells that may be a target for cytotoxic antibodies in acute KS plasma. Experimental evidence suggests a direct role of TNF-α in endothelial cell dysfunction and apoptosis.

Reduced endothelial nitric oxide synthase (eNOS) production, which is essential for vasodilatation in response to numerous stimuli, is a marker for endothelial cell dysfunction. TNF-α blocks production of eNOS by preventing the phosphorylation and activation of the serine/threonine protein kinase, Akt, and by directly degrading eNOS mRNA. In addition, TNF-α mediates endothelial cell apoptosis via specific signaling pathways leading to activation of the caspase cascade.

The role of TNF-α blockade in improving endothelial cell function has been demonstrated in two different patient populations. In adults with rheumatoid arthritis, infliximab improved endothelial cell function as assessed by eNOS-dependent vasodilation in response to acetylcholine. In

### Table. (Continued)

<table>
<thead>
<tr>
<th>Infliximab dose; Illness Day at treatment</th>
<th>Echocardiography results†</th>
<th>CRP‡ (mg/dL) pre-/post-infliximab</th>
<th>Comments</th>
</tr>
</thead>
<tbody>
<tr>
<td>5 mg/kg Day 11</td>
<td>3 RCA aneurysms, 6 mm each; LAD aneurysm 6.5 mm; Circ aneurysm 4.3 mm</td>
<td>17.0/11.0</td>
<td>Died at home on Illness Day 70, no autopsy</td>
</tr>
<tr>
<td>5 mg/kg Day 34</td>
<td>LAD ectasia 6.6 mm RCA ectasia 5.8 mm</td>
<td>15.3/1.5</td>
<td>Prednisone taper over 6 wk</td>
</tr>
<tr>
<td>5 mg/kg Day 17</td>
<td>LAD aneurysm 4.8 mm RCA aneurysm 4.5 mm</td>
<td>3.8/ND</td>
<td>Complete response³</td>
</tr>
<tr>
<td>5 mg/kg Day 8</td>
<td>Normal</td>
<td>17.4/ND</td>
<td>Complete response</td>
</tr>
<tr>
<td>10 mg/kg Day 35</td>
<td>dilated CA → resolved</td>
<td>4.3/2.5</td>
<td>Infliximab given for persistent arthritis, not for fever</td>
</tr>
<tr>
<td>5 mg/kg Day 47</td>
<td>RCA aneurysm 5 mm LMCA ectasia 4.4 mm</td>
<td>7.9/ND</td>
<td>Complete response</td>
</tr>
<tr>
<td>5 mg/kg Day 14</td>
<td>LMCA dilated</td>
<td>0.4/ND</td>
<td>Complete response</td>
</tr>
<tr>
<td>5 mg/kg Day 20</td>
<td>LCA ectasia 4.4 mm LAD ectasia 4.4 mm</td>
<td>20.4/6.8</td>
<td>Complete response</td>
</tr>
<tr>
<td>5 mg/kg Day 11</td>
<td>normal</td>
<td>ND/ND</td>
<td>Complete response</td>
</tr>
<tr>
<td>5 mg/kg Day 11</td>
<td>RCA aneurysm 3.5 mm</td>
<td>2.8/1.3</td>
<td>Complete response</td>
</tr>
<tr>
<td>5 mg/kg Day 22</td>
<td>normal</td>
<td>8.4/ND</td>
<td>Complete response</td>
</tr>
<tr>
<td>5 mg/kg Day 53</td>
<td>RCA ectasia 4.3 mm LMCA ectasia 5.4 mm</td>
<td>14.4/4.4</td>
<td>Afebrile × 8 d post-infliximab infusion, then relapsed with fever; responded to 2nd course of MP with oral prednisone taper over 6 mo</td>
</tr>
<tr>
<td>5 mg/kg Day 11</td>
<td>normal</td>
<td>4.2/ND</td>
<td>Complete response</td>
</tr>
<tr>
<td>10 mg/kg total (5 mg/kg on Day 15 and 16)</td>
<td>dilated post. descending artery → resolved</td>
<td>30.5/15.7</td>
<td>Severe arthritis</td>
</tr>
<tr>
<td>5 mg/kg Day 19</td>
<td>dilated CA → resolved</td>
<td>3.5/1.4</td>
<td>Infliximab followed by plasmapheresis × 3 d</td>
</tr>
<tr>
<td>5 mg/kg Day 25</td>
<td>dilated CA → resolved</td>
<td>10.7/3.3</td>
<td>Prednisone given after infliximab infusion, tapered over 8 wk</td>
</tr>
<tr>
<td>5 mg/kg Day 30</td>
<td>normal</td>
<td>5.7/3.7</td>
<td>Complete response</td>
</tr>
</tbody>
</table>
a study of adults with anti-neutrophil cytoplasmic antibody-associated systemic vasculitis, treatment with infliximab improved both clinical indicators of inflammation and endothelial cell vasomotor function as measured by forearm blood flow response to intra-arterial infusion of acetylcholine. Thus, anti-TNF-α therapy not only reduces inflammation in these patient populations, but it also directly improves endothelial cell function.

Other considerations regarding infliximab therapy of refractory KS include cost and safety. The cost of infliximab therapy (average wholesale price $692 for a single-use, 100-mg vial) compares favorably with the cost of IVIG treatment (average wholesale price approximately $85/g). Thus, the average cost for treating a 15-kg patient would be $692 versus $2,550 for infliximab and IVIG, respectively. In addition, there is no risk of contamination with unknown infectious agents, which is a lingering concern with use of IVIG. The safety profile of infliximab deserves further discussion. In adults the most common minor side effects are headache, nausea, and upper respiratory infections. Infusion reactions, ranging from flushing and dyspnea to anaphylaxis, are thought to be related to formation of antibodies directed against infliximab in patients receiving repeated doses for chronic diseases. Such reactions are associated with 4% to 13% of infusions in adults and adolescents receiving chronic therapy but have not been observed following the first infusion. No data are available regarding the rate of infusion reactions or other side effects in infants and young children. No infusion reactions were noted in our series despite the inconsistent use of premedication with acetaminophen and diphenhydramine. Other complications of infliximab administration are related to immunosuppression and include re-activation of latent tuberculosis, histoplasmosis, and coccidiodomycosis, increased risk of bacterial sepsis, increased risk of lymphoma, and development of IgM and IgA anti-nuclear antibodies. Many patients who receive infliximab are on other immunosuppressive agents, so the contribution of TNF-α suppression to their infectious complication is difficult to assess. In assessing the safety of TNF-α blockade in children with KS, a population of patients with depressed myocardial contractility, it is important to consider the occurrence of new-onset heart failure in 10 adults (19–48 years of age) following anti-TNF therapy for rheumatoid arthritis or Crohn’s disease. Whether or not a causal relationship exists between TNF-α blockade and the occurrence of heart failure is complicated by the fact that heart failure is a known complication of rheumatoid arthritis. Indeed, a retrospective review of more than 13,000 patients with rheumatoid arthritis found a 3.9% incidence of heart failure and a significant protective effect of anti-TNF therapy among the 5800 patients receiving this therapy. Conversely, clinical trials designed to test the hypothesis that TNF-α blockade might be beneficial in adults with ischemic heart failure were terminated prematurely because of either no benefit of anti-TNF therapy or worsening heart failure and death. Although virtually all patients with KS have subclinical myocarditis, no adverse effect on myocardial contractility was observed in this small series of patients.

Limitations of this study include its retrospective nature, small number of patients, and the administration of multiple different therapies following the first IVIG infusion failure. Different IVIG preparations were used at different centers and concomitant or sequential anti-inflammatory therapies administered to several of these patients precluded a clear assessment of the effect of infliximab infusion. The pharmacodynamics, pharmocokinetics, and safety of infliximab in children <5 years of age has not yet been established, but a randomized, prospective, phase I clinical trial of infliximab treatment versus repeat IVIG infusion for refractory KS is in progress (J. C. B.). Similarly, the appropriate dose of infliximab to control inflammation in acute KS has not been established. A dose of 5 mg/kg was used in the majority of patients in this series by extrapolation from data generated in pediatric patients with juvenile rheumatoid arthritis and Crohn’s disease in whom 5 mg/kg was safe and effective.

Trials of new treatment can lead to insights into disease pathogenesis, and the success of TNF-α blockade in most patients in this small series further supports the central role of TNF-α in KS pathogenesis. Future controlled, randomized clinical trials will define the role of anti-TNF-α therapy in KS.

The author wishes to thank Dr Hiroko Shike and Dr Alan Mendelsohn for helpful discussion.

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POLICY ISSUES FOR EXPANDING NEWBORN SCREENING PROGRAMS: THE CYSTIC FIBROSIS NEWBORN SCREENING EXPERIENCE IN THE UNITED STATES

BENJAMIN S. WILFOND, MD, AND SARAH E. GOLLUST, BA

Objective To describe the screening approaches and implementation strategies for cystic fibrosis newborn screening in the 12 programs that were offered in 11 states in 2002.

Study design Telephone interviews conducted in the spring of 2003 with program representatives in the 11 states. Screening approaches were defined in four overlapping categories: state mandated screening, state-wide offering, hospital based screening, and screening with informed consent.

Results Screening was state mandated in seven states but was routinely offered to most infants in nine states. The primary care provider or hospital determined if screening was done in three states (four programs). Informed consent was explicitly documented in two states. In five programs, immunoreactive trypsinogen exclusively was used to identify at risk infants. In seven programs, a second tier DNA test was also used, but these programs each had distinct strategies. In only two programs where DNA testing was performed and normal sweat tests indicated carrier status, were results routinely provided to parents “in person” at a CF center.

Conclusion The diversity of approaches for screening approaches and strategies has advantages for future policy decisions, provided that data about the clinical and psychosocial impact of screening from these programs are collected and disseminated. As additional states determine that the resources are available, programs can be designed with a more favorable benefit/risk balance as a result of the successes and challenges faced by other states. (J Pediatr 2005;146:668-74)

As a result of the identification of more genes associated with diseases and the development of new technologies such as tandem mass spectroscopy, there is an increasing availability of potential newborn screening (NBS) tests. States have adopted these tests to varying degrees, with states currently testing for between 4 and 36 conditions. With increasing pressure on screening programs to add tests, it can be challenging for policymakers to decide which tests to add. In some cases, the benefits and risks of NBS will depend on specific details of how the program is designed and implemented.

Consideration of these details has particular importance as screening expands from diseases such as phenylketonuria (PKU), in which a treatment affords the benefit of avoiding profound mental retardation, toward tests for conditions with less dramatic benefits from treatment, such as for Duchenne muscular dystrophy, in which there are not effective interventions that alter the clinical outcome as a direct result of newborn screening. In the muscular dystrophy case, the diagnosis offers psychosocial benefit to parents but could also be psychologically harmful. Cystic fibrosis (CF) screening falls in the middle of this spectrum: Benefits are clinically meaningful but are not as profound as PKU treatment, and there are also potential psychosocial harms.

Children with CF identified as infants can receive nutritional interventions earlier, leading to greater increases in height and weight than would be expected without early diagnosis. However, some parents of false-positive infants may have significant anxiety or confusion and worry about their infant’s results long after the test. The particular

<table>
<thead>
<tr>
<th>CDC</th>
<th>Centers for Disease Control</th>
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</thead>
<tbody>
<tr>
<td>CF</td>
<td>Cystic fibrosis</td>
</tr>
<tr>
<td>IRT</td>
<td>Immunoactive trypsinogen</td>
</tr>
<tr>
<td>NBS</td>
<td>Newborn screening</td>
</tr>
<tr>
<td>PKU</td>
<td>Phenylketonuria</td>
</tr>
<tr>
<td>PPV</td>
<td>Positive predictive value</td>
</tr>
</tbody>
</table>

From the Social and Behavioral Research Branch, National Human Genome Research Institute and Department of Clinical Bioethics, Warren G. Magnuson Clinical Center, Bethesda, Maryland.

The opinions expressed in this article are those of the authors and do not reflect the opinions or policies of the National Human Genome Research Institute, the National Institutes of Health, or the Department of Health and Human Services.

Submitted for publication Jun 25, 2004; revision received Oct 28, 2004; accepted Nov 15, 2004.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2004.11.029

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decisions about how to screen and to communicate results may influence whether the benefits will outweigh the risks.\textsuperscript{11}

Current screening approaches for CF are based on the measurement of immunoreactive trypsinogen (IRT) on dried blood spots.\textsuperscript{18} An elevated IRT level indicates an increased risk of CF. In some programs, IRT results from two samples (IRT/repeat IRT) are used to determine which infants should have a sweat test (the standard diagnostic test for CF, based on measurement of chloride in sweat). Other programs use a second tier DNA test (IRT/DNA) for the presence of one or two CF alleles to make this determination.\textsuperscript{19}

In the United States, CF NBS began in Colorado\textsuperscript{20} and Wisconsin\textsuperscript{11} in the mid 1980s, and screening was initiated in four additional states (including two programs in Connecticut) over the next decade. After a 1997 Center for Disease Control and Prevention (CDC) Workshop that recommended additional states develop “demonstration programs” for CF NBS,\textsuperscript{22} three states have implemented screening, and two more state programs were expected to add CF in 2003. In this paper, we report the results of interviews with representatives from these 12 programs to understand the policy decision to include CF, the approaches to offering testing to the population, the testing strategy, and the approach to communication and follow-up.

This information may be useful to policymakers in other states who are considering whether and how to implement CF NBS, particularly in light of a recent CDC report that concluded that the “… health benefits to children with CF outweigh the risk of harm and sufficient to justify screening for CF…. and requires consideration of costs, resources, and priorities…. and the net balance of benefits and risks is contingent on how newborn screening for CF is implemented.”\textsuperscript{23}

METHODS

A telephone interview guide was developed to address the following domains: individuals responsible for making the decision to screen, reasons for screening, whether CF NBS was mandated by the state, ability of parents to opt out of screening, the particular screening strategy used, and communication of screening results. We also asked how many infants were screened in the most recent year that complete data were available, how many positive screen infants were identified, and how many CF patients were identified. The questions were piloted with one Newborn Screening Laboratory Manager, with subsequent revisions made to improve clarity. The final interview guide contained 22 questions.

Initial e-mails were sent to the individuals in the 11 states listed with the National Newborn Screening and Genetics Resource Center.\textsuperscript{22} The e-mails requested the suggestion of an appropriate individual to address these issues. The informants included people associated with state health departments, screening programs affiliated with universities, and a commercial screening laboratory. Initial interviews lasted approximately 25 minutes and were conducted between March 5 and May 15, 2003. Information was collected from informants through multiple contacts, by telephone initially and then through follow-up interviews by telephone or e-mail. This study received an exemption of institutional review board review by the National Institutes of Health Office of Human Subjects Research.

RESULTS

Policy Decision to Screen for Cystic Fibrosis

In some cases, the decision to add a new screening test was reported to have been the result of a deliberative process, based on consideration of standard criteria for public health screening\textsuperscript{24-26} or for genetic screening in particular.\textsuperscript{27-31} In other cases, the decision was strongly influenced by an assertive advocate.\textsuperscript{32} The multiple reasons informants offered for adding CF included data suggested significant benefits (n = 3); CF fulfilled state screening criteria (n = 3); CF center resources in place (n = 3); screening test is available (n = 2); sufficient incidence of CF in state (n = 2); executive mandate (n = 1); modeled after another state program (n = 1); and, for one state, CF was ranked highest by state pediatrics on a survey listing possible conditions to screen.

Informants described a range of individuals and groups who were involved in the decision to add CF, including: newborn screening advisory committees (n = 7); the Department of Health (n = 5); executive or legislative State decision (n = 2); local physician activism (n = 3); and consumer or parent activism (n = 1). Three informants in particular described physician or parent activism as a driving force. In Connecticut, two programs were developed on the basis of professional interest within two academic institutions. In Montana, a group of physicians decided to offer the test to their patients and lobbied to make the test available via the Health Department. In Mississippi, a parent group led a campaign to make what was previously supplementary newborn screening, including CF, to be part of routine newborn screening.

Approaches to Offering Testing

Table I differentiates seven states that have programs mandated by legislative or executive actions and four states with other approaches. In the states with mandated CF NBS, every newborn is screened for CF as part of routine NBS. Massachusetts and Pennsylvania did not have mandated screening, but almost all infants were screened for CF. In Massachusetts, all parents were offered screening as part of a pilot study of expanded newborn screening, but the individual parent made the decision for CF NBS. In Pennsylvania, hospitals or physicians routinely requested supplemental screening from Pediatrix Screening, a private company that also provided state mandated screening for Mississippi.

Approximately 50% of the infants born in Connecticut and Montana were screened for CF through optional programs. In Montana, the Health Department performed CF screening. In Connecticut, University of Connecticut and Yale University both offered CF screening to hospitals and pediatricians. Parents were not routinely involved in the decision to have screening but could decline screening.
Wyoming was the only state among the 11 states with an explicit informed consent requirement for routine newborn screening. In Massachusetts, if a parent did not want to have their child tested for CF, the hospital staff would check a box on the blood spot collection form. Most states allowed parents to opt out of newborn screening entirely. In Colorado and Wyoming, parents could opt out of newborn screening for any reason. In the other states, except Montana, parents could opt out of newborn screening for religious reasons. In Montana, there were no exemptions for newborn screening explicitly stated in the state’s regulations.

**Screening Strategies**

Screening strategies were quite variable in the 10 programs that performed CF screening in 2002 (Table II). Four programs (Colorado, Wyoming, Montana, and Connecticut) were based on performing IRT assays on two samples from an infant before recommending a sweat test (IRT–repeat IRT). Additionally, South Carolina planned to implement this approach. The University of Connecticut screened for the ∆F508 allele in infants with two elevated IRT levels or in those with an initial IRT ≥ 150 ng/mL. However, a sweat test was recommended regardless of allele status for all infants with a repeat IRT level ≥ 70 ng/mL. The rationale for the DNA analysis was that it could provide the primary care physician with a better prediction of the sweat test result.

The other programs used a protocol in which DNA analysis was performed after an initial elevated IRT level before a sweat test was recommended (IRT/DNA). Programs following this general approach differed in terms of how many alleles were tested and whether children with elevated IRT levels but without an identified allele were referred for repeat IRT testing, sweat testing, or no further testing. Some programs used an absolute numerical IRT (ie, ≥120 ng/mL) cutoff, whereas other programs used a proportionate cutoff (ie, ≥96th percentile).

