CHANGE IN ROTAVIRUS EPIDEMIOLOGY IN NORTHEAST FLORIDA AFTER THE INTRODUCTION OF ROTAVIRUS VACCINE

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Abstract: Retrospective analysis done at a children's hospital showed significant decrease in infections and hospitalizations caused by rotavirus in northeast Florida after the introduction of rotavirus vaccines in 2006. The rotavirus season was delayed in onset by 8 months and duration prolonged by 2–3 months in 2008, and no definite season occurred in 2009.

Key Words: gastroenteritis, diarrhea, rotavirus vaccine, vaccine effect, Florida

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The number of deaths attributable to rotavirus in the United States is low; however, rotavirus infections still cause significant morbidity, resulting in hospitalizations and outpatient visits.[1,2] The annual rotavirus season in the United States typically occurs in the cooler months, starting in the fall in the southwest and ending in the northeast by spring.[3]

In the United States, 2 rotavirus vaccines, RotaTeq (Merck and Company, Whitehouse Station, NJ) approved in February 2006 and Rotarix (GlaxoSmithKline, Research Triangle Park, NC) approved in April 2008, are recommended for use by the Advisory Committee on Immunization Practices.[4] These 2 vaccines were expected to significantly decrease the number and severity of rotavirus infections in the United States. We conducted a retrospective analysis to examine the epidemiology of rotavirus infections and hospitalizations in northeast Florida before and after the introduction of rotavirus vaccines.

METHODS

We reviewed records at the Wolfson Children's Hospital (WCH), the only regional tertiary care center serving children in northeast Florida. WCH has 192 beds, 8000–9000 annual admissions, and a catchment area of 3 million. The study period was between January 1, 2004, and December 31, 2009, and included patients aged <18 years who were tested for and those who were hospitalized because of rotavirus infection. Prevaccine period was defined as 2004–2006, and vaccine period as 2007–2009. The study was approved by the institutional review board.

Rotavirus vaccine uptake was indirectly estimated by determining the number of vaccine doses distributed in northeast Florida. The number of doses distributed in the public sector was obtained from the Bureau of Immunization, Florida Department of Health, and in the private sector from the 2 vaccine manufacturers. Data of live births for the study period were obtained from the Florida state census online.[5]

Laboratory records were reviewed to identify patients in whom rotavirus tests were ordered on a stool specimen. Rotavirus tests were done only on bulk stool specimens. It was assumed that clinicians ordered rotavirus tests only on patients who had diarrhea, and patients were assumed to have rotavirus infection if the test result was positive.

Hospitalization because of rotavirus infection was used as a surrogate for the severity of the infection. The number of hospitalizations because of rotavirus was determined and compared with the overall number of hospitalized rotavirus-infected patients and also with all annual hospitalizations at WCH from 2004 to 2009. Children admitted secondary to rotavirus infection were identified using International Classification of Diseases, Ninth Revision, Clinical Modification code 008.61 used only as the primary or secondary discharge diagnosis.

We determined the seasonal pattern (onset, peak, and end) of rotavirus infections in northeast Florida during the prevaccine period (2004–2006) and vaccine (2007–2009) periods. Season onset was defined as >10% positive tests for at least 2 consecutive months, and season end as <10% positive tests for at least 2 consecutive months. Season peak was defined as the month(s) with the highest percentage of positive tests.

Statistical analyses were done comparing prevaccine period (2004–2006) with vaccine period (2007–2009) for rotavirus infections and hospitalizations using SAS 9.1 software (SAS Institute, Cary, NC). Categorical comparisons were done using χ^2 tests. Means were compared using 2-sample t tests.

RESULTS

Shown in Table 1 are live births, rotavirus tests done, rotavirus tests positive, total hospitalization, rotavirus hospitalizations, and vaccine doses distributed for each of the 6 study years. Monthly distribution of rotavirus tests ordered and positive results in the study period are depicted in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A440.

The number of tests performed decreased by 12.1% from an average of 758 in the prevaccine period to an average of 666 in the vaccine period ($P = 0.28$, t test statistic). There was a 57.8% decline in the absolute number of positive rotavirus tests from an annual average of 207 in the prevaccine period to 87.3 in the vaccine period ($P = 0.03$, t test statistic). The proportion of positive tests decreased significantly from 27.3% in the prevaccine period to 13.11% in the vaccine period ($P < 0.0001$, χ^2). A positive test was 2.08 times more likely in the prevaccine period compared with the vaccine period.

There was a decrease in rotavirus hospitalizations relative to total hospitalizations in the vaccine period (0.20%) compared with the prevaccine period (0.71%; $P < 0.0001$, χ^2). Among those who had rotavirus infections, the absolute number of hospitalizations decreased by 72% from an annual average 63.3 in the prevaccine period to 18 in the vaccine period ($P = 0.01$, t test statistic). The proportion of patients hospitalized because of rotavirus infection decreased significantly from 30.6% in the prevaccine period to 20.6% in the vaccine period ($P = 0.0024$, χ^2).

The rotavirus season in northeast Florida started in January and ended in May or June, with a peak in March or April for the prevaccine years and 2007. However, in 2008, the season started in September, peaked in October, and ended in April 2009 (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A440). No defined rotavirus season was appreciated in 2009.

DISCUSSION

We observed a decrease in infections and hospitalizations because of rotavirus and a change in the rotavirus season after the

TABLE 1. Live Births, Rotavirus Tests, Hospitalizations, and Vaccine Doses by Year

Year	Live Births	Rotavirus Tests	Positive Rotavirus Tests (%)	Total Hospitalizations	Rotavirus Hospitalizations	Vaccine Doses Distributed
2004	17,453	707	223 (31.5)	8757	70	N/A
2005	18,154	760	200 (26.3)	8983	58	N/A
2006	19,024	807	198 (24.5)	8915	62	8,138
2007	19,268	795	159 (20)	8567	38	34,135
2008	18,720	646	67 (10.4)	8279	9	37,910
2009	N/A*	557	36 (6.4)	8595	7	42,220

*2009 Live birth information will not be available until January 2011.

introduction of rotavirus vaccines in 2006. This is similar to the previously reported data.[6,7] However, the change in the seasonal pattern of rotavirus infection in northeast Florida after the introduction of the vaccine was different from what has recently been reported from other parts of the United States.[6]

As has been reported for the southern US,[6] our study showed that both the total number of rotavirus tests performed and reported positive decreased. We also observed that during the vaccine period there was a decrease in the proportion of positive tests relative to total rotavirus tests performed.

Our study also showed a decrease in the severity of rotavirus infections as reflected by the decline in hospitalizations. This is similar to what has been reported in a postlicensure evaluation of the effectiveness of the rotavirus vaccine.[7]

The introduction of rotavirus vaccines not only decreased the number of infections and hospitalizations but also changed the annual seasonality of rotavirus infection. National reports showed that the onset of 2007–2008 rotavirus season was delayed by 11 weeks but the duration was shorter than 2000–2006 season, whereas 2008–2009 season had an earlier season onset but longer duration compared with 2007–2008 season.[6] According to our data, the rotavirus season onset in northeast Florida during the prevaccine years and 2007 was in January, with the peak occurring in March or April and the season ending in May or June. The duration of rotavirus season in our region during the prevaccine years and 2007 was 5–6 months. However, in 2008, the duration of the season was 8 months, starting in September, peaking in October, and ending in April 2009. Compared with the prevaccine period, the onset of rotavirus season in 2008 was delayed by 8 months, the peak occurred sooner (within 1 month of season onset), and the duration was 2–3 months longer. No rotavirus season was observed in 2009. Secular variability has been reported after introduction of rotavirus vaccine.[8] Prolonged follow-up of season patterns will clarify this issue. We continue to monitor rotavirus infections and hospitalizations in northeast Florida.

There are several limitations to our study. This is a retrospective analysis subject to numerous confounders and biases. Although we believe that there were no changes in admission or testing policies at our institution, changes in clinical practice over the years may have some influences on the changes we observed. Some patients with acute gastroenteritis might not have been tested for rotavirus infection and some may have been tested at outside laboratories, leading to a lower detection rate. Individual patient information was not available before 2008, which may have resulted in counting some positive rotavirus results more than once. However, this was not very likely because we did not observe any duplication of results in 2008 and 2009 when patient-level information became available. Additionally, vaccine uptake in our study was assessed indirectly using vaccine distribution, which may not accurately represent actual vaccine use. However, vaccine uptake has been steadily increasing, with 40%–64% first-dose coverage reported for children aged 3 months.[9] In addition, use of International Classification of Diseases, Ninth Revision, Clinical Modification codes often underestimate incidence of rotavirus-related admissions.[10] However, the difference in infections and hospitalizations because of rotavirus between the prevaccine and vaccine periods is significant and seems to be temporally related to rotavirus vaccine availability.