New Jersey tested for the ∆F508 allele after an initial elevated IRT (IRT/DNA–∆F508). If no ∆F508 alleles were...
<table>
<thead>
<tr>
<th>State</th>
<th>First stage</th>
<th>Second stage</th>
<th>Third stage</th>
<th>Outcomes</th>
</tr>
</thead>
<tbody>
<tr>
<td>CO</td>
<td>≥105 ng/ml</td>
<td>Repeat IRT &gt;70 ng/ml</td>
<td>Positive screen</td>
<td></td>
</tr>
<tr>
<td>WY</td>
<td>≥105 ng/ml</td>
<td>Repeat IRT &gt;70 ng/ml</td>
<td>Positive screen</td>
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<tr>
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<td>≥100 ng/ml</td>
<td>Repeat IRT &gt;80 ng/ml</td>
<td>Positive screen</td>
<td></td>
</tr>
<tr>
<td>CT</td>
<td>≥150 ng/ml</td>
<td>Δ508 screen, ⇨ 2 alleles</td>
<td>Repeat IRT ≥70 ng/ml</td>
<td>Positive screen</td>
</tr>
</tbody>
</table>
| (U Conn) | ≥90 ng/ml   | Repeat IRT >70 ng/ml          | Δ508 screen  
|        |             | ⇨ 2 alleles                  | ⇨ 1 allele    | Positive screen |
|        |             | ⇨ 0 alleles                  |             | Positive screen |
| CT     | ≥96%        | Monthly IRT                  |             | "Presumptive CF"             |
| (Yale) |             | Δ508 screen,                |             | "Carrier"                    |
|        |             | ⇨ 2 alleles                  |             | "Negative" (Consider CF)     |
| WI     | ≥96%        | Daily IRT                   |             | CF                            |
|        |             | 25 mutation screen           |             | "Possible CF"                |
|        |             | ⇨ 2 alleles                  |             | "Remote CF"                  |
|        |             | ⇨ 1 allele                   |             |                               |
|        |             | ⇨ No alleles                 |             |                               |
|        |             | And IRT ≥ 200 ng/ml          |             |                               |
| PA     | ≥90%        | Daily IRT                   |             | "Presumptive CF"             |
| (Pediatric) |          | Δ508 screen,                |             | "inconclusive"               |
|        |             | ⇨ 2 alleles                  |             | "inconclusive" (Request repeat) |
|        |             | ⇨ 1 allele                   |             |                               |
|        |             | ⇨ No alleles                 |             |                               |
|        |             | And IRT ≥ 130 ng/ml          |             |                               |
| MA     | ≥95%        | monthly IRT                 |             | Positive screen              |
|        |             | 27 mutation screen           |             |                               |
|        |             | ⇨ 2 alleles                  |             |                               |
|        |             | ⇨ 1 allele                   |             |                               |
|        |             | ⇨ No alleles                 |             | And IRT ≥ 99.8% and No       |
|        |             |                               |             | Repeat IRT < 95%             |
| NJ     | ≥90 ng/ml   | Δ508 screen,                | Positive screen |
|        |             | ⇨ 2 alleles                  | Repeat IRT ≥ 90 ng/ml |
|        |             | ⇨ 1 allele                   | Repeat IRT ≥ 130 |
|        |             | ⇨ No alleles, IRT > 130      | Repeat IRT ≥ 90 ng/ml |
| NY     | ≥95%        | Daily IRT                   | Positive screen |
|        |             | 31 mutation screen           |             |                               |
|        |             | ⇨ 2 alleles                  |             |                               |
|        |             | ⇨ 1 allele                   |             |                               |
|        |             | ⇨ No alleles                 |             | And IRT ≥ 99.8%              |
identified, a second sample would be requested for a repeat IRT from those infants with highly elevated initial IRT levels. If the second sample had a persistently elevated IRT, a referral to a pulmonologist would be made. If an infant had a ΔF508 allele, the infant was referred to a pulmonologist for clinical evaluation and possible sweat testing or CF mutation testing.

Wisconsin, Massachusetts, and New York each tested for 25 to 31 mutations for all infants with an elevated IRT (IRT/DNA–multiple mutations). In addition, samples with IRT results $\geq 99.8\%$ were considered abnormal, regardless of mutation status. However, follow-up for these infants was different in each state. In New York, those in the top 0.2% on the first sample were referred for sweat testing. In Massachusetts, those in the top 0.2% on the first sample were referred for sweat testing unless a repeat sample was collected and had a subsequent IRT below the 95th percentile. In Wisconsin, those samples with IRT values $\geq 170$ ng/mL were reported to the primary physician as “remote possibility” of CF and were encouraged to pursue a sweat test only if there was a family history of CF or clinical symptoms.

Yale University and Pediatrisk Screening used a two-step DNA based approach (IRT/DNA–ΔF508–multiple mutations). At Yale, after screening for ΔF508, any sample with one ΔF508 allele was screened for 85 other mutations. A sweat test was recommended for infants with a second mutation identified on the expanded panel. Pediatricians were advised to consider a sweat test for infants if there were clinical symptoms, when either just ΔF508 was identified, or even with no mutations but with an IRT $\geq 200$ ng/mL.

Pediatrisk Screening used a similar approach but tested for 33 additional mutations if one ΔF508 mutation was found. Carriers of ΔF508 only were considered “inconclusive,” and no specific follow-up was recommended. Infants who did not have a ΔF508 allele but whose initial IRT exceeded a higher threshold ($\geq 130$ ng/mL) were also considered “inconclusive.” In this case, a second IRT was requested, and if this remained elevated ($\geq 90$ ng/mL), then the 33 mutation panel was performed. If two mutations were found, the test was considered positive, but if one or no mutations are found, the test was still considered “inconclusive.”

Table I shows the positive predictive value (PPV), defined as the number of positive sweat tests in each program over the number of positive screens requiring a sweat test. Because the programs made different decisions about whether to recommend a sweat test after a “positive” screen and how to interpret results, the positive predictive values listed are not indicative of one approach being superior to another in this regard. In some programs, screening results that were labeled as inconclusive or remote were recommended for sweat tests, and in other programs sweat tests were not recommended. In some programs, infants with borderline sweat tests who had two identified mutations were considered to have CF, but not in all programs.

**Communication and Follow-Up**

Representatives from each screening laboratory contacted the primary care physician to inform him or her of the infant’s results. In five states, including Connecticut, Wisconsin, New Jersey, Massachusetts and New York, a CF center or CF consultant was also contacted by the screening laboratory whenever there were positive screening results. In all states, the parent typically learned of the abnormal CF screen result through the primary care physician, not directly from the screening laboratory or CF center.

Most programs referred all positive screen patients for sweat tests to specific CF centers that were proficient at testing and interpretation. The programs in CT were each affiliated with a CF center, and the CF centers communicated with the primary care physician of all positive infants and arranged for a sweat test, often on the same day or next day. Massachusetts recommended that all infants with positive screens be seen at one of five CF centers, all of which followed a protocol that included consistent definitions for sweat test interpretation, availability of genetic counseling, and reporting of all outcome data back to the screening program. In Wyoming, parents were offered an incentive to travel to the CF centers in Salt Lake City or Denver; on receipt of the sweat test results, the health department would reimburse parents for the mileage traveled. Montana did not specify particular facilities for testing; the state simply recommended that positive screen patients seek consultation with a clinician who specializes in CF.

Sweat test results are routinely disclosed in person in Wisconsin and New Jersey and sometimes in Massachusetts and Connecticut. The result interpretation is more complex with IRT/DNA approaches since with these approaches, false-positives are CF carriers.17,33 Within the states currently using IRT/DNA approaches, follow-up genetic counseling was routinely available in Wisconsin and Massachusetts and is sometimes available in Connecticut and New York.

**DISCUSSION**

This paper demonstrates that that there is currently no single CF newborn screening model in the United States. In some states, screening for CF was offered to every infant, whereas in other states, screening depended on a particular hospital or primary care physician’s decision. States varied widely in terms of what was considered an elevated IRT screening result, whether DNA testing was done, which mutations were tested for when DNA testing was done, and how results were interpreted.

The policy decision about whether and how to offer CF newborn screening within a state was made for a range of reasons and by a range of stakeholders. In most states, the decision about CF screening followed deliberations by a state newborn screening advisory body. Screening advocates, including physicians, parents, and laboratories, also played a role in decision-making in several states.

However, the enthusiasm of an advocate should not be the primary determinant of screening. Rather, the benefits, risks, and costs must be weighed carefully by impartial policymakers before deciding to begin screening.7,34 In fact, the recent CDC report did not recommend that states begin CF NBS.23 Instead, it concluded that screening was justified.
but should be made in the context of resources and priorities and with attention to proper planning and implementation to ensure that the program benefits offset the risks and costs. These benefits, risks, and costs can vary, depending on how screening is conducted. Thus, when policy makers in other states deliberate about whether to add CF to NBS programs, the decision should be made in conjunction with decisions about the specific approach and strategies and with securing of appropriate financial and infrastructure support.

The diversity of approaches to testing illustrates the specific decisions relevant to any DNA-based newborn screening program, including which and how many mutations to screen and the provisions for counseling carriers.\(^{33,35}\) In DNA-based CF screening, for example, an increase in the number of mutations tested would improve the sensitivity of the screen but would also increase the number of unaffected carriers identified, thus decreasing the PPV of the screening program. This would increase the need for resources for appropriate communication of genetic results.

The reported PPVs (defined by the number of positive sweat tests relative to positive screens) of the CF screening programs varied. Although most of the IRT/DNA screening programs had a PPV from 9% to 11%, there were outliers of 2.6% and 100%. An additional determinant of PPV is the IRT cutoff used; the lower the cutoff, the lower the PPV. Also, those strategies that recommend that some infants with no identified mutations obtain a sweat test also reduce the PPV, because most people with CF have identifiable mutations.

However, in some programs, the PPV was increased by defining positive screens more strictly. For example, Pediatric Screening and Yale only referred those infants who had two identified alleles for sweat testing. Of course, this and other approaches to increase the PPV will decrease the sensitivity of screening. However, these programs will not be able to determine the sensitivity of their screening protocols because this requires identification of all children with CF who were not screened, as part of comprehensive surveillance.

Balancing the detection of as many patients as possible with minimizing the number of false-positives is an ethical challenge for any screening program. One advantage of DNA-based programs in addressing this challenge is the opportunity to choose not only the number of mutations to be screened for but also to select mutations based on correlations with phenotypes. This approach may have a more favorable risk/benefit ratio than approaches that maximize sensitivity. For example, testing only for alleles most closely associated with pancreatic insufficiency (ie, only class I, II, or III)\(^{36}\) rather than using the panel developed for CF carrier testing based solely on allele frequency that include class IV and V mutations such as R117H\(^ {17,37}\) could allow for detection of the patients with CF most likely to benefit from nutritional interventions.\(^ {11}\) This selective approach would also minimize the impact of false-positive identification on the families and on the health care system by reducing the number of false-positives and thereby increasing the opportunity for better communication of results. As with any screening program with expected false-negative results, it will be necessary to remind primary care physicians to continue to be alert for symptoms suggestive of CF.

However, this approach has not been used in the current seven DNA-based screening programs. Perhaps this is because newborn screening programs have traditionally focused on conditions such as PKU, in which maximizing sensitivity was the essential goal. As state policymakers consider whether to add CF to newborn screening programs, acknowledging a goal of balancing benefits and risks in the design of the program might incline policymakers to select screening strategies that strike this balance.\(^ {38}\) Further, new CF screening programs can also improve their benefit/risk ratio by learning from the experiences of current programs. Thus, developing a system to collect health-related and psychologic outcomes from screening programs could facilitate further expansion of CF screening in a uniform direction.

The results of this study are limited because all the information came from the individuals who are invested in the programs. As a consequence, the data may be biased towards descriptions of what the screening process should be in an ideal situation. To obtain a more thorough assessment of the benefits and risks of these approaches, future research should include assessments of parents and pediatricians’ perspectives.

Although a single dominant approach to screening for CF may ultimately emerge, until there are sufficient data, policymakers are likely to consider a range of approaches. State policymakers should deliberate about the best way to develop a CF NBS program, including how to approach testing in the population, to provide information to parents about screening, to select a testing strategy, to communicate test results, to provide follow-up care, and to collect data about clinical outcomes for quality assurance.

The authors thank each of the informants for their time and valuable contributions: Jan Baker, James Beebe, Anne Comeau, Marie Egan, Suzanne Ficara, Dennis Freer, Larry Goodmay, Thomas Hickey, Gary Hoffman, Cathy Kolakoski, Jerry McClure, Karen Musser, Francesca Nadeau, Regina Palazzo, Ken Pass, Kathy Tomashitis, and Dan Wright. Scott Grosse, Colleen McBride, Lainie Ross, and Marci Sontag are thanked for comments on an earlier version of the manuscript.

**REFERENCES**

PHENOTYPIC AND GENETIC CHARACTERIZATION OF PATIENTS WITH FEATURES OF “NONCLASSIC” FORMS OF CYSTIC FIBROSIS

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Objective To determine which features of incomplete or “nonclassic” forms of cystic fibrosis (CF) are associated with deleterious CF transmembrane conductance regulator gene (CFTR) mutations, and to explore other etiologies for features not associated with deleterious CFTR mutations.

Study design Clinical features were compared between 57 patients with deleterious mutations in each CFTR and 63 with no deleterious mutations. The Shwachman Bodian Diamond syndrome gene (SBDS) was sequenced to search for mutations in patients with no deleterious CFTR mutations and steatorrhea to determine if any had unrecognized Shwachman-Diamond syndrome (SDS).

Results The presence of a common CF-causing mutation, absence of the vas deferens, and Pseudomonas aeruginosa in the sputum correlated with the presence of two deleterious CFTR mutations, whereas sweat chloride concentration, diagnostic criteria for CF, and steatorrhea did not. However, sweat chloride concentration correlated with CFTR mutation status in patients infected with P aeruginosa. One patient had disease-causing mutations in each SBDS.

Conclusions Presence of a common CF-causing mutation, absence of the vas deferens and/or P aeruginosa infection in a patient with features of nonclassic CF are predictive of deleterious mutations in each CFTR, whereas steatorrhea in the same context is likely to have etiologies other than CF transmembrane conductance regulator (CFTR) dysfunction. (J Pediatr 2005;146:675-80)

Persons with classic cystic fibrosis (CF) have clinical manifestations in the pancreas, respiratory tract, male reproductive tract, and sweat gland.1 Approximately 10% of patients with CF have disease manifestations present in only some of the aforementioned organ systems. This incomplete CF phenotype has been termed pancreatic sufficient CF, atypical CF, and variant CF on the basis of organ involvement. Given the redundancy of these definitions, we and others have proposed to group the latter under the single descriptor “nonclassic” CF.2 Loss of function mutations in each copy of the CF transmembrane conductance regulator gene (CFTR) have been shown to cause classic CF, whereas mutations that causes partial loss of function in combination with loss of function mutations or other partial loss of function mutations in the other CFTR have been associated with nonclassic CF. The diagnosis of nonclassic CF can be difficult to make because of unusual presentations and borderline laboratory tests.3-6

Our laboratory has been investigating the role of CF transmembrane conductance regulator (CFTR) in patients with features of nonclassic CF. In a previous study, we found that 41% of the 74 patients studied did not have deleterious mutations identified after exhaustive scanning of the functional regions of CFTR.7 Analysis of clinical data from these patients did not reveal any significant differences in organ system involvement, degree of disease severity or sweat chloride concentration with two, one, or zero CFTR mutations. However, several trends were noted suggesting that analysis of a larger number of patients may reveal significant differences. In this study, we review findings from an expanded...
collection of 158 patients with features of nonclassic CF. We show that certain clinical manifestations are more likely to be associated with CFTR dysfunction as a result of deleterious CFTR mutations, whereas the presence of other manifestations should prompt a search for other etiologies.

METHODS

Patient Population

Patients were referred by clinicians experienced in the diagnosis of CF and were accepted into this study during a 5-year period from 1998 to 2003. At referral, each patient had one or no mutations after screening for the most common CF-causing mutations. Clinical data were collected for each patient using a form (http://www.hopkinsmedicine.org/cfgenotyping/index.html) that asked the referring clinician to describe the CF-like clinical manifestations in each of the following organ systems: (1) CF-like respiratory disease defined as one or more of the following: sputum cultures positive for Pseudomonas aeruginosa, chronic productive cough, documented pneumonia, reactive airway disease, abnormal pulmonary function tests (pulmonary function tests were unavailable in 19 children <6 years of age, and 11 adults), abnormal chest x-ray and/or computed tomography (CT) indicating bronchiectasis, infiltrates, or blebs, chronic sinusitis, and/or nasal polyposis; (2) recurrent episodes of pancreatitis or malabsorption manifesting as steatorrhea (fetal fat values >7g/day during a 72-hour period). Fecal fat levels were not available in six patients. In these cases, steatorrhea was confirmed by reduced malabsorption following pancreatic enzyme supplementation (n = 4) or significantly reduced immunoreactive serum trypsinogen levels and clinical evidence of malabsorption (n = 2); and (3) male infertility as a result of absence of the vas deferens by semen analysis, rectal ultrasonography, or physical exam. Patients who had disease manifestations in all three organ systems were excluded. Each patient had two or more measurements of chloride concentration in sweat induced by pilocarpine iontophoresis.

Genotyping of CFTR was performed for each patient using direct DNA sequencing of all 27 exons and bordering intronic regions. Details of this technique have been reported elsewhere. A mutation was predicted to be deleterious if: (1) it had been previously reported as a disease-associated mutation (http://www.genet.sickkids.on.ca/cftr/); (2) it created a nonsense codon, induced a frameshift, or altered canonical splice-site sequences; and (3) it created an amino acid substitution in a residue that was conserved in CFTR of many species or was not found in at least 100 normal CFTR. A list of all identified mutations is available from the corresponding author. All studies were approved by The Johns Hopkins Joint Committee on Clinical Investigation, and written consent was obtained for all participants.

Statistical Analysis of Clinical Features

Because the role of CFTR function in patients with only one CFTR mutation is unclear, we performed comparisons of clinical features that were limited to patients with two mutations where CFTR dysfunction was highly likely (n = 57), versus those without any mutations where CFTR function was highly likely to be intact (n = 63). Univariate comparisons were performed for nine variables among patients with two or zero CFTR mutations: sweat chloride concentration, forced expiratory volume in 1 second, forced vital capacity, age of diagnosis, documented steatorrhea, congenital bilateral absence of the vas deferens (CBAVD), abnormal chest x-ray/CT, presence of P aeruginosa in sputum, and nasal polyps. Comparison of P aeruginosa and sweat chloride levels by mutation status was performed by Fisher’s exact test. For sweat chloride concentration, forced expiratory volume in 1 second, forced vital capacity, and age at diagnosis we used one-way analysis of variance to compare values between the two groups, and for the remaining (discrete) variables we performed χ² analysis. Fisher’s exact test, analysis of variance, and χ² analysis were performed using the JMP IN statistical package (version 3.2.6, SAS Institute, Inc., Cary, NC).

Binary logistic regression was performed where the dependent variable was the presence of two CFTR mutations, or not. The seven independent variables included: average sweat chloride concentration, age at diagnosis, steatorrhea, CBAVD, abnormal chest x-ray/CT, nasal polyps, and positive cultures for P aeruginosa. Measurements of pulmonary function were not reported for 36 patients, and were therefore not included in the logistic regression analysis. The logistic regression was first performed by including all variables in the model, as well as by using forward stepwise analysis. Logistic regression was performed using the Statistical Package for the Social Sciences for Windows (11.5.0, SPSS Inc., Chicago, Ill).

Genetic Analysis of SBDS

Steatorrhea as a result of pancreatic insufficiency is a common feature of patients with Shwachman-Diamond Syndrome (SDS; online mendelian inheritance in man [OMIM] 260400), which has recently been shown to be caused by mutations in the Shwachman-Diamond syndrome gene (SBDS). SBDS was analyzed in 18 patients who did not have two CFTR mutations, and who had steatorrhea. To find mutations in SBDS, exons and bordering intronic regions were amplified from genomic DNA using polymerase chain reactions (PCR). SBDS is approximately 5.8Mb from a pseudogene (ΨSBDS) that shares 97% nucleotide identity. Thus, PCR primers were designed to discriminate between SBDS and its highly conserved pseudogene. PCR products were purified using Qiaquick purification kits (Qiagen, Valencia, Calif), sequenced using ABI dye-terminator sequencing reactions (Applied Biosystems, Foster City, Calif), and analyzed using the Sequencher analysis program (Gene Codes, Ann Arbor, Mich).

RESULTS

A total of 158 patients with manifestations compatible with nonclassic CF were enrolled in the study (Table I), 74 of whom were described in a previously published paper.
Seventy-one patients were referred with a single mutation identified by standard DNA-based tests that typically identify approximately 85% of known CF-causing alleles, whereas 87 had no common CF-causing mutations. We identified two known deleterious or putative deleterious CFTR mutations in 57 patients, a single deleterious mutation in 38 patients, and no deleterious CFTR mutations in 63 patients. At referral, 78 patients met the diagnostic criteria for CF whereas 80 did not. We then examined whether mutation status at referral or diagnostic criteria at referral predicted which patients had CFTR dysfunction (ie, two deleterious CFTR mutations).

Of the 57 patients with two deleterious CFTR mutations, 46 patients presented with a common CF-causing mutation (81% sensitivity), but only 31 met diagnostic criteria for CF (54% sensitivity). Of the 101 patients who did not have two deleterious CFTR mutations, 76 patients presented without a common CF-causing mutation (75% specificity), whereas 54 patients did not meet diagnostic criteria (53% specificity). Thus, presentation with a common CF-causing mutation ($P < .000001$), and not the CF diagnostic criteria ($P = .41$), was more predictive of CFTR dysfunction in a patient with clinical features of nonclassic CF.

To identify any major differences in clinical presentation between patients with CFTR dysfunction versus other etiologies, we compared clinical features between patients with two or zero deleterious CFTR mutations. Univariate analysis of key clinical features revealed that the mean sweat chloride concentration was near the diagnostic cutoff level for the two groups, and was neither sensitive nor specific for the presence of CFTR mutations (Figure 1). The extent of airways disease as reflected by pulmonary function tests, abnormal chest x-rays, and the frequency of nasal polyps were equally variable among the two genotype groups (Figures 1 and 2). Patients without CFTR mutations were diagnosed at an earlier age and had a higher frequency of steatorrhea than patients with 2 CFTR mutations (Figures 1 and 2). On the other hand, the frequency of congenital absence of the vas deferens and presence of P aeruginosa in the sputum on at least one occasion was significantly more common in patients with CFTR mutations (Figure 2).

We also investigated whether particular combinations of clinical features correlated with the presence of deleterious CFTR mutations. Intriguingly, patients with P aeruginosa infections and sweat chloride concentrations <40 mmol/L were more likely to have zero CFTR mutations, whereas those with P aeruginosa infection and sweat chloride concentrations greater than >mmol/L were more likely to have two CFTR mutations ($P = .003$) (Figure 3). Binary logistic regression analysis using seven presenting features as independent variables resulted in a model that predicted 71.0% of the correct outcome for the dependent variable (presence of two or zero mutations). However, an alternative model using only three of the seven variables correctly predicted 66.8% of the outcome (Table II). Thus, the majority of the variation in the total regression model could be explained by the combination of absence of the vas deferens, positive P aeruginosa culture, and absence of steatorrhea.

Because steatorrhea as a presenting feature in nonclassic patients was not correlated with CFTR dysfunction (ie, two deleterious CFTR mutations), we searched for another molecular etiology in this group of patients. Patients with SDS have steatorrhea because of pancreatic exocrine insufficiency as a result of hyposcretion of enzymes from pancreatic acinar cells, rather than obstruction of the pancreatic duct as seen in CF. Because patients with nonclassic CF or SDS can have steatorrhea, we examined the gene responsible for SDS (SBDS) in the 18 patients without CFTR mutations who had steatorrhea. Sequence analysis revealed

<table>
<thead>
<tr>
<th>Sweat [Cl-] (mmol/L)</th>
<th>Respiratory</th>
<th>Gastrointestinal</th>
<th>Male reproductive</th>
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<td>&gt;60</td>
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<td>14</td>
</tr>
<tr>
<td>Total</td>
<td>94</td>
<td>14</td>
<td>3</td>
<td>47</td>
</tr>
</tbody>
</table>

Table I. Clinical features of 158 patients enrolled in this study

**Figure 1.** Comparison of quantitative features among patients with two or zero CFTR mutations. Each data point represents a value from a single patient, and horizontal bars indicate mean values. The diagnostic cutoff for sweat chloride concentration is shown with the horizontal dotted line.
that one of the 18 patients had the most common mutation known to cause SDS (258<sup>12T→C</sup>) and a single base pair deletion mutation in exon 1 (123delC). Analysis of the SBDS in each parent confirmed that these mutations did not occur in the same gene, but were inherited independently from each parent. The patient had steatorrhea, low serum trypsinogen, chronic sinusitis, asthma, recurrent pulmonary infections with Staphylococcus aureus positive cultures, and mild digital clubbing. Sweat chloride concentrations were 44 and 49 mmol/L on 70 and 60 mg of sweat, respectively. The patient exhibited several behavioral problems and was diagnosed with attention deficit disorder and Tourette’s syndrome. Absolute neutrophil counts (ANC) over a 1-year period revealed mild, variable neutropenia (ANC 600-1700/mL), with normal platelets (158,000-190,000/mL), and hemoglobin (12.1-13.7 g/dL). There were no long bone or rib abnormalities evident upon examination of x-rays.

**DISCUSSION**

The diagnosis of CF is based primarily on clinical manifestations accompanied by evidence of CFTR dysfunction such as elevated sweat chloride concentration, a characteristic nasal potential difference profile, or two deleterious mutations.
Although the diagnostic criteria are sensitive and specific for classic CF as a result of CFTR dysfunction, the criteria were not able to accurately identify those with CFTR involvement (as defined by the presence of known deleterious or predicted deleterious mutations in each CFTR) in these patients with features of nonclassic CF. Furthermore, an ion transport defect documented by elevated sweat chloride concentration did not differentiate patients with two or zero CFTR mutations. This finding demonstrates that the “gold standard” test for classic CF was neither sensitive nor specific for CFTR dysfunction in these patients. Nasal potential difference measurement may provide a more sensitive assay for ion transport defects that could differentiate etiology in patients with nonclassic CF. Unfortunately, this test was performed on too few patients to incorporate into our analyses. However, the presence of one common CF-causing mutation was a useful predictor of those with CFTR dysfunction as the etiology of their nonclassic CF phenotype.

In addition to the presence of a common CF mutation, two clinical features, absence of the vas deferens and infection with P aeruginosa, were highly associated with the presence of two deleterious CFTR mutations in patients presenting with nonclassic CF. Although absence of the vas deferens in men with nonclassic CF is a sensitive clinical indicator of CFTR involvement, only 36% of males in this study were examined for CBAVD by either semen analysis, palpation of the vas, or ultrasonography. This is likely to be a result of the fact that many males are not screened for CBAVD until it presents as infertility in adulthood. The results of this study suggest that an evaluation for reproductive tract abnormalities should be considered for every male who presents with clinical features of nonclassic CF.

Infection with P aeruginosa is a common feature of classic CF but a highly variable feature in patients with nonclassic CF. However, two observations demonstrate a close relationship between infection with this organism, CFTR dysfunction, and abnormal ion transport in patients with nonclassic CF. First, patients with two deleterious CFTR mutations were significantly more likely to have a sputum culture positive for P aeruginosa. Second, in this study patients with two CFTR mutations and P aeruginosa were more likely to have elevated sweat chloride concentration, whereas patients with zero CFTR mutations and P aeruginosa were more likely to have a normal sweat chloride concentration. These findings are somewhat unexpected because sweat chloride concentration does not differ between genotype groups as a whole, but differs only when considering patients with P aeruginosa infection. The positive correlation between P aeruginosa infection and sweat chloride concentration in patients with CFTR defects suggests that susceptibility to infection is specifically correlated with CFTR dysfunction and an electrolyte transport defect.

Pancreatic insufficiency seen in patients with CF is typically associated with complete loss of CFTR function that also results in the development of severe lung disease and highly elevated sweat electrolytes. Most mutations that cause severe loss of CFTR function occur in the coding regions of CFTR and should be readily identified by the genetic analysis performed. Thus, the large number of patients with nonclassic CF referred with steatorrhea in the absence of CFTR mutations was unexpected. However, other studies have reported patients with atypical or mild presentations of CF with pancreatic insufficiency in the absence of two CFTR mutations. The majority of patients in our study had steatorrhea documented by elevated fecal fat levels. This method does not discriminate between fat malabsorption as a result of pancreatic exocrine insufficiency versus primary gastrointestinal absorptive defects, indicating that other causes of steatorrhea were possible.

The latter concept was evaluated by sequencing the SBDS in each of the patients with nonclassic CF with steatorrhea who had no deleterious CFTR mutations. One patient was found to have a loss of function mutation in each SBDS that would predict a classic presentation of SDS. However, the borderline elevated sweat chloride concentration contributed to a diagnosis of nonclassic CF in this patient. In retrospect, the sweat chloride concentrations were determined from low volumes of sweat, which can in some cases give erroneous quantification of electrolytes. Furthermore, the patient exhibited transient neutropenia that was not recognized as being consistent with SDS. This patient appears to be an atypical form of SDS rather than CF. Given that only 1 of 18 patients with steatorrhea had SDS, unrecognized SDS is not likely to account for a large fraction of nonclassic CF cases with steatorrhea.

Ascertaining the etiology of atypical presentations of single gene disorders can be difficult, and often cannot be based on clinical or genetic data alone. Recognizing that molecular abnormalities other than CFTR dysfunction can masquerade as nonclassic CF should trigger re-evaluation of the etiology in certain nonclassic cases. In such cases, integration of clinical, genetic and nongenetic information may help in classifying the phenotypic variability of nonclassic CF into distinct etiologic subgroups. This may be of particular importance for patients with features of nonclassic CF, as prognosis and treatment is likely to depend on the etiology of their disease.

We are indebted to the DNA Diagnostic Laboratory at Johns Hopkins and the clinicians and staff of the CF Centers who referred patients. Most of all, we would like to thank the patients and families for their willingness to participate in this study.

REFERENCES
Objectives  Cystic fibrosis-related diabetes (CFRD) has emerged as an important complication of CF. To better understand who is at risk of developing CFRD, to gain insight into the impact of CFRD on pulmonary and nutritional status, and to assess the association of CFRD with various practice patterns and comorbid conditions, we characterized the Epidemiologic Study of Cystic Fibrosis (ESCF) patient population.

Study design  Analyses were performed on the 8247 adolescents and adults who were evaluated at one of 204 participating sites during 1998. CFRD was defined as the use of insulin or an oral hypoglycemic agent at any time during the year.

Results  Previously reported risk factors for CFRD including age, gender (female), and pancreatic insufficiency were confirmed in this study. Patients with CFRD had more severe pulmonary disease, more frequent pulmonary exacerbations, and poorer nutritional status as compared with those without diabetes. CFRD also was associated with liver disease.

Conclusions  CFRD is a common complication in adolescents and adults that is associated with more severe disease. (J Pediatr 2005;146:681-7)
Taken together, a compelling case can be made that we need a more comprehensive understanding of this common complication of CF. To better understand who is at risk of developing CFRD, to gain insight into the impact of CFRD on pulmonary and nutritional status, and to assess the association of CFRD with selected practice patterns and comorbid conditions, we characterized the patient population in the Epidemiologic Study of Cystic Fibrosis (ESCF).

METHODS

The ESCF is a longitudinal, encounter-based patient registry that has been used to characterize demographics, practice patterns, and risk factors for decline in pulmonary function of the patient population in the United States. Institutional review board approval and written informed consent were obtained at institutions where required. There were 10,695 patients ≥13 years of age who were in ESCF throughout 1998 (definition: enrolled in ESCF before 1/1/98, ≥13 years of age on 1/1/98, and had not discontinued before 12/31/98). Of these, there were 8247 patients (77%) who were included in our analysis because they had at least one clinic visit at one of the 204 participating sites in 1998 and were therefore evaluable for the use of routine therapies and medical conditions. Age was defined at the beginning of the year. Diabetes was defined as the use of insulin or an oral hypoglycemic agent at any time during the year. Because of the age difference between the diabetic and nondiabetic populations, for some analyses we subdivided the patients into the following age categories: 13 to 17, 18 to 25, 26 to 35, and >35 years of age.

For the pulmonary function measure, the latest spirometry obtained during the year while the patient was in a stable condition was used for the analyses and was expressed as percent predicted using the Knudson reference equation. Pulmonary function was categorized as severe (<40% predicted), moderate (40% to 70% predicted), mild (70% to 100% predicted), or normal (>100% predicted). For weight and height measures, the values at the latest visit during the year were used in our analysis because they had at least one clinic visit at one of the 204 participating sites in 1998 and were therefore evaluable for the use of routine therapies and medical conditions. Age was defined at the beginning of the year. Diabetes was defined as the use of insulin or an oral hypoglycemic agent at any time during the year. Because of the age difference between the diabetic and nondiabetic populations, for some analyses we subdivided the patients into the following age categories: 13 to 17, 18 to 25, 26 to 35, and >35 years of age.

Gender and Age Distribution of CFRD

The gender distribution of the entire adolescent and adult population was 54.4% males and 45.6% females. However females were disproportionately affected by CFRD with a prevalence of 17.1% as compared with 12.0% in males. The mean (SD) age of the CFRD population was 25.9 (8.9) years as compared with 22.5 (8.5) years for the non-CFRD group.

Pancreatic Status and Genotype

Previous reports suggested that pancreatic exocrine insufficiency is a risk factor for the development of CFRD. We defined pancreatic insufficiency as the use of pancreatic enzymes during 1998. By this crude definition, we found that 15.0% of adolescents and adults with pancreatic insufficiency had CFRD as compared with 5.7% of patients who were pancreatic sufficient, age-adjusted P value <.001.

Genotype is highly predictive of pancreatic status. The δ F508 homozygous genotype is known to be associated with pancreatic insufficiency in nearly all patients. Patients with known genotype (n = 3160 or 38.3% of the patient population) were subdivided into three categories with respect to δ F508 status: homozygotes (n = 1586), compound
heterozygotes (n = 1263), and “other/other” (n = 311). The δ F508 homozygotes had a higher prevalence of CFRD as compared with the compound heterozygotes and “other/other” genotype groups (see Figure 1). Note that the prevalence of CFRD in the δ F508 homozygotes increased in each successive age group.

**Figure 1.** Prevalence of CFRD by age group and δ F508 genotype. This graph represents the 38.9% of the adolescent and adult population with known genotype. Note that CFRD is more common in the δ F508 homozygotes in each age group.

**Pulmonary Function, Pulmonary Exacerbations, and Sputum Microbiology**

Spirometry was available on 89.2% of the adolescent and adult population. The prevalence of CFRD was higher in patients with more severe pulmonary disease; 22.6% in patients with FEV₁ percent predicted <40%, 16.4% in patients with FEV₁ percent predicted from 40% to 70%, 9.1% in patients with FEV₁ percent predicted from 70% to 100%, and 5.9% in patients with FEV₁ percent predicted >100%.

The CFRD population had worse pulmonary function than the non–CFRD population, with a mean (SD) FEV₁ percent predicted of 55.4% (24.2) as compared with 67.5% (25.8), age adjusted P < .001. When the population was subdivided into age categories, the same pattern was observed in each category. The most notable difference was in the adolescent population, with an FEV₁ percent predicted from 40% to 70% of the CFRD group as compared with the nondiabetic population, with a mean (SD) FEV₁ percent predicted of 65.0% (25.3) in the CFRD group versus 77.4% (23.4) in the nondiabetics group.

The group with CFRD also had more pulmonary exacerbations treated with parenteral antibiotics in the preceding year than the non–CFRD group, with a mean (SD) of 1.55 (1.84) as compared with 0.78 (1.32) exacerbations, age adjusted P < .001. In the nondiabetic group, 60.7% of the patients did not have a pulmonary exacerbation during 1998 as compared with 36.4% of the CFRD group. A much greater proportion of the CFRD population (23.6%) had frequent pulmonary exacerbations (ie, ≥3) as compared with the nondiabetic population (9.5%). This striking difference in the number of exacerbations between the two groups was evident in each age category (data not shown).

Sputum microbiology, available on 84.9% of the patient population, also differed between the CFRD and non–CFRD groups. The CFRD group had a higher prevalence of *Pseudomonas aeruginosa* (84.1% vs 77.2%, P < .001), *Burkholderia cepacia* complex (10.3% vs 5.2%, P < .001), *Stenotrophomonas maltophilia* (8.6% vs 6.1%, P = .006), *Candida* (23.3% vs 16.5%, P < .001), and *Aspergillus* (18.8% vs 13.9%, P = .001). Most notable was the much higher prevalence of *B cepacia* complex in the CFRD group, which was apparent and statistically significant in each age category (data not shown).

**Table. Medical interventions**

<table>
<thead>
<tr>
<th>Therapy</th>
<th>CFRD group (n = 7,067)</th>
<th>Non-CFRD group (n = 1180)</th>
<th>Age-adjusted p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pulmozyme® (dornase α)</td>
<td>57.6%</td>
<td>49.8%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Airway clearance</td>
<td>90.7%</td>
<td>84.3%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Mast cell stabilizer</td>
<td>20.3%</td>
<td>17.5%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BD (oral)</td>
<td>21.5%</td>
<td>13.7%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>BD (inhaled)</td>
<td>91.5%</td>
<td>84.3%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>NSAID†</td>
<td>10.6%</td>
<td>9.6%</td>
<td>.206</td>
</tr>
<tr>
<td>Oral supplements</td>
<td>40.5%</td>
<td>32.7%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Enteral supplements</td>
<td>11.7%</td>
<td>7.7%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Parenteral supplies</td>
<td>2.4%</td>
<td>0.9%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Steroids (oral)</td>
<td>27.6%</td>
<td>17.9%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Steroids (inhaled)</td>
<td>48.9%</td>
<td>39.6%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Contraceptives †</td>
<td>12.7%</td>
<td>7.0%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Oxygen</td>
<td>24.2%</td>
<td>9.7%</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Diuretic</td>
<td>5.5%</td>
<td>1.0%</td>
<td>&lt;.001</td>
</tr>
</tbody>
</table>

*Bronchodilators (BDs) include β agonists, short or long acting, and anticholinergic agents.
†Nonsteroidal anti-inflammatory drugs (NSAIDs) include use of ibuprofen at any dose or other drugs in this class.
‡Contraceptives include oral or implanted agents.

**Nutritional Status**

Measures of nutritional status were available on 97.9% of the patient population. They showed greater impairment in the CFRD group as compared with the nondiabetic population, including mean (SD) height-for-age percentile, 28.9 (26.9) versus 35.3 (28.7); mean (SD) weight-for-age percentile, 28.3 (27.1) versus 32.7 (28.3); and mean (SD) body mass index, 20.5 (3.3) versus 20.6 versus (3.4) (age-adjusted P values <.001 for all three parameters).

**Medical Interventions and Comorbid Medical Conditions**

Information on medical interventions and comorbid medical conditions was available on all of the patient population. Treatments for pulmonary disease such as Pulmozyme® (α dornase), airway clearance, bronchodilators, and supplemental oxygen were used more commonly in the CFRD population (Table). This finding is not surprising, given that the patients with CFRD had more severe pulmonary disease than those without diabetes. Nutritional supplements and corticosteroids, interventions that have been associated with CFRD,19,20 as well as oral or implanted contraceptives, were used more frequently in the CFRD population.

A number of comorbid conditions also were more common in the CFRD population, including APBA (6.5% vs.
3.6%, \( P < .001 \), asthma (27.8% vs 23.8%, \( P = .002 \)), sinusitis (26.6% vs 20.4%, \( P < .001 \)), heart failure (1.5% vs 0.3%, \( P < .001 \)), and cirrhosis (4.6% vs 1.8%, \( P < .001 \)).

**Multivariate Logistic Regression Analyses**

Age, gender, and pancreatic insufficiency were associated with CFRD in a statistically significant fashion in all of the models that were developed. Pulmonary disease severity and number of pulmonary exacerbations also were associated with CFRD. Patients who showed evidence of long-standing impaired nutritional status as indicated by low height-for-age percentile also appeared to be at greater risk of having CFRD. Using a base model that included age, gender, pancreatic status, FEV\(_1\) percent predicted, number of exacerbations, and height-for-age percentile >50% for other medical conditions, organism in sputum or therapy, the group with absence of same.

3.6%, \( P < .001 \), asthma (27.8% vs 23.8%, \( P = .002 \)), sinusitis (26.6% vs 20.4%, \( P < .001 \)), heart failure (1.5% vs 0.3%, \( P < .001 \)), and cirrhosis (4.6% vs 1.8%, \( P < .001 \)).