In conclusion, our study showed a decrease in infections and hospitalizations because of rotavirus infection temporally associated with the introduction of rotavirus vaccine. In addition, the season seems to be evolving from delayed onset and prolonged duration in 2008 to no appreciable "season" in 2009. Continued surveillance over the next several years will be critical to determine whether the decrease in rotavirus infection and the seasonal differences in northeast Florida are sustained over time.

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ANTIBODY PERSISTENCE 12 MONTHS AFTER A BOOSTER DOSE OF MENINGOCOCCAL-C CONJUGATED VACCINE IN THE SECOND YEAR OF LIFE

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Abstract: We report on the results of the 12-month follow-up of children aged 14 to 18 months who received primary and booster vaccinations with either a meningococcal-C vaccine conjugated to tetanus toxoid or CRM_{197}.

Seroprotection (92.8%) and geometric mean titers/serum bactericidal activity (410.5; 95% CI: 273.4–616.3) were higher in children receiving the meningococcal serogroup C tetanus toxoid conjugate, compared with 61.5% and serum bactericidal antibody geometric mean titer of 45.1 (95% CI: 28.5–71.3) when MenC-CRM_{197} conjugate was used.

Key Words: *Neisseria meningitidis*, serotype C, immunization, secondary, meningococcal vaccines

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Waning protection after immunization with serogroup C meningococcal vaccines in infancy and early childhood has been associated with a fall in serum bactericidal antibody concentrations. Despite the immunologic memory induced by these vaccines, rapid progression of meningococcal disease would not allow the organism to mount an anamnestic immune response.[1,2] Protection correlates with the circulating antibody concentration. A booster dose, introduced in the second year of life, induces a substantial rise in bactericidal antibody titers.[3,4]

Seroepidemiologic studies have shown that 3–6 years after a single dose of conjugated meningococcal serogroup C (MenC) vaccine, the percentage of subjects protected with serum bactericidal antibody (SBA) titers ≥1:8 decreases with the age when the vaccine was given.[5,6]

The kinetics of the seroprotection after a booster dose is not well known. There are no long-term studies to assess the persistence of antibodies. In 1 clinical trial,[3] the SBA geometric mean titers (GMTs) decreased by more than 97% in the first 18 months after the booster dose. A higher rate of persisting protective SBA-MenC titers was observed in those subjects who had higher SBA-MenC GMTs after booster vaccination.[3]

We have recently described the immune response to a booster dose in children primed and boosted with 2 MenC vaccines.[4] The antibody titers reached were strongly depended on the vaccine used for priming; GMTs were 3.5 times higher if the vaccine used was conjugated with tetanus toxoid (MenC-TT) compared with CRM_{197} (MenC-CRM) conjugated vaccine formulation. Although the MenC vaccine used for priming has been described to affect the response to *Haemophilus influenzae* type b (Hib) vaccines given concomintantly,[7] we did not find any difference after boosting with either MenC vaccine.[4]

The objective of this study was to assess the persistence of the seroprotection against MenC and Hib 1 year after the booster dose and to analyze the factors that might influence seroprotection.

METHODS

This study was designed as a multicenter, randomized, open-label, clinical trial, described in a previous publication.[4]

Children aged 14 to 18 months who completed the primary vaccination series before their 8th month with either MenC-TT or MenC-CRM were randomized to receive one of these vaccines as a booster. The exclusion criteria were having an acute or severe chronic disease or hypersensitivity to any of the vaccine components including antibiotics, a history of invasive disease and being treated with any immunosuppressant drug.

The Ethics Committee of DGSP/CSISP, Valencia, approved the study, and informed written consent was signed by the parents or guardians before entering in the study.

A blood sample was collected before the booster dose, and 1 month and 12 months (±3 weeks) after the booster. The results of this last sample are described here.

Serologic Studies. Sera were analyzed at the Manchester Vaccine Evaluation Department of the Health Protection Agency, UK. Functional MenC antibody titers were determined using the SBA assay. The SBA target strain was MENC11 (C:16:P1.7–1,1), and the complement source was baby rabbit sera (Pel-Freeze Incorporated, Rodgerson, AZ). SBA titers are expressed as the reciprocal of the final serum dilution equivalent to 50% killing at 60 minutes. Hib specific antibodies (IgG) were quantified using standardized ELISA at the Immunoassay Laboratory, Health Protection Agency, Porton Down, UK. The standard sera used were the International anti-Hib Quality Control Serum Center for Biologics and Evaluation Research (CBER) 1983.

Antibody titers were log transformed; geometric mean titers (GMT) and concentrations (GMC) with 95% confidence intervals were calculated. Mann-Whitney *U* test was used to evaluate significant differences between antibody assays at each time point. Children were considered seroprotected when MenC SBA titers were ≥1:8, and anti-Hib antibodies were ≥0.15 μg/mL.

RESULTS

Of the 389 subjects randomized in the clinical trial, 334 (85.9%) had blood drawn 12 months after the booster dose. One month after the booster dose all but 2 children had reached seroprotection titers (Table 1). One year later, 61.5% of subjects primed with MenC-CRM who received a booster with the same vaccine retained seroprotective values, whereas 92.8% of subjects primed and boosted with MenC-TT retained seroprotective titers. SBA titers of ≥1:128 were measured in 49.5% and 91.3% of subjects, respectively. The seroprotection rate was dependent not only on which vaccine was used for priming (MenC-CRM versus MenC-TT), but also on which vaccine was used for the booster dose. Sixty-nine percent of the children primed with MenC-CRM had SBA titers ≥1:8, compared with 87.2% of those primed with MenC-TT ($P < 0.01$), with GMTs of 60.0 (95% CI: 43.8–82.2) and 283.8 (95% CI: 204.3–394.3), respectively.

In children initially primed with MenC-CRM, 61.5% of those who received a booster with the same vaccine, and 75.5% of those boosted with MenC-TT had seroprotective SBA titers ($P < 0.05$) 12 months after the booster dose. Among children who were primed with MenC-TT, 92.8% of those boosted with the same vaccine, and 81.9% of those boosted with MenC-CRM ($P = 0.055$) were still seroprotected 12 months after the vaccine was administered.

The SBA GMTs among groups also varied depending on which vaccine was used for booster. Children primed with MenC-

TABLE 1. Seroprotection and SBA GMTs in the 4 Study Groups Depending on the Vaccine Used for Priming and Boosting

	Priming Vaccination			
	MenC-CRM		MenC-TT	
Booster Vaccine	MenC-CRM	MenC-TT	MenC-CRM	MenC-TT
SBA ≥128				
1 month post				
N	100	107	86	81
n (%)	100 (100)	106 (99.1)	86 (100)	80 (98.8)
12 months post				
N	91	102	72	69
n (%)	45 (49.5)	60 (58.8)	55 (76.4)	63 (91.3)
SBA ≥8				
1 month post				
N	100	107	86	81
n (%)	100 (100)	106 (99.1)	86 (100)	80 (98.8)
12 months post				
N	91	102	72	69
n (%)	56 (61.5)	77 (75.5)	59 (81.9)	64 (92.8)
SBA GMT (95% CI)				
Prebooster	8.6 (6.1–11.9)	13.2 (8.9–19.5)	7.6 (5.1–11.2)	9.7 (6.4–14.9)
1 month post	1746 (1378–2213)	2061 (1599–2627)	6278 (4841–8144)	6786 (5023–9167)
12 months post	45.1 (28.5–71.3)	77.4 (50.1–119.7)	199.3 (119.8–331.5)	410.5 (273.4–616.3)

CRM had SBA GMTs of 45.1 (95% CI 28.5–71.3) if boosted with the same vaccine and 77.4 (95% CI: 50. 1–119.7) if boosted with MenC-TT ($P = 0.066$). Among children primed with MenC-TT, the SBA GMTs were 199.3 (95% CI: 119.8–331.5) if boosted with MenC-CRM compared with 410.5 (95% CI: 273.4–616.3) if boosted with MenC-TT ($P < 0.05$) (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A426).

Three of the 283 subjects from whom a blood sample was drawn for Hib antibodies did not maintain anti-Hib concentrations ≥0.15, which is considered a correlation of protection, 12 months post booster. One such case occurred in 3 of the 4 treatment groups. There was no difference in anti Hib GMC between children initially vaccinated with MenC-CRM (3.4 μg/mL; 95% CI: 2.8–4.1) versus those primed with MenC-TT (3.6 μg/mL; 95% CI: 2.9–4.4), nor was the anti-Hib concentration dependent of which vaccine was used for booster (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A427).

DISCUSSION

In the 12 months after administration of a Men C booster vaccine to toddlers (aged 14–18 months), a rapid decrease in SBA titers was observed. In blood samples taken 12 months (+3 weeks) after the booster vaccination, 1 in 4 children were not seroprotected as measured by SBA.

A booster vaccination was recommended to children who had been primed during their first year of life, as it had previously been shown that immunogenicity decreased in these children after their first birthday.[1,2] It was estimated that a single booster dose would protect children for a longer period and that additional vaccinations would not be required.