**DISCUSSION**

This is the largest analysis to date of the epidemiology of CFRD. We confirm previous reports that CFRD is associated with increased age, female gender, pancreatic insufficiency,\(^7,8\) and the \( \delta F508 \) homozygous genotype.\(^7,21\) In addition, these new data show a striking correlation between CFRD and worsened clinical status as indicated by poorer pulmonary function, increased frequency of acute pulmonary exacerbations, increased prevalence of important sputum pathogens, poorer nutritional status, and a higher prevalence of liver disease.

An association between an increased number of pulmonary exacerbations and CFRD was suggested in a previous study of 18 Danish patients,\(^13\) but the magnitude of the difference between the CFRD and nondiabetic population in the current large cohort was remarkable. Perhaps the patients with CFRD are more prone to infection. People with diabetes in the general population are at higher risk of influenza and pneumonia.\(^22\) However, it should be noted that a potential ascertainment bias might exist. Patients with more frequent pulmonary exacerbations have more contact with the healthcare team and are therefore more likely to be screened for CFRD.

There also were significant differences in sputum microbiology between the CFRD and non-CFRD groups. Most notable was the high prevalence of \( B cepacia \) complex in the CFRD group. This organism has been associated with increased morbidity and mortality in CF.\(^12,23,24\) We are not aware of any data supporting the notion that patients with diabetes are predisposed to colonization and infection by this organism. The differences in microbiology may, in part, relate to the more frequent pulmonary exacerbations in the CFRD group. Sputum cultures are typically obtained with each pulmonary exacerbation, and each additional sputum culture increases the likelihood of detecting various pathogens. More frequent exacerbations also lead to increased exposure to antibiotics, more contact with medical devices such as nebulizers, and more visits to the clinic and hospital, thus patients with CFRD may be at an increased risk of acquiring respiratory pathogens.

The CFRD group also had a higher prevalence of \( Candida \) and \( Aspergillus \) in sputum cultures. There is evidence that diabetes in the general population is a risk factor for fungal infections.\(^25\) The more intense antibiotic pressure applied to the CFRD group may put them at greater risk of acquiring these organisms. The clinical significance is suggested by the presence of nearly twice as much ABPA in the CFRD group. The most plausible hypothesis for the association between CFRD and ABPA relates to the use of oral steroids to treat ABPA. Our data do not support this hypothesis, but given the relatively small number of affected persons and the potential for misclassification of ABPA, our results should be interpreted cautiously.
Greater nutritional compromise was seen in the CFRD group as has been reported by others. Relative insulin deficiency is the major defect in CFRD and insulin is a very potent anabolic hormone. This may in part explain the nutritional impairment in CFRD. Of note, the nutritional impairment was detectable even in the adolescent group, suggesting that it was not simply related to the older age of the CFRD group. The strong negative association of CFRD with height-for-age suggests that long-term nutritional impairment with growth stunting may put patients at risk for developing CFRD or perhaps may simply reflect the fact that these are sicker patients. Evidence from the developing world suggests that malnourished infants who show improvement in nutritional status during childhood are at greater risk of developing diabetes in adolescence and adulthood. Such data may be relevant to CF, where newly diagnosed patients are often malnourished and a “catch up” growth phase occurs with the institution of a CF treatment regimen.

There was a clear-cut association between CFRD and liver disease in univariate and multivariate logistic regression analyses. Of note, other forms of chronic liver disease in the general population also have been associated with diabetes. This association has been attributed to the presence of peripheral insulin resistance in liver disease. Similar mechanisms may be operative in CFRD, but this requires further investigation.

Nutritional supplements and oral corticosteroids were used more commonly in the CFRD group. However, the associations between these interventions and CFRD were not significant when adjusted for degree of nutritional compromise and pulmonary disease severity in the multivariate logistic regression analyses. In contrast, the use of oral/implanted contraceptives was associated with CFRD in the univariate and multivariate analyses. One might speculate that patients and clinicians are aware that women with CFRD are more likely to develop CFRD or perhaps may simply reflect the fact that these are sicker patients. Evidence from the developing world suggests that malnourished infants who show improvement in nutritional status during childhood are at greater risk of developing diabetes in adolescence and adulthood. Such data may be relevant to CF, where newly diagnosed patients are often malnourished and a “catch up” growth phase occurs with the institution of a CF treatment regimen.

Another limitation of this study is that diabetes was defined by the use of diabetic medications rather than systematic diabetes screening. The 7.8% prevalence of CFRD is similar to what has been reported in other retrospective reports. However, because of the insidious nature of CFRD and the lack of systematic screening at some care centers, this figure probably grossly underestimates the true prevalence of this complication. Centers that meticulously screen for CFRD report a much higher prevalence. Based on oral glucose tolerance tests in a population of 408 patients, the University of Minnesota CF Center found a CFRD prevalence of 9%, 26%, 35%, and 43% in patients 5 to 9 years of age, 10 to 19 years of age, 20 to 30 years of age, and >30 years of age, respectively. Similarly, in a study of 191 Danish patients, 50% of the CF population >30 years of age was reported to have CFRD.

Another limitation of the study is that although the data show a clear association between CFRD and worsened clinical status, they do not prove a causal link between them. Are sicker patients more likely to develop CFRD, or does the presence of diabetes make patients with CF sicker? The answer to this question may become more apparent as this cohort is followed longitudinally.

In summary, this is the largest study to date to demonstrate that CFRD is a highly prevalent complication in the adolescent and adult CF population in the United States. It has confirmed previously reported risk factors for CFRD including age, gender (female), and pancreatic insufficiency. The current data convincingly demonstrate that patients with CFRD have more severe pulmonary disease and more severe nutritional impairment than those without diabetes. New and potentially, clinically important associations with CFRD have been uncovered, including number of pulmonary exacerbations, *B. cepacia* complex, and liver disease.

**APPENDIX: DEFINITION OF COMORBID MEDICAL CONDITIONS**

**Allergic Broncho pulmonary Aspergillus (ABPA)**

For this study, a physician’s diagnosis of ABPA required two of the following three criteria: positive skin test to *Aspergillus*, a positive serum precipitins to *Aspergillus*, a total serum IgE >1000 IU/mL and at least two of the following criteria: bronchoconstriction, pulmonary infiltrates, elevated serum IgE/IgG specific for *Aspergillus*, peripheral blood eosinophilia (>1000/μL), recovery of *Aspergillus* from sputum or hyphae present on smear, or response to steroids.

**Asthma (Reactive Airways)**

If in the treating physician’s opinion, asthma contributed significantly to the patient’s lung disease, the patient was considered as having asthma. The diagnosis of asthma is suggested by the following: episodes of acute airway obstruction reversed by bronchodilators (especially if seasonal), a strong family history of asthma and/or evidence of atopy (such as eczema or hay fever), or laboratory evidence of allergy such as eosinophilia, or elevated IgE.

**Nasal Polyposis**

Evidenced by the formation of polyps in the nasal cavity.
Sinusitis (Symptomatic)

Symptomatic sinusitis for this study was defined as sinusitis severe enough to cause respiratory tract symptoms. Symptoms include headache attributable to sinus pain, increased nasal discharge or postnasal drip, and increased respiratory symptoms (eg, cough).

Atypical Mycobacterium Disease (Treated)

Includes pulmonary diseases that are severe enough to require drug treatment and are caused by Mycobacterium other than M tuberculosis or leprosy bacilli, such as M avium-intracellulare, M kansasii, M xenopi, M szulgai, M scrofulaceum, M fortuitum, and M chelonei.

Heart Failure

Evidence or knowledge of symptoms described as dyspnea, fluid retention, low cardiac output secondary to cardiac dysfunction; or rales (thought related to heart failure), jugular venous distention (JVD), pulmonary edema, or cardiogenic shock.

Liver Disease

Cirrhosis. Diffuse disorganization of normal hepatic structure by regenerative nodules that are surrounded by fibrotic tissue.

Portal Hypertension. Increased pressure in the portal venous system, usually inferred by the presence of collateral circulation, splenomegaly, ascites (free fluid in the peritoneal cavity), or portal-systemic encephalopathy in a patient with chronic liver disease.

Elevated Liver Function Tests. For this study, a liver function test was considered elevated if the serum alkaline phosphatase was greater than 3 times the upper limit of normal for age and gender, or an SGPT/ALT was greater than 2 times the upper limit of normal or a prothrombin time was greater than 1.5 times the upper limit of normal.

REFERENCES


50 Years Ago in The Journal of Pediatrics

VIRAL HEPATITIS AND ITS RISK FROM BLOOD AND PLASMA TRANSFUSIONS


Physicians practicing a half-century ago knew about hepatitis, but they didn’t know much. This well-written review article describes the state of knowledge, vintage 1955. There was “epidemic” or “infectious” hepatitis and “homologous serum” hepatitis. Distinctive risk factors and differences in natural history were recognized, but little else was known. Serum hepatitis could spread by blood transfusion and use of nonsterile needles and syringes. However, it was not appreciated then that what later came to be known as hepatitis B could be contracted by intravenous drug abuse, sexual intercourse, or the transplacental route. No tests were available to detect the virus in asymptomatic individuals. The “Australia antigen” expressed by the hepatitis B virus was not described until 1963. Also, the heterogeneity of serum hepatitis could not be appreciated until non-A non-B hepatitis (which is usually caused by the agent now known as hepatitis C) began to be reported in the 1970s.

What a long way we have come! The blood supply in developed countries is now virtually free of hepatitis viruses. Extremely sensitive tests for diagnosis and disease monitoring are available, and a vaccine against hepatitis B now saves thousands if not millions of lives. However, we must not become too complacent. Hepatitis A and B vaccines are underutilized in the United States and are unavailable to most recipients in developing countries. Treatment of chronic hepatitis B and C infection—although much better than a decade ago—is still extremely costly and often ineffective. Pediatricians should be thankful for investigative successes during the past 50 years but should also continue to strongly endorse and support research involving the pathogenesis, epidemiology, management, and ultimately prevention of all forms of viral hepatitis.

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YMPD1424
10.1016/j.jpeds.2005.01.037
Objectives To determine incidence, outcomes, and risk factors for pediatric cerebral edema with diabetic ketoacidosis (CEDKA) in Canada.

Study design This was a case-control study nested within a population-based active surveillance study of CEDKA in Canada from July 1999 to June 2001. Cases are patients with DKA <16 years of age with cerebral edema. Two unmatched control subjects per case are patients with DKA without cerebral edema.

Results Thirteen cases of CEDKA were identified over the surveillance period for an incidence rate of 0.51%; 23% died and 15% survived with neurologic sequelae. CEDKA was present at initial presentation of DKA in 19% of cases. CEDKA was associated with lower initial bicarbonate ($P = .001$), higher initial urea ($P = .001$), and higher glucose at presentation ($P = .014$). Although there was a trend to association with higher fluid rates and treatment with bicarbonate, these were not independent predictors.

Conclusions CEDKA remains a significant problem with a high mortality rate. No association was found between the occurrence of CEDKA and treatment factors. The presence of cerebral edema before treatment of DKA and the association with severity of illness suggest that prevention of DKA is the key to avoiding this devastating complication. (J Pediatr 2005; 146:688-92)

Diabetic ketoacidosis (DKA) occurs in 15% to 67% of patients with new-onset diabetes, depending on geographic location, and has a frequency of 1 to 10 episodes/100 patient-years in those with established diabetes. Cerebral edema is an uncommon but serious complication of DKA associated with high morbidity and mortality rates. Estimates of the incidence of cerebral edema in DKA (CEDKA) range from 0.40 to 3.1. Recent reports from Britain and the United States have shown a mortality rate of 21% to 24% in CEDKA and significant neurologic sequelae in 21% to 35%. A population-based study from the British Paediatric Surveillance Unit (BPSU) reported one of the lowest incidences for CEDKA, at 0.68%. This suggests that the true incidence probably is lower than that estimated from retrospective reviews, based on data from tertiary care centers.

There is no consensus regarding the pathophysiology of CEDKA. In a retrospective case-control study from 10 pediatric centers, Glaser et al reported that CEDKA was associated with lower partial pressure of arterial CO$_2$ and higher serum urea nitrogen concentration at presentation as well as treatment with bicarbonate and a smaller increase in the serum sodium concentration during treatment. Younger age, new-onset diabetes, and rate and type of fluid administration have also been identified as risk factors.

We report the results of a Canada-wide 2-year prospective surveillance program for CEDKA and a case-control analysis of identified cases. Additional cases were identified retrospectively for the case-control analysis. This study was undertaken to (1) determine the
Incidence and outcomes of cerebral edema associated with DKA in children <16 years of age in Canada; and (2) assess risk factors for CEDKA.

METHODS

Prospective Surveillance for CEDKA

Surveillance for cases of CEDKA was conducted through the Canadian Pediatric Surveillance Program (CPSP) from July 1, 1999, to June 30, 2001. The CPSP coordinates surveillance of Canadian pediatricians for a number of rare conditions of childhood.15 All pediatricians in Canada were mailed monthly report cards and asked to report, among other conditions, any cases of CEDKA in patients <16 years of age. To increase the likelihood of identifying all cases, participants were also asked to report any deaths in pediatric diabetes. CEDKA was defined as sudden or unexpected deterioration in level of consciousness (LOC) associated with pH < 7.35 and/or low bicarbonate with diabetes and ketonuria. Cases of neurologic deterioration in association with hypoglycemia were excluded. After reports of CE (confirmed by CT scan) occurring before treatment of DKA, the definition was expanded to include patients with suspected CE at initial presentation to the emergency department who had a profoundly depressed LOC. Although early CE may not be evident on imaging, confirmatory neuroimaging was required for cases with suspected CE at presentation to avoid inclusion of cases of decreased LOC due only to severity of DKA. Cases with normal neuroimaging presenting after onset of DKA treatment were retained in the analysis, as CEDKA is considered a clinical diagnosis and CE may occur in the absence of radiologic evidence.16

On receipt of a report of a possible case of CEDKA, a detailed report form was sent to the reporting physician(s) and reviewed by the three investigators to confirm the diagnosis. The response rate for the monthly report forms to the CPSP for the study period was 82% for 2300 participants. (CPSP report, 2001) The response rate for the detailed CEDKA report forms was 100%.

Incidence of CE in DKA

The numerator for incidence calculations was the number of confirmed cases of CEDKA identified during active surveillance. For the denominator, the Canadian Institute for Health Information (CIHI) Discharge Abstract Database was searched to determine all patients <16 years of age with DKA coded as the primary or a contributing determinant to hospital admissions. Up to 16 diagnosis fields were searched for any of the following ICD-9 codes related to diabetes: 250.1 (DKA), 250.2 (diabetes with hyperosmolar state), and 250.3 (diabetes with coma). The CIHI data are available based on fiscal year (April 1 to March 31); therefore, cases of DKA from April 1, 1999, to March 31, 2001, served as the denominator for the incidence calculation. The Discharge Abstract Database contains information on 85% of all hospital discharges from acute care facilities in Canada, excluding the province of Quebec.17 We previously reported 83% accuracy in discharge codes for DKA for the CIHI database.18

Case-Control Study

All cases identified prospectively and judged to be true cases were reviewed by a trained research assistant who traveled to each reporting site. To increase the number of cases for risk factor analysis, cases of CEDKA occurring from 1995 to 1999, at reporting institutions only, were identified by medical records searches and reviewed. Two control subjects per case were randomly selected from all cases of DKA not complicated by CE obtained by medical records searches occurring at each reporting institution in the 12 months before the reported case of CEDKA. Control subjects were matched only to treating institution. DKA was managed according to the protocols of the individual institutions or treating physicians and was not standardized. This study was approved by the research ethics boards at each of the institutions reporting a case of CEDKA.

Demographic information, concurrent medical conditions, laboratory data, CT/MRI reports, treatment data, and outcomes were obtained by a single trained reviewer for all English language institutions. To ensure accuracy of data extraction, the first three charts were reviewed by one of the investigators. Three cases from francophone institutions were reviewed by a second bilingual reviewer who was trained by the first reviewer. Both reviewers abstracted three charts to ensure agreement between the reviewers. Hospital course and treatment variables were obtained for CEDKA cases up to the point of development of cerebral edema with matching duration of data collection for 2 control subjects per case. Effective osmolality was calculated by using the formula 2(Na+K) + glucose (mmol/L).

Statistical Analysis

The Wilson score method19 was used to calculate 95% confidence intervals around incidence estimates. Baseline group comparisons are presented as means with standard deviations, unless otherwise indicated. Mann-Whitney and Fisher exact tests, when appropriate, were used to compare baseline characteristics of subjects who presented to the emergency department with cerebral edema versus those who had CEDKA after treatment onset. Differences in demographic and baseline laboratory values between cases and control subjects were assessed by using Student t tests and the Fisher exact test. Treatment and demographic values differing at a significance level of 0.1 or less in univariate analyses were included in two different logistic regression models to assess their impact on the risk of development of CEDKA and severity of illness. All reported P values are 2-sided and were declared statistically significant when they reached a .05 probability level (after Bonferroni adjustment for multiple testing).
RESULTS

Prospective Surveillance for CEDKA

During the 2-year surveillance period, 43 potential cases of CEDKA were reported to the CPSP; 12 were duplicates. Eighteen cases were excluded: 6 occurred before the start of the reporting period, 1 was >16 years of age, 2 had hyperosmolar hyperglycemic syndrome, 8 did not meet the established criteria for CEDKA, and 1 case of CE at presentation of DKA lacked neuroimaging. Thus, 13 cases of CEDKA were documented in Canada during the study period. Of these, 3 occurred in the province of Quebec and were excluded from the incidence calculation. CIHI Discharge Abstract Database data for the study period reported 1960 cases of DKA in patients <16 years of age. Of these, 13 were also coded as having cerebral edema and 3 were coded as having died. The balance of 3 cases unreported to the CPSP may represent either missed reports or cases excluded by the investigators. The incidence of CEDKA was therefore 0.51%, or 5.1 episodes of cerebral edema per 1000 cases of DKA (95% CI, 2.4, 9.4).

Outcome of CEDKA in Prospective Surveillance

Of the 13 prospective cases, 3 (23.1%; 95% CI, 8.2, 50.3) died, 2 (15.4%; 95% CI, 4.3, 42.2) recovered with neurologic impairment, and 8 (61.5%; 95% CI, 35.5, 82.3) recovered without any documented sequelae. Of the two surviving with sequelae, one had seizures and posturing and the other had new facial tics and slow speech at discharge from the hospital. The overall DKA mortality rate from cerebral edema during the prospective surveillance period was 0.15% (95% CI, 0.1, 0.4).

Case-Control Study of Prospectively and Retrospectively Identified Cases

All 13 cases of CEDKA identified through the prospective study were reviewed. Eight of 11 additional cases identified through the retrospective medical records searches at the reporting institutions were included. Access to 1 chart was denied for medicolegal reasons and 2 cases with clinical cerebral edema at initial presentation of DKA were excluded for lack of confirmatory neuroimaging.

Demographic and initial laboratory data from cases identified prospectively and retrospectively were similar, and therefore the data from the all cases were pooled for the case-control study (n = 21).

Description of Cases

The age at presentation of cases of CEDKA ranged from 1.1 to 15.3 years, with a mean (SD) of 9.0 (4.5) years; 38% were male and 76% occurred in patients with new-onset diabetes. Four (19%) patients were reported to have CE at initial presentation of DKA. In general, those presenting with CEDKA tended to be more acidic, with lower pH and bicarbonate, more hyperglycemic, and have a higher BUN, although none of these differences reached statistical significance. For those cases with development of CE after treatment was started (n = 17), the median time of onset was 5.8 hours, with a range of 1.5 to 20 hours (Figure). Ten of the 17 cases had neuroimaging; in 5 there was evidence of cerebral edema; 4 were reported as normal, and 1 report was not located.

Risk Factors for CEDKA

Table I shows the demographic and baseline laboratory values of CEDKA cases and control subjects. Cases of CEDKA were more acidic and dehydrated, with lower pH (P = .004), lower bicarbonate levels (P = .001), and higher urea (P = .001). Higher glucose levels (P = .014) did not reach significance after Bonferroni adjustment. Regression analysis for the baseline laboratory values was not performed, as not all values were available in every subject. Details of treatment factors are presented in Table II. There was a tendency toward an association between CEDKA and higher fluid (P = .020) and sodium (P = .012) infusion rates and treatment with bicarbonate (P = .037), but after Bonferroni adjustment, there was no statistical difference between the groups in any factors. In a logistic regression analysis, CE tended to occur more often in patients with new-onset diabetes (P = .085), those who received higher rates of fluid infusion (P = .090), and those who were treated with bicarbonate (P = .093).

To determine if the higher infusion rates in cases was a reflection of severity of illness, rates of fluid infusion and BUN value at presentation were examined in a logistic regression analysis. The small number of index cases (n = 12) for whom we have complete set of information limits the number of variables included in the regression model. Only initial BUN level remained a significant risk factor for CE, with an odds ratio of 1.42 (95% CI, 1.08 to 1.88; P = .013).

DISCUSSION

Cerebral edema complicated 0.51% of episodes of DKA occurring in individuals <16 years of age in Canada. Previous data based on case series and retrospective chart reviews reported CE in 0.68% to 3% of episodes of DKA in the pediatric population.8,9 The low incidence rate found in our surveillance program is comparable to that reported from the United Kingdom by Edge et al,9 who used similar...
Table I. Demographic and baseline laboratory values in CEDKA cases and control subjects

<table>
<thead>
<tr>
<th>Factor</th>
<th>No. of cases</th>
<th>CEDKA cases</th>
<th>No. of control subjects</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (y)</td>
<td>21</td>
<td>9.0 (4.5)</td>
<td>42</td>
<td>9.6 (4.5)</td>
</tr>
<tr>
<td>New-onset diabetes n (%)</td>
<td>21</td>
<td>16 (76.2)</td>
<td>42</td>
<td>23 (54.8)</td>
</tr>
<tr>
<td>PH</td>
<td>15</td>
<td>7.1 (0.1)</td>
<td>39</td>
<td>7.2 (0.1)</td>
</tr>
<tr>
<td>PCO₂ (kPa)</td>
<td>14</td>
<td>2.9 (1.7)</td>
<td>34</td>
<td>3.4 (1.3)</td>
</tr>
<tr>
<td>HCO₃⁻ (mmol/L)</td>
<td>16</td>
<td>5.9 (2.7)</td>
<td>37</td>
<td>10.2 (5.5)</td>
</tr>
<tr>
<td>Na² measured (mmol/L)</td>
<td>19</td>
<td>135.9 (9.0)</td>
<td>38</td>
<td>138.8 (5.4)</td>
</tr>
<tr>
<td>BUN (mg/dL)</td>
<td>13</td>
<td>37.3 (15.1)</td>
<td>32</td>
<td>19.6 (8.1)</td>
</tr>
<tr>
<td>Glucose (mg/dL)</td>
<td>20</td>
<td>931 (515)</td>
<td>42</td>
<td>607 (277)</td>
</tr>
<tr>
<td>Osmolarity</td>
<td>19</td>
<td>335.6 (38.1)</td>
<td>36</td>
<td>319.5 (20.4)</td>
</tr>
</tbody>
</table>

Values are means (SD) except where indicated. Level of significance after Bonferroni adjustment = .006.

Table II. Treatment factors in CEDKA cases and control subjects

<table>
<thead>
<tr>
<th>Factor</th>
<th>n</th>
<th>Cases</th>
<th>Control subjects</th>
<th>P  value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fluid infusion rate (mL/kg per h)</td>
<td>17</td>
<td>9.16 (6.17)</td>
<td>28</td>
<td>5.20 (2.49)</td>
</tr>
<tr>
<td>Na infusion rate (mmol/kg per h)</td>
<td>17</td>
<td>1.41 (0.75)</td>
<td>28</td>
<td>0.88 (0.60)</td>
</tr>
<tr>
<td>Rate of change in Na measured (mmol/L per h)</td>
<td>12</td>
<td>2.26 (5.70)</td>
<td>17</td>
<td>0.96 (1.18)</td>
</tr>
<tr>
<td>Rate of change in Na corrected (mmol/L per h)</td>
<td>12</td>
<td>1.17 (5.25)</td>
<td>18</td>
<td>0.19 (1.13)</td>
</tr>
<tr>
<td>Rate of change of glucose (mg/dL per h)</td>
<td>13</td>
<td>−6.47 (5.93)</td>
<td>23</td>
<td>−4.32 (5.27)</td>
</tr>
<tr>
<td>Bicarbonate use, (n (%))</td>
<td>17</td>
<td>4 (23.5)</td>
<td>34</td>
<td>1 (2.9)</td>
</tr>
</tbody>
</table>

Values are means (SD) except where indicated. Level of significance after Bonferroni adjustment = .008.

ascertainment methods through the BPSU. The lower incidence in these population-based studies compared with series reported from tertiary care centers is not surprising, since the latter are subject to referral bias. Data from CIHI, the national Discharge Abstract Database, indicate that the surveillance ascertainment was high. The 23% (95% CI, 8.2, 50.3) mortality rate of CEDKA is similar to reports from both Britain (24%) and the United States (21%). Only 2 patients (15.4%) had persistent neurologic sequelae at the time of hospital discharge. This morbidity rate is lower than the rate of 35% reported by both the British Pediatric Surveillance Unit and the American study. Since the cases were not extensively evaluated, it is possible that the rate of less severe neurologic sequelae may be underestimated. There is, however, a possibility that the heightened awareness of CEDKA created by the monthly mail out from the CPSP may have led to earlier detection and treatment of CEDKA, which may lead to better outcomes. However, despite a similar surveillance system, the British Pediatric Surveillance Unit found a higher morbidity rate. The management of CEDKA in Canada in 1999 to 2001 may have been different from that in the United States from 1982 to 1997 or in Britain from 1995 to 1998. Intubation with hyperventilation to a pCO₂ <22 mm Hg has been associated with poorer outcomes. As a result, this approach to cerebral edema management has been less widely used.

All cases of CE occurred within 20 hours after presentation with DKA, at a median time of approximately 3.5 hours, with a range of 1.5 to 20 hours. Since the vast majority of cases of CEDKA previously reported occurred after presentation to the emergency department, there has been a major emphasis on management issues of DKA when assessing risk factors for CEDKA. Interestingly, 19% of cases in this series had CE at initial presentation of DKA, suggesting that events before presentation to medical attention, or individual susceptibility factors, may play an important role in the treatment of CEDKA. In keeping with this, our other recent reports have found that factors at presentation may be more important than treatment factors in the development of cerebral edema. Similar to the Glaser and BPSU studies, age was not found to be a risk factor for CEDKA. However, patients who had CE were more likely to have a greater degree of dehydration, as indicated by elevated urea, to have more severe acidosis with a lower initial bicarbonate, and to be more hyperglycemic than control subjects. This suggests that severity of illness at presentation to the emergency department is a risk factor for CE. Unlike the Glaser report, pCO₂ was not significantly lower in cases than control subjects. Rapid changes in serum osmolality with the onset of treatment have been implicated in the theoretical pathophysiology of CEDKA and thus a more rapid decline in glucose or a failure of the measured serum sodium to rise as the glucose decreases could be important risk factors. This was not evident in this study, and, in fact, the serum sodium showed a greater rise in cases than in control subjects, although this difference was not statistically significant. Rapid administration of hypotonic fluids has been found by some to be an important risk factor for CEDKA. However, more recent papers have failed to confirm this. A retrospective-prospective study by Mel and Werther showed no difference in the frequency of CE after changing treatment protocols for a more conservative fluid rate but using hypotonic fluid. The finding of increased CEDKA with higher sodium infusion rates should not be interpreted to mean that the use of hypotonic saline would lower the risk, as it covaries with the rate of fluid administration. Although the rate of fluid and
sodium administration appeared to be important on univariate analysis, regression analysis suggests that this was related to the degree of dehydration. Similarly, the use of bicarbonate approached significance on univariate but not regression analysis. This may be due to the small number of patients who received bicarbonate and, as such, lends support to the cautions raised about use of bicarbonate in the management of DKA. \(^{8,25}\)

The incidence of cerebral edema in DKA in our study was lower than that anticipated from reports available at the time of study design. This resulted in fewer cases for risk factor analysis, thus limiting the power to find associations. Although the active surveillance appeared to be effective and response rates were high, it is still possible that a small number of cases were missed. Another limitation relates to the difficulty in confirming the diagnosis of case reports, as one must rely on descriptions of clinical events to define cases. In many but not all cases, cerebral edema was confirmed by neuroimaging. Exclusion of all cases not confirmed by neuroimaging would not present a true picture of the spectrum of CEDKA.

The results of this study emphasize the importance of prevention of DKA in limiting morbidity and mortality rates of CEDKA. For the 19% of cases who had cerebral edema at initial presentation to the emergency department, prevention of DKA is the only strategy that will be effective. For those who have development of CE during treatment of DKA, factors already present at diagnosis of DKA are extremely important: Children and adolescents presenting with a greater degree of dehydration, acidosis, and hyperglycemia appear to be at higher risk. Thus, the key to reducing the incidence of CE, which is responsible for most of the morbidity and mortality associated with DKA, lies in DKA prevention.

The authors acknowledge the invaluable support of the Canadian Pediatric Surveillance Program and in particular the hard work of Andrea Medaglia and Dr Danielle Grenier. This work would not have been possible without the cooperation of all the reporting pediatricians and the support of the Canadian Pediatric Endocrine Group. This work was facilitated by the Chalmers Research Group and in particular Candice McGahern.

REFERENCES


THE GLOBAL SPREAD OF TYPE 2 DIABETES MELLITUS IN CHILDREN AND ADOLESCENTS

ORIT PINHAS-HAMIEL, MD, AND PHILIP ZEITLER, MD, PHD

The rising prevalence of type 2 diabetes mellitus (T2DM) in children and adolescents was initially recognized in the United States in the 1990s. T2DM, which 15 years ago accounted for less than 3% of all cases of new-onset diabetes in children and adolescents, today accounts for up to 45% of new-onset cases among adolescents. Though the diagnosis was initially regarded with skepticism, T2DM is now a serious diagnostic consideration in all young people who present with signs and symptoms of diabetes. Subsequent studies conducted in Asia and Europe revealed a similar pattern, and, more recently, reports on T2DM in children and adolescents have begun to mount worldwide.

The review of the North American experience with youth–onset T2DM published by Fagot-Campagna et al in 2000 has proved to be an important resource for both clinicians and investigators addressing this problem. The growing number of reports of this problem in an ever-expanding list of countries and its increasingly important implications for international public health prompted the present effort to collate published reports of case-series and epidemiology to complement the previous review of the North American literature.

METHODS

The Medline database and Cochrane Library online were searched for articles on T2DM in children and adolescents published between September 1978 and May 2004 in all languages. Searches were performed by using the following key words alone and in combination: non–insulin-dependent diabetes mellitus, type 2 diabetes mellitus, children, adolescents, and youth. A total of 1902 publications were identified; however, only 110 contained epidemiologic data on T2DM in children and adolescents, including publications in English, French, and Hebrew. However, the vast majority of publications were in English, and this is primarily a review of English language reports. The remaining 1792 articles addressed T2DM in adults with references to their children and thus were not relevant. Twenty-two additional reports were identified from abstracts or conference summaries. There were no documents identified in the Cochrane Library relevant to epidemiology of T2DM in children.

For the sake of clarity, studies reviewed are classified as either population studies (PS) if they have clearly defined population numerators and denominators or case studies (CS) if they are clinical series or surveys.

RESULTS

The apparent prevalence and incidence of T2DM determined from population-based studies in specific countries or regions are presented in Figure 1 and Figure 2, respectively. The percentages of new cases of diabetes in children and adolescents reported to have T2DM are presented in Figure 3.

The recent recognition of T2DM in children and the recent proliferation of reports in the literature from around the world is illustrated by the number of publication included in our report: Before 1990, there were only 2 reports; between 1990 and 1994, 4 reports; 1995 to 1999, 12 reports; and between 2000 and 2003 53 reports.

Because of the nature of the search parameters, the reports varied extensively; some of the identified reports were from public health organizations with population-based data, whereas others were series from diabetes clinics. Furthermore, there was important variation in the age range of the patients reported, the details of which are provided in the summary of each report. Finally, there were variations in the definition of T2DM used in the various reports over time, though the diagnoses in all studies were generally based on either American Diabetes Association (ADA) or World Health Organization guidelines.

<table>
<thead>
<tr>
<th>Acronym</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>ADA</td>
<td>American Diabetes Association</td>
</tr>
<tr>
<td>BMI</td>
<td>Body mass index</td>
</tr>
<tr>
<td>CS</td>
<td>Case studies</td>
</tr>
<tr>
<td>DKA</td>
<td>Diabetic ketoacidosis</td>
</tr>
<tr>
<td>PS</td>
<td>Population studies</td>
</tr>
<tr>
<td>SDS</td>
<td>Standard deviation score</td>
</tr>
<tr>
<td>T1DM/T2DM</td>
<td>Type 1/type 2 diabetes mellitus</td>
</tr>
</tbody>
</table>

From the Pediatric Endocrinology and Diabetes Unit, Sheba Medical Center, Tel Hashomer, Ramat-Gan and Maccabi Juvenile Diabetes Center, Rananna, Israel; and the Division of Endocrinology, Department of Pediatrics, University of Colorado Health Science Center, Denver, Colorado. Submitted for publication Sep 13, 2004; revision received Nov 11, 2004; accepted Dec 20, 2004. Reprint requests: Dr Orit Pinhas-Hamiel, Maccabi, Juvenile Diabetes Center, 4 Bar-Ilan St, Ranana, Israel. J Pediatr 2005;146:693-700. 0022-3476/ - see front matter
Copyright © 2005 Elsevier Inc. All rights reserved. 10.1016/j.jpeds.2004.12.042
and supported by a combination of antibody testing, insulin/c-peptide measurement, or disease natural history during follow-up. However, given the limitations of a retrospective analysis of this type, we report the results of all reports identifying patients as having T2DM.

Asian-Pacific Region

**Tokyo, Japan (PS).** In Japan, 80% of all new cases of diabetes in children and adolescents are diagnosed as type 2.\(^3\) The incidence of T2DM among Japanese primary school children in Tokyo increased 10-fold, from 0.2 per 100,000 in 1976 to 2 per 100,000 in 1995.\(^4,5\) In junior high school children (age not specified in report), the reported incidence in 1995 was 13.9 per 100,000, almost twice that in 1976. By contrast, the incidence of type 1 diabetes mellitus (T1DM) in Japanese children and adolescents was 1.5 cases per 100,000 in 1976 and did not change appreciably over the next 20 years.\(^6\) The adolescent population affected with T2DM showed a strong female predominance of 2.1:1, and approximately 80% of the children with T2DM were obese. Accordingly, researchers noted that the increase in the diagnosis of T2DM was paralleled by both an increase in childhood and adolescent obesity in Japan and a shift from the traditional Japanese diet to consumption of more animal fat and protein.\(^7\)

**Taipei, Taiwan (PS).** T2DM appears to be the leading cause of childhood DM in Taiwan. A nationwide screening program for diabetes among schoolchildren aged 6 to 18 years was undertaken in 1992 to 1999. Screening was done by urine and blood testing. Individuals were considered to have T2DM if the fasting plasma glucose level was >126 mg/dL, current treatment was an oral hypoglycemic drug or diet control with no insulin injection, and there was no recurrent diabetic ketoacidosis (DKA). Of newly diagnosed diabetes, 54.2% of cases were diagnosed with T2DM, with an incidence of newly identified T2DM of 6.5 per 100,000 (compared with 1.5 per 100,000 T1DM). Factors associated with the diagnosis of T2DM were hypercholesterolemia, elevated blood pressure, and family history of T2DM.\(^8\)

**Singapore (PS).** In general, T2DM is on the rise in Singapore, accounting for ~10% of all new cases of childhood diabetes.\(^9\) The KKH Diabetes registry, a multicenter audit of diabetes diagnoses in Singapore, reported >50 T2DM cases in 2000 versus 10 cases in 1997.\(^10\) The mean reported age of children with T2DM in Singapore was 12 years, and mean body mass index (BMI) was 25.6 kg/m\(^2\). Anti-GAD antibodies and ICA were negative; family history of T2DM was noted in 80% of cases.

**Hong Kong (PS).** According to the Hong Kong childhood diabetes registry, T2DM accounted for 7% of all identified cases of childhood diabetes in all districts of Hong Kong in 1996.\(^11\) The annual age-standardized incidence of T1DM and T2DM in southern Chinese children (<15 years old) was 1.4 per 100,000 and 0.1 per 100,000, respectively.

**Bangkok, Thailand (CS).** At an academic diabetes center, the proportion of new cases of T2DM in children 0 to 14 years of age rose from 5% in 1987 to 1996 to 17.9% in 1997 to 1999.\(^12,13\) The mean age of onset was 11.6 ± 2.1 years, and BMI was 29 ± 6.1 kg/m\(^2\). Here too, the increase in the disorder occurred concomitantly with an increase in the prevalence of obesity in the population from 5.8% in 1990 to 13.3% in 1996.
In Children And Adolescents

SHANGHAI, CHINA (CS). A survey at the Children’s Hospital of Fudan University in 2001 revealed that among 83 patients with diabetes <18 years of age, 2.4% had T2DM.  

INDIA (CS). Eighteen children (5 boys and 13 girls), 9 to 15 years of age, with insidious onset of nonketotic diabetes and responsiveness to oral antidiabetic agents for periods from 2 months to 12 years were reported from a diabetes specialty center in Chennai. All of the children were tested for the presence of anti-GAD antibodies and for pancreatic beta-cell reserve. A positive family history of diabetes was present in all cases.  

A larger series of 545 patients <30 years of age in South India evaluated at a diabetes center in the mid-1980s yielded 314 (58%) with T2DM, including a small but unspecified number of patients who were younger than 20 years.  

NEW ZEALAND (CS). Trends in T2DM among adolescents were studied in a diabetes clinic serving Maori of the Pacific Island ethnic group in Auckland, New Zealand in 1996 and 2002. T2DM accounted for 12.5% of new cases of diabetes in the years 1997 to 1999 and 35.7% of new cases in the years 2000 to 2001. Mean age at diagnosis was 15 years, and the mean BMI was 34.6 kg/m². Family history of T2DM and risk factors for cardiovascular disease were common in the subjects with T2DM: 85% had dyslipidemia, 58% had increased albumin excretion rates, and 28% had systolic hypertension.  

These findings are in agreement with other reports of an increasing rate of diabetes among young Maori. In 1998, 55% of all patients registered with the Northland New Zealand Diabetes Services, diagnosed with diabetes before the age of 30 years, had T2DM, accounting for 2.7% of the population; 61% of the patients had been diagnosed before age 20. Studies in the 1980s have shown a relatively lower incidence of nonketotic diabetes as compared with T2DM: 85% had dyslipidemia, 58% had increased albumin excretion rates, and 28% had systolic hypertension.  

A prospective, national, population-based epidemiologic study of T2DM from 1999 to 2001 yielded 8 cases (7 girls, 1 boy) among 529 children with diabetes <15 years of age (1.6%), for a calculated incidence of 0.25 per 100,000. The diagnosis of T2DM was according to the ADA diagnostic criteria. The age of onset was 12 to 15 years, and all affected children were overweight. Half of the patients were of Pakistani, Turkish, or South American origin, and half of Austrian origin.  