In a previous study we showed the immunologic effect 4 weeks after the booster dose,[4] in the current article we describe the follow-up of these children. One month after the booster vaccine, most of the subjects had seroconverted to SBA ≥1:128 and had attained high SBA titers, with differences depending on which vaccine had been used for the primary vaccination series; subjects who had been primed with the MenC-TT vaccine attained SBA titers 3.5 times higher than those primed with the MenC-CRM vaccine.[4] One year later, the SBA titers were shown to have decreased substantially, although the SBA GMTs remained higher than before the booster vaccination and a large percentage of subjects retained seroprotective levels. A decrease in the percentage of children with SBA ≥1:8 might reflect a decrease in the number of children protected with advancing age.

At 12 months postbooster vaccination, seroprotection rates and SBA titers were dependent on which vaccine had been used for the primary and booster vaccinations. The highest percentage of subjects was seroprotected when MenC-TT vaccines had been used for all (prime and booster) vaccinations.

The decrease in seroprotection has also been described in another study in which the investigators used a combination vaccine (Hib-MenC[3]) and found that SBA titers fell up to 95% in the first 18 months after administration of a booster dose given in the second year of life. Also, although 91% to 100% of the children who received a single vaccine dose in their second year of life[8] became seroprotected with an SBA titer of ≥1:8, at 4 years of age the seroprotection rates in these children were low.[9] These findings seem to indicate that seroprotection decreases over time after a single dose of MenC vaccine in preschool-aged (1–2-year-old) children.

Findings from this study may influence future MenC vaccination policies. If SBA titers and seroprotection rates decrease in this way, a large proportion of young children could be at risk for developing MenC infection. However, if herd immunity is established, this will not translate into increased infection rates. Most of the adolescents in Europe, in countries where a catch-up program was established, are still seroprotected[6]; however, as children vaccinated in infancy, with or without a booster dose, reach adolescence, a possible resurgence of MenC circulation among this group is possible, as the protective herd immunity disappears. A booster dose during early adolescence could be required[6]; however, this was not predicted in a mathematical model indicating that high levels of indirect protection against meningococcal group C diseases are likely to persist even if the vaccine only provides 3 years of protection against carriage.[10]

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CONCOMITANT RESPIRATORY VIRAL INFECTIONS IN CHILDREN WITH KAWASAKI DISEASE

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Abstract: The role of respiratory viruses in the pathogenesis of Kawasaki disease (KD) remains controversial. In this study, we showed that 8.8% of patients with KD had documented respiratory viral infections. Patients with concomitant viral infections had a higher frequency of coronary artery dilatations and were significantly more often diagnosed with incomplete KD. The presence of a concomitant viral infection should not exclude the diagnosis of KD.

Key Words: Kawasaki disease, incomplete, respiratory viruses

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Kawasaki disease (KD) is an acute self-limited multisystem vasculitis that represents the most common cause of acquired heart disease in children in the developed world.[1,2] Although the epidemiology and clinical features of KD suggest an infectious origin, and many viral infections share similar clinical characteristics, its etiology remains unknown.[3–5]

The diagnosis of KD requires the presence of fever for 5 days, 4 of 5 characteristic clinical features, and the lack of another diagnosis that could explain the findings.[6,7] In this context, the identification of a microbial pathogen in children with suspected KD could delay diagnosis and treatment.[8] The present study was designed (1) to determine the frequency of concomitant respiratory viral infections in children with KD, and (2) to compare the clinical features and outcomes of KD patients with and without documented viral respiratory infections.

METHODS

Medical records from patients hospitalized at Children's Medical Center Dallas with the diagnosis of KD from January 1, 1999, through December 31, 2008, were identified by International Classification of Diseases-9 codes (446.1). Records were reviewed to determine which patients with KD had been tested for respiratory viruses. Subsequently, KD patients were classified as cases and controls according to the results of their virology tests. Cases were defined as patients with KD in whom a viral respiratory pathogen was identified, and controls were those KD patients who tested negative for those viruses. Each case was matched with 2 controls for age, sex, and date of hospitalization (±3 weeks). Viral testing was performed at the discretion of the attending physician in all patients by direct fluorescent-antibody (DFA) assay for 7 respiratory viruses (respiratory syncytial virus [RSV], parainfluenza virus [PIV] types 1, 2, and 3, influenza virus A and B, and adenovirus). If the DFA showed negative results, a viral culture was automatically performed. Patients' demographic characteristics, clinical presentation, course, response to treatment with intravenous immunoglobulin (IVIG), laboratory results, and echocardiographic findings were compared between the groups. Refractory disease was defined by the presence of persistent or recrudescent fever 36 hours after completion of IVIG infusion.[6,9] Incomplete KD was defined by fever for at least 5 days, and less than 4 of classic KD clinical criteria at presentation documented by the attending physician, and before any echocardiographic studies were performed.[6] The study was approved by the Institutional Review Board of the University of Texas Southwestern Medical Center at Dallas (IRB #052009-004).

Descriptive analyses were performed using frequency distributions and rates. Means (±SD) or medians (percentiles 25th to 75th) were used to summarize patient's demographic and baseline characteristics. Groups were compared using Student *t* test or Mann-Whitney *U* test for continuous variables. χ^2 tests with Yates correction for continuity or Z-test were used for associations between categorical variables and proportions. A 2-tailed *P* value <0.05 was considered significant. Sigma Stat 2003 software (SPSS Science, San Rafael, CA) was used for analyses.

RESULTS

During the study period, 394 patients were hospitalized at Children's Medical Center Dallas with KD. Viral testing was performed in 251 (63.7%) patients. The proportion of patients tested for respiratory viruses significantly increased from 24% in 1999 to 84% in 2008 ($P < 0.0001$). From 1999 to 2001, 24%–43% of KD patients were tested for respiratory viruses, all with negative results. Thereafter, the percentage of KD patients tested increased as well as the percentage of positive viral results ranging from 6.6% in 2006 to 16.6% in 2004 (overall rate of detection 11.4% per year). Twenty-two (8.8%) patients had a respiratory virus identified, including rhinovirus (n = 6), adenovirus (n = 6), influenza A or B (n = 5), PIV 1–3 (n = 3), and RSV (n = 2). Although RSV

(2 of 2) and influenza (4 of 5) were mostly identified by DFA, all rhinovirus isolates (6 of 6) and the majority of PIV (2 of 3) and adenoviruses (4 of 6) were negative by DFA and diagnosed by viral culture.

There were no significant differences in age, sex, and race or ethnicity between KD patients with viral infections (cases) and those who tested negative for viruses (controls). The median (75%–25% percentiles) age of cases and controls were 3.4 (1.2–4.5) versus 2.7 (1.2–5.7) years, respectively ($P = 0.76$). Sixty percent (13 of 22) of cases and 68% (30 of 44) of controls were male, and white (63% of cases vs. 52% of controls) followed by Hispanic (23% in each group), black (5% vs. 16%), and other races (9% in each group) were the most common cases and controls' race/ethnic groups.

The clinical criteria, clinical course, and outcomes of cases and controls are summarized in Table 1. Except for emesis, which was documented more frequently in KD patients with viral infections; the rest of factors evaluated, including fever for at least 5 days, the classic clinical features of KD,[6] and the presence of respiratory and gastrointestinal symptoms at the time of hospitalization, were similar between groups. On the other hand, children with viral infections were diagnosed more often with incomplete KD by the attending physician before echocardiographic studies were performed than were those with negative viral results (cases 36% [8 of 22] vs. controls 11% [5 of 44]; $P = 0.036$).

Except for serum alanine aminotransferase values before IVIG administration, which were significantly lower in cases than in controls, the rest of laboratory indices evaluated, including white blood cell and platelet counts, serum albumin concentrations, other liver function tests (aspartate aminotransferase, γ-glutamyl transpeptidase), and pyuria, were not significantly different between the groups. Acute-phase reactants (erythrocyte sedimentation rate and C-reactive protein) showed a trend toward lower values in children with KD and concomitant viral infections (Table 2).

TABLE 1. Demographics, Clinical Characteristics, and Outcomes of Cases and Controls

Variable	Cases (n = 22)	Controls (n = 44)	*P*
Clinical criteria and nonspecific symptoms, n (%)			
Bilateral conjunctival injection	16 (73)	41 (93)	0.06*
Mucosal changes†	16 (73)	37 (84)	0.46*
Polymorphous rash	17 (77)	37 (84)	0.72*
Changes of extremities§	6 (27)	19 (43)	0.32*
Cervical lymphadenopathy	10 (45)	20 (45)	0.79*
Rhinorrhea	10 (46)	19 (43)	0.97*
Cough	8 (36)	13 (30)	0.83*
Diarrhea	5 (23)	5 (11)	0.35*
Emesis	9 (41)	3 (7)	0.02*
Sore throat	0	2 (5)	0.73*
Clinical characteristics and outcomes			
Days of illness at presentation, median (IQ range)	5.5 (5–7)	5 (4–7)	0.13‡
Days of fever before IVIG, median (IQ range)	7 (5–8)	6 (5–7)	0.12‡
Length of stay, median (IQ range)	4 (3–5)	3 (3–5)	0.48‡
IVIG resistance, n (%)	4 (18)	8 (18)	1*
Abnormal echocardiography findings, n (%)	12 (55)	18 (41)	0.41*
Coronary aneurysms	2 (9)	4 (9)	0.64*
Coronary dilation¶	9 (42)	6 (14)	0.02*
Coronary ectasia	1 (4)	4 (9)	0.81*
Pericardial effusion		4 (9)	0.37*

*Z-test for proportions.
†Strawberry tongue or dry, cracked, erythematous lips.
‡Mann-Whitney *U* test.
§Change of extremities, including induration of hands and feet with erythematous palms and soles and periungual desquamation.
¶Z-scores.

TABLE 2. Laboratory Values on Admission of Cases and Controls

Laboratory Values, Median (Interquartile Range)	Cases (n = 22)	Controls (n = 44)	*P**
WBC count, $10^3/\mu L$	12.7 (8.1–14.5)	13.2 (10.4–18.7)	0.1
PMN, %	52.5 (41–67)	59.5 (45.5–72)	0.36
Bands, %	10 (3–12)	6 (1–16.5)	0.29
Lymphocytes, %	20.5 (15–32)	21 (12–30.5)	0.6
Platelet count, $10^3/\mu L$	299 (233–362)	331 (278.25–396.75)	0.09
ESR, mm/h	54.5 (32–68)	71 (58.25–91)	0.05
CRP, mg/dL	7.9 (5.5–14.2)	11.05 (6.8–17.7)	0.32
Albumin, g/dL	2.75 (2.25–3.65)	2.9 (2.8–3.2)	0.71
ALT, U/L	36 (27–78.5)	66 (33–127.5)	0.03
AST, U/L	31.5 (25–66)	46 (26.75–80.25)	0.31
GGT, U/L	54 (15.5–112)	77 (20.5–210.5)	0.22
Pyuria (>10 WBCs per hpf), %	15 (14.5–22.5)	25 (21.5–48.25)	0.38

*Mann-Whitney *U* test.
WBC indicates white blood cell; PMN, polymorphonuclear leukocytes; ESR, erythrocyte sedimentation rate; CRP, C-reactive protein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, γ-glutamyl transpeptidase.

Regarding clinical outcomes, there were no differences in days of fever before hospitalization or days of fever to IVIG administration, frequency of refractory disease, and length of hospitalization between the groups. Overall, echocardiographic examinations performed during the course of the acute hospitalization showed similar frequency of cardiac abnormalities between the groups, with the exception of coronary artery dilations defined by Z-scores, which were more frequently diagnosed in KD patients who tested positive for respiratory viruses and were independent of the timing of IVIG administration (cases 42% [9 of 22] vs. controls 14% [6 of 44]; $P = 0.02$; Table 1).

There was follow-up information for 16 (72%) cases and 35 (79%) controls. One patient in each group with previous normal echocardiograms developed cardiac abnormalities at the first follow-up visit 2 weeks after discharge. One was a patient with adenovirus infection who developed a right coronary artery saccular aneurysm and left coronary artery dilation, and the other was a control patient who developed left anterior descending coronary artery dilation.

DISCUSSION

The purpose of our study was to determine the frequency and the clinical impact of respiratory viral infections in almost 400 children diagnosed with KD during a 10-year period. We found that the detection of a respiratory virus by DFA and culture was not uncommon in children with KD. Children with KD and a concomitant viral infection were diagnosed significantly more often with incomplete KD at presentation and had a higher frequency of coronary artery dilations than those KD patients who tested negative. This observation was independent of the timing of IVIG administration after onset of fever.

Multiples studies have evaluated the role of various infectious pathogens as potential agents for KD[10–13]; however, despite more than 40 years of active research, the cause of KD remains unknown. Because of its seasonality, different studies have at-

tempted to establish a link between KD and specific respiratory viruses, including adenovirus, human coronavirus, and human bocavirus, with inconclusive results.[10,12,14] In agreement with Shike et al[14] we did not find a predominant virus associated with the disease; rather, a similar proportion of children with KD who had a variety of respiratory viruses, including adenovirus, rhinovirus, PIV, or influenza. In a study conducted during 2 outbreaks of KD in the United States during the 1980s, a preceding respiratory illness documented by questionnaire was found in 83% and 56% of children with KD, respectively.[15] The investigators measured convalescent-phase antibodies to 33 microbial agents, but there was no information as to whether the presence of a concomitant viral infection was associated with differences in clinical presentation and outcomes.[15]

Currently, in the absence of a specific test, the diagnosis of KD relies on clinical characteristics, experience of the clinician,[6] and exclusion of other illnesses that could mimic the disease.[7,16] In an earlier retrospective study conducted in an emergency department to assess the burden of adenovirus infections in children, treatment with IVIG was withheld and no echocardiograms were performed in 4 of 5 children with adenovirus infection and suspected KD.[8] In our study, 3 of 6 patients with KD and adenovirus infections had abnormal echocardiograms initially, and 1 of the 6 patients developed cardiac abnormalities on follow-up examination. Moreover, children with KD and a concomitant viral infection had coronary artery dilations significantly more frequently than those in the control group. Except for this observation, there were no other significant differences in terms of outcomes between cases and controls. In addition, 18% of patients in each group did not respond to the first dose of IVIG, and 9% in each group developed coronary artery aneurysms. Evidence suggests that delaying therapy for KD is associated with increased risk of treatment failure and for development of coronary artery aneurysms.[17,18] Our results underscore the need for considering IVIG therapy in children with a high index of suspicion for KD even in the absence of all classic symptoms and with documented respiratory viral infections.

Our study has a number of limitations. Its retrospective design makes it difficult to draw definitive conclusions. We cannot demonstrate that all patients diagnosed with incomplete KD and a concomitant viral infection had indeed KD, or that the diagnosis of incomplete KD itself prompted clinicians to order viral testing more frequently, which may have biased our results. Nevertheless, this is an inherent limitation of this disease, whose cause is still unknown. In addition, the fact that nonmolecular techniques were used for the diagnosis of respiratory viruses most likely underestimated the real incidence and contribution of respiratory viral infections in these patients and warrants future prospective studies to address the role of respiratory viruses in the pathogenesis of KD.

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MYCOBACTERIAL INFECTIONS IN TEXAS CHILDREN

A 5-YEAR CASE SERIES

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Abstract: The epidemiology of nontuberculous mycobacterial infections is poorly understood, particularly in regions where tuberculosis (TB) is endemic. In 5 years, 75 children had nontuberculous mycobacterial disease (30 lymphadenopathy, 17 pulmonary, 17 soft tissue, and 11 bacteremia) and 30 had TB. Divergent antibiotic susceptibility profiles and the persistence of disease caused by TB emphasize the importance of microbiologic diagnosis for suspected mycobacterial disease.

Key Words: nontuberculous mycobacteria, tuberculosis, child

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Nontuberculous mycobacteria (NTM) are ubiquitous environmental organisms long dismissed as nonpathogenic. Knowledge of NTM epidemiology, diagnosis, and treatment has been hampered by geographic variation in species distribution,[1] variability in laboratory growth requirements, interspecies variation in antimicrobial susceptibility patterns,[2] and a lack of clinical trials demonstrating efficacy of individual treatment regimens. Data regarding the epidemiology of NTM species can guide empiric therapy in immunocompromized patients. This series is from a

region of the United States that manages large numbers of cases of children with both NTM and tuberculosis (TB) disease.

METHODS

This was a retrospective case series of all patients with culture-proven mycobacterial disease seen at Texas Children's Hospital in Houston, TX, from June 1, 2003, to May 31, 2008. Antimicrobial susceptibility testing by minimal inhibitory concentrations for NTM isolates, ordered at physician discretion, was performed by the University of Texas Health Center at Tyler and for all *M. tuberculosis* isolates was performed at the City of Houston Mycobacteriology Laboratory. Medical, radiologic, and microbiologic records were reviewed after the approval by our Institutional Review Board. Statistical analyses were performed using STATA 10 (Stata, Inc., College Station, TX).

American Thoracic Society guidelines[2] were used to evaluate NTM respiratory tract isolates. Clinical criteria for NTM pulmonary disease included pulmonary symptoms with radiographic findings (eg, nodules, cavities, and multifocal bronchiectasis). Microbiologic criteria included positive cultures from ≥2 separate upper respiratory tract specimens or at least 1 lower respiratory tract specimen. For other sites, disease was defined as a single positive culture from an ordinarily sterile site.