ENGLAND. The first study on T2DM in children and adolescents from the United Kingdom was conducted in West Midlands and Leicester in 2000 (CS). Eight girls 10 to 16 years of age of Pakistani, Indian, or Arabic origin were identified. Their BMI was 2.1 to 3.4 SDS above the mean for age and sex. A subsequent paper (CS) from the southern and western regions of England reported T2DM in 4 obese white children, 3 girls and one boy, 13 to 15 years of age, who were diagnosed over a 2-year period. According to a crude estimate (PS), the prevalence of T2DM in the under-18 population in England is 0.038 per 1000, with an annual incidence of 1.52 per 100,000. In Leeds in 2000, the prevalence of T2DM was 0.05 per 1000 for patients 10 to 19 years of age; 40% of the subjects were south Asians. A cross-sectional survey of all pediatric diabetes centers in the United Kingdom (PS) yielded a 0.2% prevalence of T2DM in children <16 years of age.  

FRANCE (CS). A study of the distribution of T1DM and T2DM from 1993 to 1998 in a large diabetes center in Paris revealed 8 cases (2%) of T2DM, 6 of which were diagnosed with an equal distribution of male and female subjects. The median BMI standard deviation score (SDS) was 2.3 (−1.9 to 9.7). The ethnic distribution was white (27%), Asian (22%), Aboriginal (19%), and Middle Eastern (11%). Urban/rural and socioeconomic status were evenly distributed; 75% had a family history of T2DM.
in 1998 in children 8.5 to 14.9 years of age. One child was of Caribbean origin and the rest were white. Four were obese.

**SWEDEN (PS).** A national, retrospective, population-based case study, detecting all known cases of T2DM in Sweden in 2001, suggested that T2DM represents only 0.5% of all cases of diabetes in the age group of 0 to 18 years.35

**OTHER EUROPEAN COUNTRIES.** Additional cases of T2DM are accumulating from various parts of Europe. Forty-two young patients were identified in a study in Budapest, Hungary.36 In a multicenter study evaluating the safety and efficacy of metformin in adolescents, 20 of the 82 participants were from Russia (Moscow, Volgograd, Kazan), Belarus (Minsk), or Poland (Warszawa).37 Several cases have been reported from Bulgaria (Sofia),38 Italy (Naples),39 and the Netherlands.40

**East/Middle East**

**ISRAEL (CS).** Three cases of T2DM in obese female subjects were reported in 2000.41 Among 101 obese adolescents who underwent an oral glucose tolerance test, asymptomatic T2DM was discovered in 3%, whereas 8% had impaired glucose tolerance.42 According to the Israeli Registry Study Group of Diabetes, in 1997, T2DM accounted for 0.6% of newly diagnosed diabetes in patients <18 years of age, compared with 1.9% in 2000. This can be translated to an estimated incidence of 0.05/100,000 in 1997, compared with 0.17/100,000 in 2000 (personal communication, Silva Koton, PhD, Israel Center for Disease Control, June 2004).

**SAUDI ARABIA (CS).** A total of 25,337 Saudis were screened for diabetes mellitus and impaired glucose tolerance, using World Health Organization criteria for diagnosis. The reported prevalence of T2DM in the Saudi population under-14-year age group is 0.12%, and 0.79% for the 14- to 29-year age group.43

**UNITED ARAB EMIRATES (CS).** Between 1990 and 1998, T2DM was diagnosed in 12.5% of all patients 0 to 18 years of age with new-onset diabetes at Al-Ain general hospital. Affected patients were superbese and had a positive family history of T2DM.44

**South America**

**BUENOS AIRES, ARGENTINA.** The percentage of new cases of diabetes in the diabetes unit in Hospital General de Ninos Pedro that were T2DM increased from 0% in 1992 to 4.16% in 2001.45 Mean age was 12.9 ± 2.8; the majority of subjects were obese, with acanthosis nigricans and family history of diabetes.

**North America**

**UNIQUE AND INDIGENOUS POPULATIONS.** The Pima Indians of Arizona (PS) have the world’s highest reported incidence of diabetes. Since 1965, this population has participated in a longitudinal study of diabetes and its complications. The report of this longitudinal study was included in the review of T2DM in North American children in 2000.2 By the 1990s, the prevalence of T2DM in the 15- to 19-year age group had increased to 51 per 1000, and the disease had emerged also in the 10- to 14-year age group, with a prevalence of 22 per 1000.46

At six Indian Health Service facilities in Montana and Wyoming (CS), medical records were reviewed annually for all patients with diabetes who were <20 years of age.48 T2DM was diagnosed when a child had one or more of the following characteristics: weight ≥95th percentile, acanthosis nigricans, elevated C-peptide or insulin, family history of T2DM, treatment with oral agents with or without insulin, or no hypoglycemic therapy after 1 year of follow-up. From 1999 to 2001, 53% of prevalent cases and 70% of incident cases were categorized as probable T2DM. The average annual prevalence of probable T2DM was 1.3 per 1000. The average annual incidence rates for T2DM was 23.3 per 100,000, approximately 4 times higher than T1DM.49

**CANADA.** High rates of T2DM in youth have been documented among the First Nations people, who comprise 3% of the country’s population. The majority reside in Manitoba, Southwestern Quebec, Southwestern Ontario, and Southern Alberta. The first child with T2DM was reported in 1984, and by 1998, 75 children 5 to 17 years old had been diagnosed, representing 10% to 20% of new cases of diabetes (CS).51 The recent estimated prevalence of T2DM was 1 to 2.5 per 1000 in the 5- to 14-year age group and 2.3 to 3.5 per 100052 in the 15- to 19-year age group.

The Sioux Lookout Zone Hospital is a secondary-care referral hospital for 28 remote First Nations communities in northwestern Ontario. According to the hospital’s records and Diabetes Program Registry, from 1978 to 1984 (PS), T2DM was documented in 18 First Nations youths <16 years of age,53 for an age-adjusted prevalence of 2.5 per 1000. The ratio of female to male subjects was 6:1. Typical patients were asymptomatic and obese, showed no predilection for ketosis, and had a strong family history of T2DM.

In a study of Indian children <15 years of age, with no history of DKA, who attended the diabetes clinic at the Children’s Hospital of Winnipeg between 1984 and 1990, 20 patients with a diagnosis of T2DM were identified (CS).54 These included 16 girls and 4 boys 7 to 14 years of age. All 16 children whose family history could be confirmed had at least one parent with T2DM. Five of the 20 children reported having polyuria or nocturia; the remainder presented with asymptomatic glycosuria.
A cross-sectional survey of school children from northern Ojibwa-cree in 1996 to 1997 found a 1.1% prevalence of T2DM in the 4- to 19-year-olds and 3.6% prevalence in the 10- to 19-year-olds (PS). 55

OTHER REPORTS FROM THE UNITED STATES. Since the mid-1990s, centers from various parts of the United States have reported growing numbers cases of T2DM among adolescents. These reports were summarized in an extensive review in 2000. A few additional reports that have appeared since the publication of that review are included in this report.

COLUMBIA, SOUTH CAROLINA. A population-based surveillance for diabetes prevalence among 0- to 19-year-old black and non-Hispanic white youth was reported in a two-county region in South Carolina. 56 In this population, T2DM accounted for 26% of prevalent black case subjects and 10% of non-Hispanic white case subjects. Among black female subjects 10 to 19 years of age, 46% of new cases of diabetes were classified as T2DM. The total estimated cases of T2DM per 1000 youth >10 years of age were 0.6 (1.5 black female subjects, 0.5 black male subjects, 0.4 non-Hispanic white male subjects, 0.3 non-Hispanic white female subjects).

NEW YORK, NEW YORK (CS). A 10-fold increase of the number of pediatric patients <18 years of age diagnosed with T2DM was documented over a 10-year period (1990 to 2000) at the Montefiore Medical Center, Bronx, NY. 57 In 1990, T2DM accounted for 12% of all new cases of pediatric diabetes, whereas by 2000, almost 50% of patients diagnosed with diabetes had T2DM. Diagnosis was based on the National Diabetes Data Group. All patients had negative islet cell antibodies, anti-insulin, or anti-GAD antibodies. Patients were black, Caribbean–Hispanic, and Asian Indian children. At presentation, the mean age was 14 ± 2.3 years, BMI was 34.4 ± 9 kg/m², female/male ratio was 1.6:1, and all the patients were pubertal. Acanthosis nigricans was present in 89% of the patients; nearly 30% of the patients were asymptomatic at diagnosis. DKA occurred in 5 of 89 patients.

Similarly, at Mount Sinai Pediatric Diabetes Center, 48 adolescents with T2DM were diagnosed between 1987 and 2001; 48% were Hispanic, 40% were black, 8% were white, 2% were Asian American, and 2% were Lebanese. 58

FLORIDA. An increase in the percentage of patients with new-onset diabetes, diagnosed with T2DM, was observed at three university-based diabetes centers in Florida, rising from 9.4% in 1994 to 20.0% in 1998. 59,60

PHILADELPHIA, PENNSYLVANIA (CS). The Children’s Hospital of Philadelphia has recorded 143 cases of pediatric T2DM diabetes since 1990, compared with only 2 cases in the previous decade. 61 During the year from 1999 to 2000, 29 (16%) of the 180 new cases of diabetes were type 2, with 34.5% of the affected children being white, 52% black, 10% Asian, and 3.4% Hispanic. 62

DISCUSSION

This review confirms the rise over the last two decades in the global recognition and report of T2DM in children and adolescents. Historically, the first cases were reported in 1979 and 1984 among Native Americans and Canadian First Nation People, who were regarded as homogenous groups with a genetic susceptibility to T2DM. The second wave of reports appeared in the mid-1990s and involved predominately ethnic minorities, namely blacks and Hispanic Americans and some white populations in the United States. At about the same time, reports began to appear from Japan as well. About a decade later, cases from Europe were reported. The North American experience with youth-onset T2DM was reviewed by Fagot-Campagna et al 4 in 2000. Now that this phenomenon has been reported extensively outside of North America, we believed that a similar collation of the available international data would be helpful in underlying emerging patterns.

This review is limited to published data; therefore information from regions in which there has not yet been systemic data collection is not reflected. Moreover, the nature of the available searchable databases necessarily limits the ability to identify reports only to those journals, predominantly in the English language, which are indexed. Finally, the variation of the reports, from public health surveys to clinic series and including varying definitions of T2DM, render the data hard to compare directly across time and across different countries. However, despite these limitations, we believe that this review demonstrates that the problem of T2DM is not limited to certain ethnic groups nor to particular regions but has now become nearly universal.

The major observation highlighted by the present study is that from a worldwide perspective, there appears to be a close relation between rates of T2DM in adults and the eventual appearance of the disorder in adolescents. Thus, T2DM in children was reported earliest in those countries with the highest rates of adult T2DM in the world. For example, according to estimates of the prevalence of diabetes from e-Atlas (http://www.idf.org/e-atlas) for the years 2000 and 2001 for IDF member countries, the prevalence of T2DM among adults was Hong Kong (12.10%), Singapore (11.30%), Taiwan (9.10%), and Japan (7.40%); and the pacific region, New Zealand (8.00%) and Australia (5.90%). The repetition of this pattern in many geographic regions, ethnic and cultural groups, suggests that attention to the epidemiology of T2DM in adult populations may be helpful in predicting the emergence of T2DM in adolescent populations. For example, in the Asia-Pacific region, the incidence of diabetes in adults continues to grow at an alarming rate, and by 2025, China is expected to show an increase of up to 68%, followed closely by India with 59%, and the other Asian countries and the Pacific Islands (41%). These data appear to have implications for screening programs among youngsters and public obesity prevention programs.

The fact the T2DM among adolescents was also reported from regions where the prevalence of T2DM in
adults is lower than in Asia and North America (England, 3.5%; France, 4.00%; Germany, 4.20%; Austria, 3.80%; Sweden, 6.40%) may reflect the more inclusive nature of data collection in European with a National Health Service, such as the United Kingdom, than in countries without universal insurance. Additionally, countries with population registries (eg, Sweden) may have more sensitive population data.

In each of the regions affected, there was also an increase in obesity in the general population and, specifically, among children. For example, the source for the first reports of an increase in T2DM in children was the Pima Indians from the Gila River Indian Community in central Arizona, who have the world’s highest recorded prevalence and incidence of T2DM in adults, along with high rates in the prevalence of obesity. Although the data differ in terms of definition (overweight and obesity) and age ranges used, a growing trend toward obesity in the Asia-Pacific, Europe, and the United States in both adults and children has been documented during the past decade, in parallel with the emergence of T2DM in children.

Among adults, studies suggest that minority populations and immigrants have increased obesity. Asian-American and Hispanic adolescents born in the United States are more than twice as likely to be obese as first-generation residents of the 50 states. Immigrants are also disproportionately affected by T2DM. Similarly, adolescents from minority groups in the United States (Pima Indians, blacks, Hispanic Americans), Canada (First Nations people), Australia (Aboriginal) and New Zealand (Maori) have a high prevalence of T2DM. Data from Austria, the United Kingdom, New York, and Australia indicate that up to 40% to 50% of the reported patients are from immigrant groups (Pakistan, Turkey, South America, South Asia, India, Middle East), and analyses of social trends suggest that adoption of a Western lifestyle is strongly associated with T2DM in these populations.

Careful evaluation of the available reports emphasizes several limitations to the current literature in T2DM in children and adolescents. First, the divisions by age are usually informal and inconsistent. For example, in some studies, the upper range limit was 15 years. Therefore, a large proportion of patients may have been missed, leading to underestimations in some areas (Germany, Pacific, and Hong Kong). At the same time, other studies (New Zealand, Libya) included patients up to 30 years of age and did not strictly report adolescent prevalence. It would be helpful in the future if researchers would adopt a standard age breakdown and limit admissions can also be expected to be higher in this age group due to the long disease duration, it is particularly important that appropriate screening measures be implemented in these areas.

Third, the growing awareness of adolescent T2DM over time may have affected prevalence rates in the more recent studies. On the other hand, as clinicians in affected areas have become more adept at identifying and treating the disease, fewer affected children are being referred to academic centers. This may have led to an underestimation in prevalence studies based on referral patterns. The combined effect of these two forces cannot be accurately determined.

Finally, the epidemiologic studies reviewed in this report almost exclusively reported diagnosed cases of T2DM. However, if pediatric T2DM mirrors the adult experience, many affected individuals go undiagnosed. This assumption is supported by a recent study of obese children, wherein asymptomatic T2DM was identified in 4% of obese adolescents at screening. A small number of population-based studies in the United States, Australia, and Canada suggest that the true prevalence T2DM in children is at least twice that of the known cases a proportion similar to that seen in adults.

Despite these limitations, the studies reviewed in this report confirm that T2DM is fast being recognized as a health problem of international scope in children and adolescents, following much the same pattern as in adults. Our findings suggest that adolescents living in areas characterized by a rise in T2DM in adults should be considered at risk of diabetes. Since the life-long occurrence diabetes-associated complications can also be expected to be higher in this age group due to the long disease duration, it is particularly important that appropriate screening measures be implemented in these areas.

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GENETIC SYNDROMES MIMIC CONGENITAL INFECTIONS

A. Sanchis, MD, PhD, L. Cervero, MD, PhD, A. Bataller, MD, J. L. Tortajada, MD, J. Huguet, MD, Y. J. Crow, PhD, M. Ali, PhD, L. J. Higuët, MD, and M. L. Martínez-Frias, BS, PhD

Genetic syndromes that mimic congenital infection are important to recognize because of the associated risk of recurrence. We describe two brothers born to consanguineous parents with clinical features suggestive of intrauterine infection but with negative serologic investigations. Our observations suggest that Aicardi-Goutiéres syndrome (AGS) and pseudo-TORCH syndrome likely represent the same disorder. (J Pediatr 2005;146:701-5)

The association of intrauterine growth retardation, microcephaly, intracranial calcification, nonspecific hematological anomalies, hepatosplenomegaly and hydrops in a newborn infant is suggestive of intrauterine infection. However, since 1983 when Baraitser et al first described two brothers with microcephaly and intracranial calcification and negative TORCH analysis, a number of authors have reported children in whom detailed investigation has failed to identify objective confirmation of an intrauterine infective agent. These cases have been considered to define a distinct autosomal recessive disorder named pseudo-TORCH syndrome (OMIM 251290). Aicardi-Goutiéres syndrome (AGS) shares many features with pseudo-TORCH syndrome, although the conditions are said to differ by the presence in the former of cerebrospinal fluid (CSF) lymphocytosis with raised levels of interferon α (IFN-α) and in the latter of an early onset microcephaly with neonatal elevation of hepatic transaminases and thrombocytopenia. Herein, we describe two boys born to consanguineous parents demonstrating the phenotypic overlap of pseudo-TORCH syndrome and AGS.

CLINICAL REPORTS

Case 1

This male infant was the first child born to a 19-year-old mother and 22-year-old father. The parents are second cousins of white Spanish ancestry. There is no family history of note. The boy was delivered at 37 weeks gestation after an uncomplicated pregnancy. Birth weight was 2.06 kg (−2 SD) from the mean, length 41.5 cm (−3 SD), and occipital frontal circumference (OFC) 29.5 cm (−3 SD). Apgar scores were 10 and 9 at 1 and 5 minutes, respectively. Physical examination identified microcephaly, left peripheral facial paralysis, and dysmorphic features including a hooked nose with low-set anteriorly rotated ears. He had a petechial rash on the face and upper half of the body that intensified during the first hours after delivery. He had mild dyspnea requiring oxygen administration. A grade III/VI precordial systolic murmur was detected. Abdominal examination revealed hepatomegaly of 6 to 7 cm and splenomegaly of 5 to 6 cm. Moro and press reflexes were present. The child exhibited spontaneous tremors with muscular tone shifting from hypertonic to hypotonic. He did not cry. Neurologic examination demonstrated reduced reflexes with absent sucking reflex. A few hours after delivery he developed clonic seizures poorly controlled with anti-epileptic medication.

Hematologic investigation showed a normal red cell count (4.6 × 10⁶ red cells/mm³), hemoglobin 17.1 g/dL, leukopenia with neutropenia (white cells 4.7 × 10³ cells/mm³; absolute neutrophil count 94 cells/mm³), and thrombocytopenia (50,000/mm³). Calcium,

| AGS | Aicardi-Goutiéres syndrome | IFN-α | Interferon α |
| ALT | Alanine aminotransferase | MRI | Magnetic resonance imaging |
| AST | Aspartate aminotransferase | OFC | Occipital frontal circumference |
| CT | Computed tomography | TORCH | Toxoplasmosis, rubella, cytomegalovirus, herpes simplex virus, and other viruses |
| CSF | Cerebrospinal fluid | | |

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Submitted for publication Jul 13, 2004; last revision received Nov 15, 2004; accepted Jan 19, 2005.

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0022-3476/$ - see front matter

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10.1016/j.jpeds.2005.01.033

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Table. Results of testing for infectious agents in mother and both boys

<table>
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<tr>
<th>Mother Case 1</th>
<th>Mother Case 2</th>
<th>Case 1 At birth</th>
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<th>Case 1 At 3 months</th>
<th>Case 2 At 1 week</th>
<th>Case 2 At 30 days</th>
</tr>
</thead>
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<tr>
<td>Syphilis</td>
<td>RPR negative</td>
<td>VDRL negative</td>
<td>VDRL negative</td>
<td>VDRL negative</td>
<td>VDRL negative</td>
<td>VDRL negative</td>
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<tr>
<td>Hepatitis B</td>
<td>HBsAg negative</td>
<td>HBsAg negative</td>
<td>HBsAg negative</td>
<td>HBsAg negative</td>
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<tr>
<td>Hepatitis C</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
</tr>
<tr>
<td>Human immunodeficiency (1 + 2)</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
<td>Antibodies negative</td>
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<tr>
<td>Rubella</td>
<td>IgG 149, IgM (−)</td>
<td>IgG 182, IgM (−)</td>
<td>IgG 153, IgM (−)</td>
<td>IgG 148, IgM (−)</td>
<td>IgG 229, IgM (−)</td>
<td>IgG 218, IgM (−)</td>
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<tr>
<td>Toxoplasma</td>
<td>IgG and IgM negative</td>
<td>IgG and IgM negative</td>
<td>IgG and IgM negative</td>
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<td>IgG and IgM negative</td>
<td>IgG and IgM negative</td>
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<tr>
<td>Herpes simplex (1 + 2)</td>
<td>IgG (+), IgM (−)</td>
<td>IgG (+), IgM (−)</td>
<td>IgG (+), IgM (−)</td>
<td>IgG (+), IgM (−)</td>
<td>IgG (+), IgM (−)</td>
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<tr>
<td>Cytomegalovirus</td>
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<tr>
<td>Epstein-Barr</td>
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IgG and IgM measured by ELISA. HIV assayed by Western blot.