RESULTS

One hundred sixty-two mycobacterial isolates were cultured from 150 patients during a 5-year period; 30 children had culture-confirmed TB (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A430). Of the 75 children meeting American Thoracic Society criteria for NTM disease (Table 1), 31 (42.3%) had comorbid medical conditions, the most common of which were cystic fibrosis (CF) (11, 14.7%) and malignancies (8, 10.7%). In contrast, only 20% (6/30) of children with TB disease had medical comorbidities (3 autoimmune disorders, 2 malignancies, and 1 congenital heart disease).

NTM lymph node disease occurred in 30 young children (mean age 2.7 years); *Mycobacterium avium* complex (MAC) was the predominant cause (Table 1). All were previously healthy; 60% were Hispanic. Tuberculin skin tests (TSTs) were performed on 11 children and were ≥10 mm in 3 (27%). The 4 children with TB lymphadenopathy tended to be older than children with NTM lymphadenopathy (11 years versus 2.7 years, $P = 0.11$); TSTs were positive in all 4, and chest radiographs were abnormal in 2 of the 4 children.

NTM skin and soft tissue infections were seen in 17 children. The mean age was 8.7 years (range: 6 months to 20.6 years). Thirteen (76%) children were previously healthy. The most common risk factor was sustaining a puncture wound, seen in 13 of 17 children (76.5%) and occurring a mean of 4.5 weeks before presentation. *Mycobacterium fortuitum* was significantly more likely to be associated with skin and soft tissue infections than other NTM species ($P < 0.001$) (Table 1).

NTM bacteremia was seen in 11 patients; 7 had central venous catheter-associated bacteremia (6 of whom had malignancies). Four children had peripheral bacteremia (all with MAC); 3 of these 4 were HIV infected, and 1 had an ill-defined immunodeficiency. The latter child was a 2-year-old girl who died of disseminated MAC disease (bacteremia, lymphadenopathy, hepatosplenomegaly, and skin nodules).

Seventeen children had NTM pulmonary disease. When children with (11) and without (6) CF were compared, the former group were older (13.7 years versus 6.2 years, $P = 0.03$), more likely to have *M. abscessus* or *chelonae* (8 versus 0, $P = 0.006$), and less likely to have MAC (2 versus 5, $P = 0.02$). Except for worsening bronchiectasis seen only in CF patients, there were no differences in radiographic findings between these groups. Twenty-three children had culture-confirmed pulmonary TB. The mean age was 7.5 years (range: 2 weeks–17 years). The most common radiographic findings were lobar infiltrates (9, 39%), pleural effusion (5, 22%), intrathoracic lymphadenopathy (4, 17%), miliary TB (4, 17%), and cavitary lesions (1). There were no significant differences in race/ethnicity, symptoms, signs, or radiographic findings in children with pulmonary TB and non-CF children with pulmonary NTM disease.

The drug susceptibility patterns for all 63 (47.7%) NTM isolates for which susceptibilities were obtained are shown in Table, Supplemental Digital Content 2, http://links.lww.com/INF/A431. Resistance to fluoroquinolones tended to be uniform across that antibiotic class. Fluoroquinolone susceptibility was seen widely in only 3 species: *M. fortuitum*, *M. mucogenicum*, and *M. simiae.* In contrast, resistance to the aminoglycosides tended to be disparate, with many isolates having tobramycin resistance but retaining amikacin susceptibility; most species remained susceptible to at least 1 of the aminoglycosides. The lack of predictability of drug susceptibility patterns also was observed for the rapidly growing mycobacteria. One *M. tuberculosis isolate* was isoniazid resistant; the remainder was susceptible to the commonly used agents.

DISCUSSION

Several patterns in the epidemiology and clinical presentation of NTM and TB infections emerged. First, *M. tuberculosis* remained the cause of many of our patients with mycobacterial disease: 18.5% of all mycobacterial isolates, 28.6% of all cases of mycobacterial disease, and the most common species isolated from the respiratory tract and the third most common cause of mycobacterial lymphadenitis in this case series. Prior studies examining the relative burden of NTM and TB had shown ~90% of disease in children in industrialized nations was caused by NTM species[1,3]; this was not observed

TABLE 1. Mycobacterial Species by Site of Disease

Species	Total (%)	Pulmonary	Lymph Node	Skin/Soft Tissue	Bacteremia	Meningitis
M. avium complex (MAC)	31 (29.5)	7	20	0	4	0
M. tuberculosis	30 (28.6)	23	4	0	0	3
M. fortuitum	14 (13.3)	0	3	9	2	0
M. abscessus or *chelonae*	12 (11.4)	8	0	3	1	0
M. simiae	5 (4.8)	0	5	0	0	0
Other mycobacterial species*	13 (12.4)	2	2	5	4	0
Total	105	40	34	17	11†	3

**M. peregrinum*, *M. mucogenicum*, *M. kansasii*, *M. gordonae*, *M. bovis*, and *M. marinum.*

†The 4 cases of MAC bacteremia were all peripheral bacteremia, whereas the other 7 episodes of bacteremia were central venous catheter associated.

here. Houston/Harris County has 1 of the highest rates of TB in the United States, with a case rate of 8.5 per 100,000, almost double the national rate.[4] The high rate of TB disease in this population made empiric therapy and public health considerations for mycobacterial lymph node and pulmonary disease challenging.

Second, a number of children with puncture wounds grew a NTM species, with *M. fortuitum* being the most associated with disease after skin/soft tissue inoculation. NTM species should be considered in infected puncture wound refractory to conventional antibiotics. Empiric therapy for suspected mycobacterial skin/soft tissue infections should target the rapidly growing mycobacterial species.

Third, children with NTM lymphadenopathy tended to be younger than children with TB lymphadenopathy. In a preschool-aged child lacking TB risk factors, an enlarged peripheral lymph node in combination with a positive TST was much more likely to be caused by a NTM species. This epidemiologic pattern has public health and clinical importance. As NTM species are not transmitted person-to-person, no contact investigation is needed for a child with NTM disease. Excision of the involved node is usually curative for NTM,[5] but systemic antibiotic therapy is necessary for tuberculous lymphadenopathy. Additionally, empiric antibiotic selection would differ markedly based on whether TB or NTM was suspected.

Fourth, pulmonary disease was the second most common site of NTM disease. In contrast to children with NTM lymphadenopathy or skin/soft tissue infections, almost all children with NTM pulmonary disease had comorbid medical conditions. This made differentiating colonization from true infection difficult, as many of these children had underlying pulmonary pathology.[6] Relying on a radiographic criterion was difficult because differentiating worsening CT findings due to NTM infection versus progression of underlying disease was challenging. Further complicating clinical management was that children with CF tended to have disease with NTM species (*M. abscessus* or *chelonae*), which were less susceptible to oral agents than non-CF children with NTM pulmonary disease.

Finally, the importance of securing a microbiologic diagnosis was emphasized by the divergent drug susceptibility patterns. Although lack of standardization in susceptibility testing and small numbers of isolates for certain species precluded a powered statistical analysis, resistance to fluoroquinolones tended to be uniform, whereas resistance to aminoglycosides tended to be disparate. The relatively large burden of *M. tuberculosis* in this population also made empiric therapy more challenging for pulmonary and lymphatic disease, as few NTM species apart from *M. kansasii* are susceptible to the same antibiotics as *M. tuberculosis*.

There were limitations to this study. First, the information was collected retrospectively; consequently, not all data were evaluable for all subjects, and the diagnostic and treatment regimens were not standardized. Second, this study was conducted in a tertiary care pediatric facility with many immunocompromized children, a group not reflective of the general population. Third, there was selection bias as to which children with suspected TB were admitted for cultures; cultures are sought more often in sicker children or children in whom the source case is unknown. Fourth, using only culture-proven cases of TB disease in children underestimated the true disease burden in this population.[7] The issue of low-culture yield is applicable to NTM species as well—negative cultures are common; therefore, a review of only positive culture cases might introduce bias, because children who have positive cultures may not be reflective of all cases.

Tuberculosis comprised a large proportion of mycobacterial disease in this series of children and was the most common specimen isolated from respiratory tract isolates. Wide variation in antimicrobial susceptibility patterns among NTM species, together with the large percentage of disease caused by TB, emphasizes the importance of obtaining a microbiologic diagnosis, particularly in children presenting with pulmonary findings and/or lymphadenopathy.

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ASSOCIATION BETWEEN LIPODYSTROPHY AND LEPTIN IN HUMAN IMMUNODEFICIENCY VIRUS-1-INFECTED CHILDREN RECEIVING LOPINAVIR/RITONAVIR-BASED THERAPY

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Abstract: Highly active antiretroviral therapy might lead to the development of dyslipidemia and lipodystrophy (LD) syndrome. We carried out a multicenter prospective study of 22 human immunodeficiency virus (HIV)-1-infected children treated during 48 months with lopinavir/ritonavir-based highly active antiretroviral therapy to evaluate the trend of serum lipids and adipokines. Increase in plasma leptin levels and leptin/adiponectin ratio was associated with LD. These adipokines may be surrogate markers of LD.

Key Words: protease inhibitor, adipokines, lipids, antiretroviral therapy, adverse effects

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Highly active antiretroviral therapy (HAART) has achieved reduction in mortality and morbidity in human immunodeficiency virus (HIV)-infected children.[1] Nevertheless, long-term HAART exposure could lead to the development of dyslipidemia and lipodystrophy (LD) syndrome.[2–4] In particular, lopinavir/ritonavir (LPV/r; Kaletra; Abbott Laboratories, Abbott Park, IL), a combination of HIV-1 protease inhibitors (PIs), has shown potent antiviral activity and clinical effectiveness[5,6] but its benefits must be balanced against potential adverse events.[7]

LD is characterized by adipose tissue redistribution and metabolic disorders (hypercholesterolemia and insulin resistance [IR]),[8,9] both being associated with increased proinflammatory cytokine values. Moreover, HAART and inflammatory cytokines are associated with a decrease in adiponectin and an increase in leptin.[10,11]

Leptin regulates proinflammatory immune responses by controlling tumor necrosis factor-α production and macrophage activation[12]; tumor necrosis factor-α and interleukin-6 are capable of stimulating adipocyte leptin production.[12] Moreover, adiponectin has been found to be inversely associated with metabolic syndrome,[11] and this adipokine has anti-inflammatory activity,[11] which could be involved in a compensatory mechanism to cushion the inflammatory effect induced by leptin and resistin. To date, few studies on plasma kinetics of these adipocyte-secreted hormones (adipokines) in HIV-infected children have been published. This study evaluates the trend of serum lipids and adipokines in HIV-1-infected children treated with LPV/r-based HAART as salvage therapy.

PATIENTS AND METHODS

A multicenter prospective study of vertically HIV-1-infected children of the HIV Spanish Pediatric Cohort on LPV/r-based salvage therapy was done. The study was approved by the hospitals' ethical committees. The inclusion criteria were as follows: (*a*) $\geq$2 years of follow-up, (*b*) age $>$1 year, (*c*) previously treated with antiretroviral therapies (ARTs) and having records of virologic failure with PI or non-nucleoside analog, and (*d*) starting salvage HAART with LPV/r.

Children were monitored every 3 months with interviews, physical examinations, and blood sample collection for percentage of $CD4^+$ and $CD8^+$ T-cells and HIV-1 RNA measurements. Fasting serum samples were frozen at -70°C for further assays. Total cholesterol, triglycerides, high-density lipoprotein cholesterol, low-density lipoprotein cholesterol, and glucose concentrations were available for routine clinical use. The lipid panel was performed on fasting specimens. Several biologic samples were provided by the Spanish HIV BioBank belonging to the Spanish AIDS Research Network.[13]

Pediatricians administered the appropriate ART following international guidelines.[14] The ART previous LPV/r-based therapy was classified as monotherapy of a nucleoside reverse transcription inhibitor, on combined-therapy of 2 nucleoside reverse transcription inhibitors, or HAART (combinations of 3 or more drugs). Adherence was evaluated recording the dose taken and interviewing the parents or tutors during follow-up, and it was summarized as a single percentage for each patient. LPV/r-based HAART initiation was defined as the first time children took LPV/r with 2 or more antiretrovirals. Subsequent regimen changes were ignored in terms of statistical analysis whereas it still included LPV/r.

LD was diagnosed by clinical examination at the most recent visit[15] and was standardized across participating hospitals. The degree of lipoatrophy or lipohypertrophy in every part of the body was categorized as absent (score of 0), mild (score 1), moderate (score 2), or severe (score 3). Patients with scores $\geq$2 were classified in the LD group and patients with scores $<$2 in the no-LD (N-LD) group. Height, weight, and body mass index were expressed as z-scores and were adjusted for age and sex.

The adipokines panel was performed from fasting serum samples frozen at -70°C. Multiplex suspension bead array immunoassay was performed using the Luminex 100 analyzer (Luminex Corporation, Austin, TX) Multiplex kit (LINCOplex; LINCO Research, St. Charles, MO) to identify protein expression in plasma. The degree of IR was estimated using the homeostatic model assessment (HOMA) method using the formula: plasma glucose (mmol/L) $\times$ serum insulin (mU/L)/22.5.

The 2-sided Fisher exact test was used to compare categorical variables. Continuous variables were compared longitudinally either within groups in comparison with baseline (Wilcoxon test) or between groups (Mann-Whitney *U* test). Medians were estimated with interquartile range; SPSS (v.12; SPSS Inc., Chicago, IL) was used. *P* values were 2-tailed, and significance was defined as $P < 0.05$.

RESULTS

We recruited 22 vertically HIV-1-infected children in 2 tertiary hospitals, and they were followed up from October 1, 2000, until January 1, 2003. Eleven (50%) patients were assigned to the LD group and 11 (50%) to the N-LD group. One patient had moderate lipohypertrophy; 1 had mild lipoatrophy and lipohypertrophy; 1 had mild lipoatrophy and moderate lipohypertrophy; 4 had moderate lipoatrophy; 3 had moderate lipoatrophy and moderate lipohypertrophy; and 1 had moderate lipoatrophy and severe lipohypertrophy. The 2 groups (N-LD and LD) showed similar age, anthropometric values, adherence to antiretrovirals, as well as clinical and immunologic status (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A438). Neither gender nor age at baseline was associated with LD, but LD patients were slightly older than N-LD patients. Patients had been similarly exposed to antiretrovirals previous LPV/r administration but a shorter duration of d4T administration as part of the salvage regimen was observed in the LD group ($P = 0.021$) (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A438).

The medians of $CD4^+$, $CD8^+$, HIV-1 RNA, and lipids were comparable between groups during follow-up (data not shown). The N-LD group had an increase in adiponectin values, but it was not statistically significant (Fig. 1A). The LD group had a significant increase in leptin concentrations at weeks 24, 36, and 48 and had higher values of leptin than in the N-LD-group ($P = 0.016$; Fig. 1B). The leptin/adiponectin ratio was similar between groups at baseline; the LD group had higher values than the N-LD group at weeks 36 and 48 (Fig. 1C). Patients of the 2 groups had similar resistin, C-peptide, and HOMA values, which slightly increased during follow-up in both groups (data not shown).

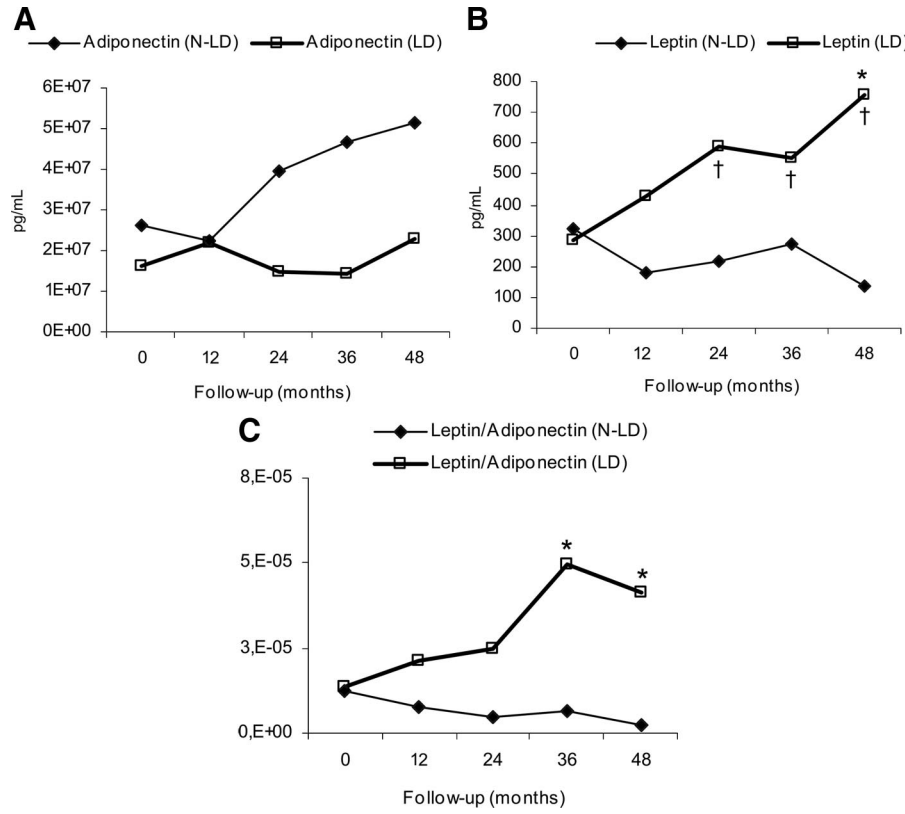

FIGURE 1. Trend of plasma adiponectin (A), leptin (B), and adiponectin/leptin (C) levels during follow-up of vertically HIV-1-infected children on salvage HAART with LPV/r. Values are expressed as median. *Significant differences ($P < 0.05$) between groups by Mann-Whitney *U* test. †Significant differences ($P < 0.05$) within group by Wilcoxon test. LD indicates children with lipodystrophy; N-LD, children without lipodystrophy.