Serology Testing
- HBV surface antigen, anti-HCV, and anti-HIV 1-2 antibodies using microparticle enzymatic immunoassay (MEIA) (HbsAg [V2] AsXYM HCV AsXYM; HIV 1-2 AsXYM).
- IgM- and IgG-specific antibodies study by EIA for the following pathogens: CMV (IgG/IgM EIAGEN cytomegalovirus, Corméda); EBV (ETI VCA-G/ETI VCA-M, DiaSorin); Toxoplasma gondii (Toxo PLATELIA IgG/IgM, BioRad); Rubella (Rubella PLATELIA IgG/IgM, BioRad); VHS 1-2 (Enzygnost Anti HSV 1-2 IgG/IgM, Dade-Behing); and VZV (Enzygnost Anti VZV IgG/IgM, Dade-Behing).
- Presence of reaginic antibodies for Treponema pallidum (Test Card RPR-BD).

CMF, Cytomegalovirus, EBV, Epstein-Barr virus, VZV, varicella zoster virus, HBV, hepatitis B virus, HCV, hepatitis C virus, RPR, rapid plasma regain, VDRL, venereal disease research laboratory.

phosphate, immunoglobulins, amino acids, and transaminases were nearly normal (ALT 46 IU, AST 68 IU) although alkaline phosphatase (481 IU) and gamma-glutamyl-transpeptidase (1,156 IU) were both markedly increased. Bacteriologic studies and serologic tests for intrauterine infection (syphilis, rubella, hepatitis A, B, and C, HIV, herpes simplex, toxoplasma, and cytomegalovirus) were negative on a variety of repeated tests (Table). Testing for lymphocytic choriomeningitis virus was not performed. High-resolution band karyotype (550-850 bands) was 46,XY. CSF analysis on day 1 of life showed a leukocytosis of 25 white cells/mm³ with 95% lymphocytes.

Cerebral ultrasonography and computed tomography (CT) demonstrated enlarged ventricles with abundant pericallosal calcifications also affecting the thalamus, cerebellum, and brainstem (Figure 1). There were cerebral atrophy and zones of rarefaction in the white and gray substance of both temporal lobes. Cerebral magnetic resonance imaging (MRI) revealed the presence of cerebral, cerebellar, and brain stem atrophy with simplification of the gyri predominantly of the frontotemporal lobes (Figure 2). Electroencephalogram revealed asymmetric interhemispheric and subcortical paroxysmal activity. Ophthalmologic examination was normal. At 1 year of age he had severe microcephaly.

Doppler echocardiography demonstrated significant enlargement of the anterolateral wall of the left ventricle with a normal posterior wall compatible with the existence of a nonobstructive chronic cardiomyopathy. A cardiac murmur was present until 2 months of age. Oxygen therapy was administered during the first 40 days after delivery because of respiratory insufficiency. The patient developed a progressive anemia during the first 15 days of life requiring two packed red cells transfusions. The anemia, leukopenia, and thrombocytopenia resolved after 2 months of age. Seizures were refractory to treatment.

In view of the persistent hepatosplenomegaly, jaundice, and deranged hepatic enzyme tests, liver biopsy was performed at 2 months of age, showing preserved liver architecture without any sign of inflammation or focal necrosis. Abundant iron pigmentary accumulations were observed
within many hepatocytes in association with features of cholestasis.

When assessed at 7 years of age, weight was 16 kg (−3 SD) and length 102 cm (−4 SD). The patient was microcephalic with an OFC of 42 cm (−7 SD). He exhibited severe psychomotor retardation with signs of bilateral spasticity affecting the upper and lower limbs. He still experienced seizures, but these were less frequent than before. He was unable to sit, and had no speech. He died of pneumonia at 7 years of age.

Case 2

This male patient, the brother of case patient 1, was the second child born to his parents 6 years after his affected sibling. The pregnancy was uncomplicated, and the infant was delivered by forceps at 38 weeks gestation. He was discharged from the hospital at 3 days of age, having a weight of 2.4 kg (−1 SD), a length of 43 cm (−3 SD), and an OFC of 30.5 cm (−2 SD).

He was re-admitted at 6 days of age because of recurrent apneic episodes possibly related to feeding. Physical examination revealed microcephaly with a narrow anterior fontanelle and dysmorphic features similar to those of his older brother. He did not fix or follow. He had mild respiratory difficulty requiring oxygen administration. Abdominal examination revealed splenomegaly of 2 to 3 cm without hepatomegaly. He had hypertonicity mainly of the lower limbs with an absent Moro reflex. Like his brother, he did not cry. During hospitalization, he experienced recurrent apneic episodes associated with generalized hypertonia and bradycardia that persisted until 15 days of age. He also developed clonic seizures that were controlled with phenobarbital.

Investigation revealed mild anemia (red cells 4 × 10⁶/mm³; hemoglobin 13.7 g/dL), moderate leukopenia without neutropenia (white cells 5300 cells/mm³; absolute neutrophil cells 1500/mm³), and normal platelet count. Serum concentration of AST, ALT, calcium, phosphate, clotting studies, acute phase reactants, immunoglobulins, aminoacids, and organic acids were normal as was transferrin isoelectric focusing and bacteriologic cultures of blood and CSF. Testing for the most frequent intrauterine infections were repeatedly negative in blood, urine, and CSF (Table). Testing for lymphocytic choriomeningitis virus was not performed. High-resolution band karyotype was 46,XY.
At 1 month of age, in the absence of symptoms of acute infection, a CSF study demonstrated lymphocytosis with 52 white cells/mm³, 82% lymphocytes, and a protein level of 149 mg/dL. The value of interferon-α (Pierce-Endogen, Rockford, Ill) was 72.9 pg/mL (control value <10 pg/mL). IFN-α assay in serum was <10 pg/mL. At this age, an elevation of transaminases was detected (AST: 98 IU, ALT: 78 IU, and gamma-glutamyl-transpeptidase 217 IU/mL). There was mild but persistent anemia (2,880,000 red cells/mm³, hemoglobin 9.6g/dL, and reticulocytes 64.9/mm³) that did not necessitate transfusion. Platelet count was normal.

Cerebral CT demonstrated hydrocephalus (lateral, III and IV ventricles) with punctate calcifications of small size predominantly in the basal ganglia and internal capsule. Small subcortical calcifications were present in both hemispheres and also were observed, although to a lesser degree, in the cerebellar white matter and cerebellar peduncles. Cerebellar and mesencephalic atrophy were observed (Figure 3).

Doppler echocardiography revealed a small ostium secundum atrial septal defect without enlargement of the left ventricle wall. Ophthalmologic examination was normal, although the retina were pale.

At 13 months of age, this child exhibits significant psychomotor retardation with little spontaneous movement and clonic movements in response to tactile stimuli. He does not fix and follow, but he is said to know his parents. His weight is 9.7 kg (−0.7 SD), length 72 cm (−1 SD), and OFC 39 cm (−7 SD).

**Molecular Analysis**

In view of the clinical picture suggestive of AGS, the two affected children and their parents underwent genotype investigation using polymorphic markers across the AGS1 critical interval.12 Cases patients 1 and 2 were discordant across this region, having a different maternal chromosome. These results suggest linkage to another, as yet unidentified, AGS locus.

**Discussion**

Autosomal recessive genetic syndromes that mimic congenital infection are important to recognize because of risks of recurrence. By definition, they are characterized by a failure to identify an infective agent. Our own experience suggests that the possibility of a genetic disorder is frequently unrecognized until the observation of a familial recurrence. In this regard, we note that no mention was made of such autosomal recessive disorders in a recent review of congenital infection.13

More than 30 cases of pseudo-TORCH syndrome have been published to date,2-9 delineating a common clinical phenotype comprising microcephaly at birth, intracranial calcification, hypertonia, severe mental retardation, and seizures. Other manifestations such as intrauterine growth retardation and hematologic and hepatic alterations are less constant, showing variation among the described patients.

AGS (OMIM 225750) is a genetic disorder characterized by intracranial calcification, leukodystrophy, a CSF lymphocytosis, an onset of microcephaly, and negative serologic investigations for common prenatal infections. Thus, AGS also shows phenotypic overlap with the sequelae of congenital infection.10 However, the high frequency of parental consanguinity and the observation of affected children separated in birth order by unaffected siblings provides evidence against a causative role for an identified infective agent and makes it likely the disease is inherited as an autosomal recessive trait. AGS exhibits genetic heterogeneity with one locus on 3p21 in some cases,14 although no gene responsible for the disorder has been identified.

High levels of IFN-α in the serum and CSF have been identified consistently in children with the AGS.11 Raised levels of the same cytokine have been identified in a number of central nervous system infections including herpes and HIV encephalitis,15-16 and meningitis associated with mumps virus, enterovirus, varicella zoster, and congenital rubella infection.17 Of note, only one non-infectious neurologic disorder, neuro-lupus,18 has been associated with high levels of IFN-α.17-19

AGS and pseudo-TORCH syndrome are said to differ by the presence in the latter of congenital microcephaly, neonatal disturbance of serum hepatic enzymes, thrombocytopenia, and normal or mildly abnormal CSF examination.4 However, as stated by Crow et al,14 only a minority of cases

**Figure 3.** CT scan, case 2: enlarged ventricles and subcortical calcifications in both hemispheres.
reported with pseudo-TORCH syndrome include information on CSF white cell counts, and none have recorded IFN-α levels either in the CSF or serum. Moreover, in a recent review of 27 patients with AGS, 2 children showed prenatal microcephaly, 1 exhibited a neonatal thrombocytopenia, 2 had hepatosplenomegaly, and 3 developed transient elevation of hepatic enzymes with 1 child undergoing liver biopsy.\(^1\)

The two cases we describe likely have a genetic syndrome resembling the sequelae of an intrauterine infection because serologic investigations for infection were negative and the brothers were born at a 6-year interval to consanguineous parents. These two brothers must be considered to have AGS on the basis of the observed CSF lymphocytosis and raised levels of CSF IFN-α. However, the presence of hepatosplenomegaly, thrombocytopenia, deranged serum hepatic enzymes, simplified gyral pattern, and congenital microcephaly in both brothers illustrates well the overlap of AGS with pseudo-TORCH syndrome. The observation of congenital cardiomyopathy in case patient 1 is of interest because this finding has not been reported previously in AGS, although we are aware of one other case similarly affected (Y. J. Crow; personal communication), and this feature also has been observed in pseudo-TORCH syndrome.\(^6\)

Our cases add weight to the suggestion made by Crow et al\(^14\) that, in some cases at least, AGS and pseudo-TORCH syndrome are likely the same disorder. Molecular analyses of additional cases may help to determine the genetic basis of these clinical conditions.

*We thank the family for its cooperation in the preparation of this article.*

**REFERENCES**

Fatty acids play an important role in regulating insulin secretion, but the mechanisms are unclear. We report a case of a novel splice site mutation in the short-chain 3-hydroxyacyl-CoA dehydrogenase (SCHAD) gene associated with hyperinsulinism. This mutation resulted in a nearly complete absence of immunoreactive protein and a decrease in fibroblast SCHAD activity. (J Pediatr 2005;146:706-8)

Glucose is the prime regulator of insulin secretion from β-cells. Fatty acids can increase insulin secretion in vivo and amplify glucose-induced insulin secretion in vitro.1 Two major pathways are involved in regulating insulin secretion from β-cells.2 The KATP channel–dependent pathways involve the metabolism of glucose. The KATP–independent pathway involves anaplerotic input into the tricarboxylic acid cycle with generation of citrate and increases in cytosolic malonyl-CoA. Increased concentration of extramitochondrial malonyl-CoA inhibits carnitine palmitoyltransferase I blocking the entry of long-chain acyl-CoA into the mitochondria. Accumulating long-chain acyl-CoA esters then act as effector molecules regulating the activity of enzymes and channels and augmenting glucose-induced insulin secretion from β-cells through intracellular metabolism and generation of lipid-derived molecules.3

Short-chain L-3-hydroxyacyl-CoA dehydrogenase (SCHAD) is an intramitochondrial enzyme that catalyses the penultimate reaction in the β-oxidation of fatty acids, the NAD1-dependent dehydrogenation of 3-hydroxyacyl–CoA to the corresponding 3-Ketoacyl-CoA.4 We recently described a novel cause of hyperinsulinism associated with a homozygous point mutation in the SCHAD gene.5 We now report hyperinsulinism in a child with a novel splice site mutation in the SCHAD gene.

METHODS

Clinical Case History

This child was born to consanguineous first cousin parents, with a birth weight of 3.1 kg. At 4 months of age he presented with hypoglycemia and seizures. Investigations showed an increased glucose clearance rate (7 mg/kg per minute) and a biochemical picture consistent with hyperinsulinemic hypo–fatty acidemic hypoketotic hypoglycemia. This patient responded to 5 mg/kg per day diazoxide and 7.5 mg/kg per day chlorothiazide.

Measurement of 3-Hydroxyacyl-CoA Dehydrogenase Activity

Short-, medium-, and long-chain 3-hydroxyacyl-CoA dehydrogenase activity was measured in fibroblasts as described previously.6 The protein was normalized to supernatant protein rather than total cellular protein. Proteins were separated, electro-botted overnight, and detected by Western blotting as described previously.6 Exon-specific PCR amplification of SCHAD sequences was performed with the appropriate primer pair for each exon, as described in Reference 5. DNA from the patient, his parents, and a control subject were amplified by using forward primer 5’AGTGCTGCGGCTTCTCCAT3’ and reverse primer 5’GGTAACCCTCTTCC3’, which introduce a

SCHAD Short-chain 3-hydroxyacyl-CoA dehydrogenase
restriction site for NCO1 in combination with the IVS6-2 a/g mutation but not with the normal sequence. mRNA was isolated from the patient and control fibroblasts. A fragment encompassing exon 7 was amplified by reverse transcriptase–PCR by using forward primer GGTAGACTTTAGCAAGCCC and reverse primer TCTTCTGCGACATTG. Reamplification of the obtained PCR products was performed by using forward primer GGTAGACTTTAGCAAGCCC and reverse primer GTAATGGGTTCTCTGATCC. Amplification products were excised from a 2% agarose gel, purified, reamplified by using the same primer pair, and sequenced as described above.

RESULTS

The results of the diagnostic fast were similar to the first patient we described. During one fast with a blood glucose level of 1.9 mmol/L, insulin level was <1 mU/L and NEFA (1.49 mmol/L) and 3-hydroxybutyrate (0.53 mmol/L) were elevated. During a second fast with a blood glucose level of 2.9 mmol/L, the insulin level was elevated at 3.0 mU/L, with suppressed NEFA (0.50 mmol/L) and ketones (<0.05 mmol/L).

Hydroxybutyrylcarnitine was persistently elevated (0.70 to 1.69 mmol/L). Urine organic acids showed increased 3-hydroxyglutarate (16 to 28 μmol/mmol creatinine) during the two fasts.

Assay of short-, medium-, and long-chain 3-hydroxyacyl-CoA dehydrogenase activity indicated significantly decreased activity with the short-chain substrate, approximately 10% of control activity (Table).

Western blotting with an anti-SCHAD antibody indicated a decrease in the amount of immunoreactive protein in fibroblasts from the patient consistent with the observed decrease in enzyme activity, although some residual protein could be detected in some fibroblasts (Figure).

The patient was found to be homozygous for an IVS6-2 a→g mutation. Both parents are heterozygous for the IVS6-2 a→g mutation. The mutation was not found in 100 control subjects. Electrophoresis of the DNA fragments from the restriction fragment length analysis indicated that the IVS6-2 a→g mutation, in combination with NCO1 digestion, yields two bands of 59 and 17 nucleotides compared with 76 nucleotides in the control (undigested PCR product).

Amplification of SCHAD cDNA from patient’s fibroblast mRNA yielded no detectable products of exon 27, and a decreased amount of product when regions of exon 2 or exon 4 sequences were amplified. Reamplification of exon 7 by half-nested PCR yielded various PCR products from the patient's mRNA, including 269 nt, which could not be detected in control fibroblast. On reamplification and sequencing, the 270 nt product was seen to consist of at least three different sequences. The normal SCHAD cDNA sequence could be deduced from the mix of sequences, indicating that at least some normal SCHAD mRNA can be formed in patient fibroblasts.

DISCUSSION

This is the third reported case of a mutation in the SCHAD gene in a patient with hyperinsulinism. All reported cases have presented with increased 3-hydroxyglutarate in urine and hydroxybutyrylcarnitine in blood, diagnostically useful markers for SCHAD deficiency. The clinical presentation is heterogeneous, with either mild late onset hypoglycaemia or severe neonatal hypoglycaemia. The mechanism of how a defect in SCHAD leads to dysregulated insulin secretion is unclear at present. In the pancreas, the activity of SCHAD is highest in the Islets of Langerhans and more specifically in the pancreatic β-cells, suggesting that fat oxidation may have a role in regulating insulin secretion.

<table>
<thead>
<tr>
<th></th>
<th>Short (U/U CS)</th>
<th>Medium (U/U CS)</th>
<th>Long (U/U CS)</th>
<th>Short (mU/mg protein)</th>
<th>Medium (mU/mg protein)</th>
<th>Long (mU/mg protein)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control Mean (n = 5)</td>
<td>0.66 ± 0.06</td>
<td>0.62 ± 0.08</td>
<td>0.68 ± 0.05</td>
<td>46.99 ± 4.39</td>
<td>43.56 ± 4.51</td>
<td>48.50 ± 3.70</td>
</tr>
<tr>
<td>Mean patient (n = 5)</td>
<td>0.06 ± 0.01</td>
<td>0.43 ± 0.03</td>
<td>0.45 ± 0.04</td>
<td>4.78 ± 0.32</td>
<td>33.35 ± 1.76</td>
<td>35.63 ± 2.86</td>
</tr>
<tr>
<td>Mean SCHAD (n = 2)</td>
<td>0.07</td>
<td>0.39</td>
<td>0.47</td>
<td>5.02</td>
<td>29.81</td>
<td>36.07</td>
</tr>
<tr>
<td>Mean LCHAD (n = 2)</td>
<td>0.45</td>
<td>0.09</td>
<td>0.18</td>
<td>38.28</td>
<td>8.56</td>
<td>14.88</td>
</tr>
</tbody>
</table>

CS, Citrate synthase activity.

The patient is the patient described in the current article, SCHAD is our previously described case, and LCHAD is a patient with established long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency.
Fatty acids increase insulin secretion by affecting the concentrations of long-chain fatty acyl derivatives as a result of the inhibitory effect of citrate and malonyl-CoA on the rate controlling enzyme CPT1,9 stimulation of G-protein–coupled receptors, GPR40,10 and by activation of L-type Ca2+ channels.11 The effect of L-hydroxybutyrate, L-hydroxybutyryl-CoA, or L-hydroxybutyryl-carnitine on these mechanisms remains uncertain.10

The mutation in this patient in the SCHAD gene leads to the formation of abnormally spliced mRNAs. The decreased amount of total SCHAD mRNA in the patient’s fibroblasts is probably due to premature nonsense sequences and subsequent removal of the mRNA by the nonsense mediated mRNA decay system. This can be deduced from the relative difficulty to amplify exon 2 and 4 sequences.