DISCUSSION

HIV-associated LD syndrome (HIV-LS) has been associated with the use of PIs in children.[2–4,15] Although it is well characterized in adults,[16] there are few pediatric data available on HIV-LS, and the role of adipokines has been previously highlighted only in cross-sectional studies. Verkauskiene et al[17] and Kim et al[18] found decreased adiponectin levels in HIV-infected children with LD, whereas Papaevangelou et al[19] and Dzwonek et al[4] found no difference in leptin values between HAART-treated and untreated patients. The main goal of our longitudinal study was to monitor plasma metabolic changes and adipokines levels associated with LD in HIV-infected children on LPV/r-based HAART as salvage therapy.

All patients reached virologic suppression during 48 weeks of follow-up, consistent with previously reported evaluation of LPV/r effectiveness.[6,20] Nevertheless, virologic failure was observed in some patients without LD at the end of the study. Despite this, the $\%CD4^+$ values were similar in both study groups.

Unlike other studies, we did not find a significant increase in cholesterol, triglycerides, low-density lipoprotein, and high-density lipoprotein; however, only a small percentage of children in our cohort had significant hyperlipidemia during follow-up. The prevalence of LD was higher than in other reports,[3,4,21] probably because of previous long-term ART exposure.

Overall, 50% of the patients experienced LD and showed changes in plasma adipokines that have been found to be associated with IR.[10,22] The increase in leptin levels may be the direct effect of PI on adipose tissue, which may contribute to an overall adipose imbalance and the development of LD and metabolic syndrome in HIV patients.[23] Serum leptin concentrations reflect body fat content and are associated with IR.[11] Moreover, the increase in leptin that we observed might be explained by the so called leptin resistance[24] and might support the hypothesis that leptin (as a marker for body fat) could play a key role in the pathogenesis of LD in HIV-infected children. By contrast, children without LD had an increase in adiponectin levels and a decrease in leptin/adiponectin ratio, which might represent a compensatory response to antiretroviral-induced metabolic syndrome.[10,25] Adiponectin has been found to be inversely associated with metabolic syndrome[11] and changes in adipose tissue may lead to hypoadiponectinemia in the later stages of metabolic syndrome or LD in HIV-infected children.[11,18]

Disturbance of lipid metabolism was observed in all patients and included moderate hypercholesterolemia and hypertriglyceridemia but all the children had HOMA and C-peptide values below the lower limit of normal, this being associated with absence of type II diabetes. The high leptin values observed in patients with LD might suggest that these subjects meet criteria close to the IR.

The limitation of our study arises from the small number of patients enrolled. Moreover, a significant percentage of children had already received PI-based HAART before the salvage regimen, which could have played an important role in metabolic syndrome and LD. In conclusion, the increases in plasma leptin concentrations and in the leptin/adiponectin ratio were associated with LD, and these adipokines may be surrogate markers for the risk of diabetes and

cardiovascular disease in HIV-infected children on HAART. Future prospective studies that involve larger cohorts of children on LPV/r treatment as salvage therapy are necessary to confirm our findings and to further elucidate the complexity of HIV-LS.

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NATURALLY FLUCTUATING LOW INCIDENCE OF INVASIVE PNEUMOCOCCAL INFECTIONS NOT AFFECTED BY LARGE-SCALE *HAEMOPHILUS INFLUENZAE* TYPE B VACCINATION

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Background: Since many pathogens colonize the child's oro/nasopharynx in a similar manner, elimination of diseases such as those caused by *Haemophilus influenzae* type b (Hib) has a potential of augmenting other serious infections. *Streptococcus pneumoniae* is an agent of special interest now that Hib conjugates have been used widely for more than 2 decades.
Patients and Methods: All blood and cerebrospinal fluid isolations of Hib and *S. pneumoniae* were collected prospectively from 85,000 Finnish children at age 0–15 years by one central laboratory during 27 years.
Results: Hib vaccination, launched in 1986–1988, led to a quick decline of cases until the last was detected in 1991. In the next few years, the incidence of bacteremic *S. pneumoniae* infections increased, but now for 15 years, the numbers of cases have been slowly declining. This finding is not explained by less active sample-taking because the number of blood cultures have almost doubled in the past years.
Conclusions: Large-scale Hib vaccination does not increase the incidence of pneumococcal diseases which continue their year-to-year fluctuation at low levels. Only a years-long follow-up permits conclusions on a vaccination's potential influence on the epidemiology of other diseases.

Key Words: *Streptococcus pneumoniae,* pneumococcus, *Haemophilus influenzae*, incidence, epidemiology, vaccination
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Because certain competition likely prevails among the bacteria residing in the human oro/nasopharynx, an introduction of large-scale vaccination affecting the colonization may potentially increase the incidence of infections caused by other respiratory pathogens.[1] The rationale is that some less-common pathogens may find a new niche because of the changed balance in carriage.[2]

For *Haemophilus influenzae* type b (Hib), this concern was raised along with conjugate vaccines and diseases such as those caused by the non-type b *H. influenzae* strains[3–5] and *Streptococcus pneumoniae*.[6,7] Replacement of Hib infections by other severe *H. influenzae* infections seems unlikely,[8] whereas for the nonvaccine-type *S. pneumoniae* strains, this phenomenon is well documented in several countries.[9–16] However, few prospective long-term data have been available to address the question whether an interaction exists between Hib vaccination and invasive pneumococcal infections.

In 1995, it was reported that bacteremic *S. pneumoniae* infections were becoming more common in Finland,[7] likely because large-scale vaccination had eliminated Hib diseases. However, an association does not prove causation,[17] and the follow-up was only 3 years. We now have continued it prospectively for 15 more years and are convinced that the fear was unfounded.

PATIENTS AND METHODS

The Pirkanmaa region, located 100 miles north from Helsinki (capital), has a population of almost half a million, of which 85,000 are children aged 0 to 15 years. No major fluctuation has occurred over the last years. The district is served by 1 tertiary hospital (Tampere) whose central laboratory is the only 1 in the region and obtains all bacterial isolates. The center handles more than 30,000 blood cultures a year. Because of its large size, Pirkanmaa is representative of the whole country in which the interregional differences in primary health care are remarkably small.

All blood and cerebrospinal fluid (CSF) isolations of Hib or *S. pneumoniae* were collected and computerized during the 27-year period of 1983 to 2009. Following the concept of the earlier report,[7] we examined children treated in the Tampere University Hospital. Through years, the clinicians were encouraged to take the blood and, if necessary, CSF cultures to disclose the causative agent of all potentially invasive infections. Standard techniques were used to identify Hib and *S. pneumoniae*.

RESULTS

Between 1995 and 2009, the number of blood cultures varied (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A439). Fewer CSF cultures were obtained, but only 1 child (in 1997) with *S. pneumoniae* meningitis did not yield the agent also from the blood. Almost 3000 cultures per year have been performed in the past several years.

The last Hib isolation in 1991 was soon followed by an increase in bacteremic *S. pneumoniae* cases (Fig. 1). However, none of the years during the last 15 years has reached the peak of 1994 when 23 cases were identified. Pneumococcal infections, if anything, have been slowly declining towards the pre-Hib vaccination levels (Fig. 1), and this favorable trend has not been due to less active sample-taking.

In this population, as in Finland in general,[18] bacterial meningitis has become rare; 4 to 8 cases per year in the whole country. Annually, 11 bacteremic *S. pneumoniae* cases and <1 pneumococcal meningitis have been diagnosed in Pirkanmaa in the last 5 years. No other agent has replaced Hib or *S. pneumoniae* isolations. *Neisseria meningitidis* has been even a rarer one; 19 cases from 1995 to 2009, of which 13 were meningitis (all except 1 were of group B).