Elucidating the biochemical mechanisms that lead to hyperinsulinism in patients with mutation in the SCHAD gene will provide further insights into β-cell physiology.

REFERENCES
Prophylactic ibuprofen in premature infants: a multicentre, randomised, double-blind, placebo-controlled trial

**Context** Ibuprofen is used for treatment and prevention of patent ductus arteriosus (PDA) in low-birthweight infants. Its effects on regional circulations differ from those of indomethacin.

**Objectives** To study the efficacy of early ibuprofen in reducing the frequency of severe intraventricular haemorrhage (IVH) and PDA.

**Design** Double-blind, randomized, multicenter trial.

**Setting** Neonatal intensive care units at seven hospitals in Belgium.

**Participants** 415 low-birthweight infants (gestational age <31 weeks).

**Interventions** Within 6 hours after birth, infants were randomly allocated ibuprofen-lysine (10 mg/kg then two doses of 5 mg/kg after 24 hours and 48 hours) or placebo intravenously.

**Main outcome measures** The primary outcome was occurrence of severe IVH; secondary outcomes were occurrence of PDA and possible adverse effects of ibuprofen.

**Results** 17 (8%) of 205 infants assigned ibuprofen and 18 (9%) of 210 assigned placebo developed severe IVH (relative risk 0.97 [95% CI 0.51–1.82]). In 172 (84%) infants in the ibuprofen group, the ductus was closed on day 3 compared with 126 (60%) of the placebo group (relative risk 1.40 [95% CI 1.23–1.59]). No important differences in other outcomes or side effects were noted; however, urine production was significantly lower on day 1 and concentration of creatinine in serum was significantly higher on day 3 after ibuprofen.

**Conclusions** Ibuprofen prophylaxis in preterm infants does not reduce the frequency of IVH, but it does decrease occurrence of PDA.

**Comment** This is a carefully designed and implemented study to determine whether prophylactic ibuprofen would prevent IVH in premature neonates. The authors found no protective IVH effect and are to be complimented on their reporting of negative results.

Their primary objective disproven, the authors describe a secondary effect of prophylactic ibuprofen on PDA closure. The crux of this effect is based on an increased ductal closure rate on day 3 of life in the treated group. However, there are several potential pitfalls inherent in this comparison. At this age, the infants in the ibuprofen group have already benefited from exposure to prostaglandin synthetase inhibitor therapy. On the other hand, infants in the placebo group have not yet been exposed to any PDA closure medication. Ductal closure rates can be validly compared only after all infants have been provided medical treatment (ie, following an attempt at therapeutic closure in those placebo-treated infants whose ductus did not close spontaneously). In the placebo group, it will generally be later than day 3 of life by the time clinically significant PDAs are diagnosed and treated therapeutically. If the definitive comparison, performed after such an attempt at therapeutic closure has been offered, reveals a comparable number of infants requiring surgical ductal ligation, and if long-term morbidity remains similar in the two groups, then essentially the same clinical result will have been achieved in both groups. Further, the observation that the PDA was closed by day 3 in 60% of the placebo group implies that an equivalent 60% of PDAs in the ibuprofen group would have been expected to close spontaneously; thus, these infants may have been unnecessarily exposed to a drug. Finally, even if treatment is required for a PDA, using ibuprofen therapeutically, rather than prophylactically, delays exposure to a drug whose toxicity (pulmonary hypertension, oliguria, increased creatinine) may be increased when given in the first hours of life.

In conclusion, before we consider routine implementation of prophylactic ibuprofen, we must consider whether it is justified to close PDAs prophylactically if treatment confers no long-term benefit and unnecessarily exposes infants to a drug with a small but documented potential toxicity. This may
be one case in which an ounce of prevention is not worth a pound of cure.

Oral probiotics reduce the incidence and severity of necrotizing enterocolitis in very low birth weight infants


Context Necrotizing enterocolitis (NEC) is a worldwide problem in very low birth weight (VLBW) infants, with highly variable incidence affecting 2.6% to 28% of these infants.

Objectives To evaluate the efficacy of probiotics in reducing the incidence and severity of NEC in VLBW infants.

Design Prospective, masked, randomized control trial.

Setting A level III neonatal center in the central part of Taiwan.

Participants 367 VLBW (<1500 g) infants who started to feed enterally and survived beyond the 7th day after birth.

Interventions Infants in the study group were fed with Infloran (Lactobacillus acidophilus and Bifidobacterium infantis) 125 mg/kg with breast milk twice daily until discharge. Infants in the control group were fed with breast milk alone.

Main outcome measures Death or NEC (≥ stage 2).

Results The incidence of death or NEC (≥ stage 2) was significantly lower in the study group (5% vs 12.8%, \(P = .009\), number needed to treat [NNT] = 13). The incidence of NEC (≥ stage 2) also was significantly lower in the study when compared with the control group (1.1% vs 5.3%, \(P = .04\), NNT = 24). There were six cases of severe NEC (Bell stage 3) in the control group and none in the study group. None of the positive blood cultures grew Lactobacillus or Bifidobacterium species.

Conclusions Infloran as probiotics fed enterally with breast milk reduces the incidence and severity of NEC in VLBW infants.

Comment There is a strong rationale to prevent NEC with probiotic agents. Premature infants may be colonized with pathogenic bacteria and develop an aberrant fecal flora that contributes to the pathogenesis of NEC. Probiotics may improve intestinal immune regulation, enhance mucus production, produce antibacterial agents, stimulate IgA production, block mucosal binding, reduce mucosal permeability, and produce anti-inflammatory cytokines.

The well-designed study by Lin and co-workers demonstrates the efficacy of probiotics in preventing NEC. Unfortunately, the study did not have the power to define the risk of serious infections from probiotic bacteria. Although most probably rare, sepsis, meningitis, endocarditis, pneumonia, and abscesses have been reported in immunocompetent and immunocompromised adults and children treated with probiotics.

Further studies in premature infants should confirm efficacy but must address safety. Based on the literature, it would be wise to avoid prophylactic use of probiotics in premature infants with congenital or acquired immunodeficiencies, congenital heart disease, or gastrointestinal compromise (ileus, mucositis, diarrhea, suspected or documented NEC). The probiotic organism should be the least virulent and have a favorable antibiotic susceptibility profile (this needs to be rechecked during hospitalization because resistance may develop). In the meantime, premature infants should be fed human milk because it encourages the growth of endogenous probiotic organisms.

Randomized, controlled trial of slow versus rapid feeding volume advancement in preterm infants


Context Controversy exists regarding when feedings for preterm infants should be started, whether minimal enteral feedings should be used routinely in small preterm infants, and how fast to advance enteral feedings.

Objectives To determine an optimal enteral method for preterm infants.

Design A randomized, controlled, single-center trial.

Setting A Neonatal Intensive Care Unit of a community-based county hospital in Houston, Texas.

Participants 155 infants between 1000 and 2000 g at birth, gestational age ≤ 35 weeks, and weight appropriate for gestational age.

Interventions Infants received feedings of expressed human milk or Enfamil formula starting and advanced at either 30 mL/kg per day or 20 mL/kg per day. Infants remained in the study until discharge or development of stage IIA necrotizing enterocolitis (NEC).

Main outcome measures Primary outcome measures were days to full feedings, incidence of feeding complications, and NEC. Secondary outcomes included: time to regaining...
be one case in which an ounce of prevention is not worth a pound of cure.

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REFERENCE


Randomized, controlled trial of slow versus rapid feeding volume advancement in preterm infants


Context Controversy exists regarding when feedings for preterm infants should be started, whether minimal enteral feedings should be used routinely in small preterm infants, and how fast to advance enteral feedings.

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Design A randomized, controlled, single-center trial.

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Participants 155 infants between 1000 and 2000 g at birth, gestational age ≤35 weeks, and weight appropriate for gestational age.

Interventions Infants received feedings of expressed human milk or Enfamil formula starting and advanced at either 30 mL/kg per day or 20 mL/kg per day. Infants remained in the study until discharge or development of stage ≥IIA necrotizing enterocolitis (NEC).

Main outcome measures Primary outcome measures were days to full feedings, incidence of feeding complications, and NEC. Secondary outcomes included: time to regaining
Results  Infants in the intervention group achieved full-volume feedings sooner (7 vs 10 days, median), regained birth weight faster (11 vs 13 days, median), and had fewer days of intravenous fluids (6 vs 8 days, median). Three infants in the intervention group and two control infants developed NEC for an overall incidence of 3.2% (relative risk: 1.73; 95% confidence interval: 0.30–10.06).

Conclusions  Among infants between 1000 and 2000 g at birth, starting and advancing feedings at 30 mL/kg per day seems to be a safe practice and results in fewer days to reach full-volume feedings than using 20 mL/kg per day. This intervention also leads to faster weight gain and fewer days of intravenous fluids.

Comment  On first thought it seems obvious that the group with the more rapid increase in feeding volume would reach the enteral feeding goal of 150 mL/kg/day more quickly. However, if the rapid schedule precipitated feeding intolerance, perhaps that group would actually reach the goal more slowly. The benefits of reaching “full” enteral feedings more rapidly include not only fewer days of intravenous administration but also fewer days of deep line usage. If such benefits were extended to large populations, a diminution in nosocomial sepsis would be expected, as would reduced costs and shorter hospital stays. However, before such extrapolations are made it should be kept in mind that this study demonstrated the feasibility of this approach, but estimates of the benefits and risks will remain imprecise until larger numbers are studied.

There are three caveats to these findings. First, readers should be cautioned not to apply these findings to neonates <1000 g birth weight. In fact, marked differences in feeding tolerance can exist between 1000-g neonates and 2000-g neonates. To allow for these differences, the feeding guidelines we use separates 1000- to 2000-g neonates into three categories: 1000 to 1250 g; 1251 to 1500 g, and 1501 to 2000 g; and each has a different set rate of volume increase. Second, it should be emphasized that feeding tolerance is better with human milk than with formula. It was disappointing to see that only one third of the patients in this study received human milk. Additionally, the authors fed 20 cal/oz Enfamil (rather than a premature formula) during the first days of life. It is not clear whether results would differ if a premature formula were used. Finally, this report points out the important unresolved issue of when to “hold” or temporarily discontinue feedings. Indomethacin use and large prefeeding gastric residuals were the two most common reasons for holding feedings, together accounting for 65% of the feeding discontinuations. Certainly, additional clinical research is needed regarding means of avoiding or compensating for these missed feeding opportunities.

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Preventing substance use and disordered eating: initial outcomes of the ATHENA (Athletes Targeting Healthy Exercise and Nutrition Alternatives) program


The objective of this well-designed study of 928 female high school athletes was to use a school-based, team-centered model of health promotion in order to reduce disordered eating habits and discourage the use of body-shaping substances. The intervention group had 8 weekly 45-minute discussions on key health topics incorporated into the usual practice schedule. Following the intervention, the authors found less use of diet pills and less use of athletic-enhancing substances (amphetamines, anabolic steroids, and sport supplements). Other risks were also reduced (eg, less riding with an alcohol-consuming driver, more seat belt use, and less new sexual activity). The intervention group also reported less inclination to use unhealthy weight control measures in the future.

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Gaps in the evidence for well-child care: a challenge to our profession


Given the focus in this section on the current best evidence, it makes sense to review this article that highlights many of the gaps in the evidence for well-child care. This systematic review examined the interventions recommended by seven major North American organizations. Forty-two interventions (noted by two or more organizations) related to behavioral counseling, screening, and prophylaxis were reviewed in more detail. Although not surprising to those of us in practice, the evidence supporting the recommended interventions is generally lacking, especially for common screening interventions. Some evidence exists for the effectiveness of behavioral counseling, particularly for injury prevention. There also is evidence that prophylaxis with folate prevents neural tube defects and iron supplementation can prevent iron deficiency. This article should be read in its entirety, especially by general pediatricians and health services researchers, as we strive to provide high-quality care for children.
HYPEREOSINOPHILIA IN RED SCALY INFANTS WITH SCABIES

Two 3-month-old, otherwise healthy infants came to medical attention with appearance of “red scaly baby” and hypereosinophilia. The rash (erythroderma with multiple, discrete, yellow to red-brown macules and papules on the trunk, extremities, scalp, face, palms, and soles) started at 1 year of age and at 3 weeks of age, respectively. The infants were reported to be mildly irritable and had poor feeding. Each was treated before diagnosis with oral antihistamines and topical corticosteroids. In both, normal blood count with hypereosinophilia (3.1 and 26.9 \( \times 10^9/L \)) was documented. Skin scrapings showed Sarcoptes scabei mites. In one infant, abnormal leukocyte chemotaxis was found. In the other infant, eosinophil peroxidase deficiency and mild elevation of IgE were found. Both infants were treated with permethrin 5% cream. Skin appearance and blood counts returned to normal 1 month after initiation of treatment.

Scabies incognito is a rare disorder in the neonatal period with an unusual presentation of numerous mites.\(^1,2\) Scabies may cause significant hypereosinophilia in the presence of mild reduction of immunity (eg, as from treatment with potent topical corticosteroid, abnormal leukocyte chemotaxis, and immature immune system in newborns). The presence of eosinophil peroxidase deficiency, a rare inherited anomaly, without evidence of disease\(^3\) is probably unrelated to scabies.

Scabies should be considered in the differential diagnosis of presentation of “red scaly infant.” Complete blood count and microscopic examination of the skin should be performed. Infants with prolonged hypereosinophilia, regardless of etiology, may develop organ damage, especially cardiac.\(^4,5,6\) Treatment for scabies might be considered even without clear evidence of \( S \) scabei mites in the skin scraping.

REFERENCES
Letters

Selecting families for successful insulin pump therapy

To the Editor:

DiMeglio et al offer important evidence that continuous subcutaneous insulin infusion may be safe and well tolerated in preschool age children with type I diabetes. Based on their findings, DiMeglio et al conclude that the initiation of pump therapy should be based upon physician selection of appropriate patients and the family's preference. It is noteworthy that DiMeglio et al included patients in their study “based on a history of compliance” with physician recommendations. This inclusion criterion suggests that the authors have an a priori hypothesis about which patients may be best suited to pump therapy.

A history of adherence to physician recommendations is one predictor of those patients likely to succeed with pump therapy. Additional variables elucidated in our work with children with diabetes using insulin injections include diabetes-specific family factors such as parental warmth, parental criticism or nagging, and responsibility-taking for diabetes care tasks. In a recent study examining the relationship between family factors and metabolic control in 109 youths with type I diabetes, we found that poorer metabolic control was associated with critical or unsupportive parental behaviors and a lack of child or parent responsibility for diabetes tasks. In contrast, better metabolic control was associated with higher ratings of parental warmth and caring. Without considering family factors, previous examinations of the relationship between adherence and metabolic control have not found as strong a relationship.

Although the DiMeglio study suggests the efficacy and safety of pump therapy, we highlight the importance of empirically examining family and sociodemographic factors that predict pump response. We suggest that diabetes-specific family factors, as well as past adherence behaviors, are likely important predictors of success using pump therapy. The development of a more standardized method of selecting patients well suited to pump therapy would be beneficial for physicians and families alike.

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YMPD1315

References


Reply

To the Editor:

We appreciate the comments of Williams et al. As noted, we chose to study patients who had a history of adherence with recommended diabetes therapy and of attendance at scheduled clinic visits. This was done because we felt that it was essential to pick families that would likely complete the study protocol safely because insulin pumps were an untested method of diabetes management in toddlers. We agree that there is a need to continue to empirically examine patient and family-specific factors that may predict success with pump therapy. We have conducted a study investigating predictors of good diabetes control in 94 diabetic children and adolescents on pump therapy for over 1 year. After examining a series of factors including: gender, family structure, years with diabetes, prior education, pre-pump diabetes regimens, pre- and post-pump hemoglobin (Hgb)A1Cs, age at pump initiation, pump type, duration of time on pump, number of basal rates, clinic visit type and frequency, and diabetes camp attendance, the only variables that were predictive of good control on continuous subcutaneous insulin infusion (CSII) were pre-pump HgbA1C, younger age, and number of basal rates.

Assessing family variables in the clinical setting remains enigmatic. Additional tools for these evaluations, such as those referred to in the letter by Williams et al, are needed. Given the cost and complexity of CSII, efforts aimed at identifying optimal candidates must continue.

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YMPD1316

10.1016/j.jpeds.2004.11.012
Obesity and Asthma

To the Editor:

We read with interest the article by To et al titled, “Is Obesity associated with asthma in young children?”1 Baseline data from the National Longitudinal Survey of Children and Youth (for Canadian children) were used in this cross-sectional study. The outcome measure was current asthma (based on historic information from parents), and the independent variable of primary interest was obesity as measured by body mass index (BMI).1

The authors state that they used the generally accepted definition of obesity as BMI ≥85th percentile, and they cite two references.2,3 In reference 2 (Epstein et al) the data were based on the Second National and Nutrition Examination Survey (NHANES II) published in 1987, in which obesity was indeed defined as a BMI ≥85th percentile. However, the definitions of overweight and obesity have been revised as age and gender-specific BMI growth charts that provide a statistical definitions of weight status for children 2 to 20 years of age have been published.4,5 With the use of the current recommendations, at risk for overweight is defined as a BMI at the 85th through the 94th percentile, and overweight is defined as a ≥95th percentile BMI.4,5

We find it puzzling that the authors defined obesity as a BMI ≥85th percentile considering the current definitions used in the literature on this subject,3 and we would appreciate clarification from the authors. Additionally, the pages of reference 15 are incorrectly stated.

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YMPD1317

10.1016/j.jpeds.2004.11.013

REFERENCES


Reply

To the Editor:

The decision to use body mass index (BMI) ≥85th percentile as our “exposure” variable of interest and, hence, the definition of obesity in our study was made after careful review of the literature and expert consultation. The rationale for our decision was twofold. First, we chose BMI ≥85th percentile to be comparable with previous population-based research examining the association between asthma and obesity. Percentile cutoffs obviously depend on the baseline population. Over the last 30 years, absolute BMIs in children have increased. The cutoffs used in our study are based on Canadian population-based data collected from 1994 to 1995. Our data4 show similar BMIs to the NHANES III data (see our article, Table I, BMI values in kg/m²) but significantly higher BMIs than NHANES II data or previous Canadian data.2 Thus, a cutoff at the 85th percentile in our data is similar in absolute BMI values to a BMI cutoff of 95th percentile in 1981 Canadian data.2 Previous research examining asthma and obesity has used a range of BMI cutoffs based on different baseline populations, as can be seen in Table IV in our article.1

Second, BMI quintiles were used, as noted in the text of the article, to ensure sufficient numbers within each category. BMI ≥85th percentile was the highest cutoff in which the number of children in each group remained sufficiently large to allow for meaningful multiple variable analyses. Furthermore, secondary analyses were conducted using higher cut-points, including the 95th percentile, and there were no significant differences observed (ie, no association between asthma and BMI ≥95th percentile). Because of Statistics Canada’s publication guidelines, we were unable to report results of children with BMI ≥95th percentile as the unweighted number of children in each group became too small.

We do recognize that the definition of obesity in our study may not coincide exactly with clinical definitions of obesity. The definition of an exposure variable for any epidemiological study is difficult and may be slightly different than a definition used for clinical purposes. If we had used a cutoff value of 95th percentile, it would be hard to argue that this could be responsible for the large increase in asthma prevalence. Furthermore, whereas Barlow and Dietz3 indicate BMI ≥95th percentile should be used as the definition of obesity, they state that children with BMI ≥85th percentile should still be evaluated carefully for complications of obesity but should not be called “obese” to avoid the potential psychological and physical harm of the potential misclassification.3 Because the children with BMI ≥85th percentile are at risk of complications of obesity it is reasonable to hypothesize that they could be at risk for asthma.