DISCUSSION

Our 27-year follow-up of a large childhood population in Finland elucidated 2 important issues. First, the incidence of invasive *S. pneumoniae* infections has been low, despite the disappearance of Hib diseases,[19] and despite these 2 agents perform rather similarly in the respiratory tract. Our epidemiologic data fit well with those from the whole country (population 5.4 million) in which the annual incidence of all invasive *S. pneumoniae* infections at age 0 to 4 years was 23.5/100,000. Not all of these cases are preventable with the existing vaccines.[20]

Second, the incidence of pneumococcal (and many other) infections fluctuate from year to year. In Pirkanmaa, it first looked as invasive *S. pneumoniae* infections would explode once Hib

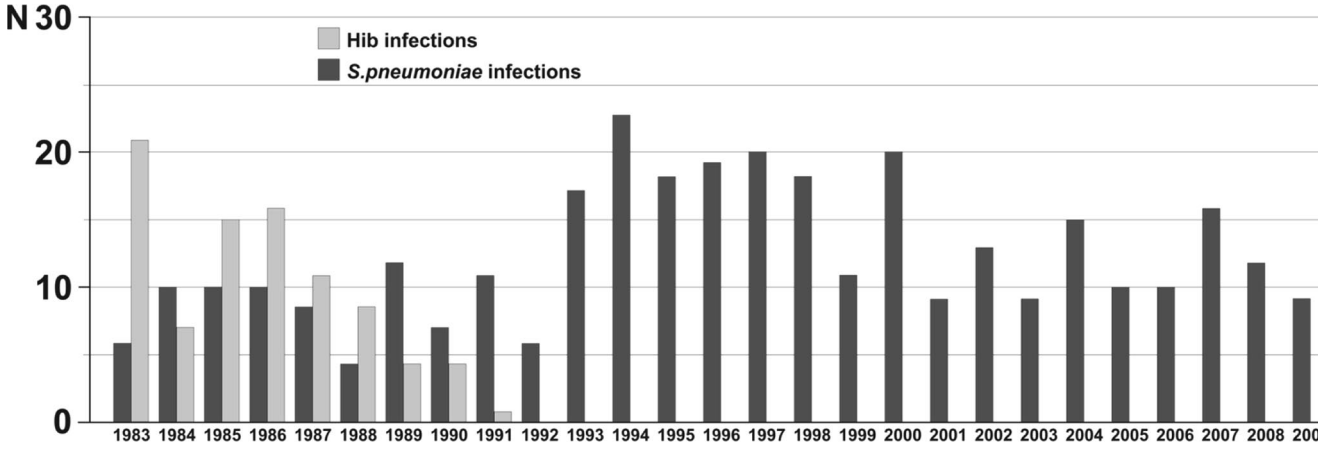

FIGURE 1. Annual cases of invasive *H. influenzae* type b and *S. pneumoniae* infections in 0- to 15-year-old children during the 27-year period of 1983 to 2009 in the district of Pirkanmaa, Finland.

diseases were eliminated,[7] until a follow-up of many more years showed that this was not the case. Conclusions on epidemiologic trends should be drawn only after a long enough (several years) monitoring of the situation.

In the United States, pediatric vaccination has surprisingly reduced invasive pneumococcal diseases of the elderly people by herd immunity.[21] This effect has not been seen in Europe (vague evidence comes from Catalonia, Spain).[22] The obvious reason is that the prevaccination incidence in western Europe was approximately one sixth of that in the United States; the annual rate at age 0 to 1 years was 20 to 35 versus 160 to 180 cases per 100,000 children in Europe[23] and the United States,[21] respectively. With a difference of this magnitude, one cannot expect the US experience to repeat in most of Europe, although the vaccine-type infections have expectedly declined.[24,25] On the other hand, nonvaccine-type infections have increased alarmingly in countries such as Spain, France, and United Kingdom.[11,12,14,16,22]

In the developing world, and among risk populations and industrialized countries (the United States), where the incidence of pneumococcal infections is or was high, swift introduction of vaccination is well justified—despite it doubles the costs of pediatric vaccines. In the industrialized low-incidence countries, the situation is less clear, specially that the replacement phenomenon has become an issue.[9,14] Some 40 affluent countries have included pneumococcal vaccination in their calendar, and Finland joins the orchestra in 2010. As a consequence, 10 to 15 severe and many more nonsevere *S. pneumoniae* infections per year will likely be prevented, but these figures should be projected against the overall costs and the 180,000 vaccinations which the 3-dose schedule requires. If the new conjugate is also capable of preventing otitis media,[26] the scenario changes drastically.

Our data demonstrate that the launch of large-scale Hib vaccination does not increase the incidence of pneumococcal diseases. However, local epidemiology, and the severity of manifestations[20] should also be taken into account. If severe manifestations are rare, as they are in most of industrialized Europe, a balance between the clinically meaningful benefits, costs, and the risks of augmenting more serious nonvaccine-type diseases[14,16,22] should be sought. Warning signs[11,12,14,16,27] have been signaled.

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POLYMORPHISM IN THE P2X7 GENE INCREASES SUSCEPTIBILITY TO EXTRAPULMONARY TUBERCULOSIS IN TURKISH CHILDREN

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Abstract: The P2X7 gene polymorphisms have been linked to increased risk for pulmonary and extrapulmonary tuberculosis in some popula-

tions. In this study, the genotype and allelic frequencies 1513A→C variant within the P2X7 gene was significantly higher than in the healthy controls ($P = 0.035$, $P = 0.041$). This is the first study demonstrating that the 1513A→C polymorphism is associated with extrapulmonary tuberculosis in children.

Key Words: childhood, extrapulmonary tuberculosis, single nucleotide polymorphism

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Tuberculosis in children and adults are 2 distinct genetic disease. Childhood tuberculosis has been relatively neglected and remains a public health priority, particularly in developing countries. *Mycobacterium tuberculosis* is a facultative intracellular pathogen. Macrophages are the principal host cells for intracellular replication of mycobacteria and are responsible for killing internalized bacilli through the generation of reactive nitrogen and oxygen intermediates and the destructive action of lysosomal enzymes. The P2X7 gene is highly expressed on human macrophages, and it is activated mainly by adenosine-5-triphosphate. Activation of P2X7 opens a cation channel causing induction of apoptosis and the activation of the phospholipase D(PLD) promoting phagosome-lysosome fusion and thus leading to mycobacterial death. Several single nucleotide polymorphisms (SNPs) in P2X7 lead to loss of receptor function. The most common of these, the 1513A→C polymorphism, leads to almost complete loss of the P2X7 function with lack of ATP-induced mycobacterial killing in these individuals.[1] On the other hand, the −762T→C SNP in the promoter region of P2X7 has been reported to have a protective effect against tuberculosis in the Gambian population.[2] In contrast, no significant association of the P2X7 −762→C SNP polymorphism with tuberculosis has been found in different populations.[3,4] To determine whether polymorphisms of P2X7 confer susceptibility or resistance to extrapulmonary tuberculosis in Turkish children, we performed a case-control study comparing the frequency of polymorphisms in P2X7 in children with extrapulmonary tuberculosis and healthy controls.

MATERIALS AND METHODS

The study population consisted of 74 extrapulmonary patients admitted to Sisli Etfal Training and Research Hospital and Sureyyapasa Chest Diseases and Thoracic Surgery Teaching Hospital. Extrapulmonary tuberculosis was diagnosed by isolation of *M. tuberculosis* in culture or through pathologic evidence of tuberculosis disease in biopsy materials and radiographic findings. The control group of 192 unrelated subjects was assembled from the outpatient clinic by random sampling, and sex, age, ethnic ancestry, and regional distribution information was matched with that of patients with tuberculosis. The current study was approved by the institutional ethics committee. The 1513A→C and −762T→C polymorphisms in the P2X7 gene were investigated in the patients with extrapulmonary tuberculosis and in controls by the Institute of Forensic Medicine at Ankara University.

Genomic DNA Preparation. Genomic DNA was extracted from ethylenediaminetetraacetic acid anticoagulant peripheral whole blood samples employing the QIAmp blood DNA mini-kit.

Determination of P2X7 1513→AC Polymorphism. The 1513A/C single nucleotide polymorphism was genotyped by PCR-restriction fragment length polymorphism with the following primers: 5′-AGACCTGCGATGGACTTCACAG-3′ (forward) and 5′-GCCAGGTGGCGTAGCACC TG-3′ (reverse).[3] Amplification was carried out on a Techne Tc 512 PCR System in a 50-μL reaction mixture containing 200 μM of dNTPs, 20 pmol each of forward (F) and reverse (R) primers, 1 U Hot Star *Taq*DNA polymerase (Qiagen, Hilden, Germany), 1X PCR buffer (Qiagen), and 100 ng of genomic DNA. The PCR products were digested at 37°C for 3 hours with 5.0 U of *Hae*II (Promega, Madison, WI).

Determination of P2X7 –762T→C Polymorphism. The −762T/C polymorphism was genotyped using an allele-specific PCR assay.[4] Two outer primers—P2X73 (5′-GAAACAGGGCCCTGGGTCC TC-3′, forward) and P2X74 (5′-TGGTGGGGGTGGAGGGGC-30, reverse)—and the 2 inner primers—P2X75 (5′-GGTGTCCCTCACTGAATAGGTCAAT-3′, forward) and P2X76 (5′-GGCAGTCCAACAAAGTTAGGTTTG-3′, reverse)—were used. The 2 outer primers amplified a 373-bp fragment in all cases. For the −762T allele, a 186-bp fragment was amplified using primers P2X74 and P2X75; for the −762C allele, a 235-bp fragment was amplified using primers P2X73 and P2X76. The amplified PCR fragments were subjected to electrophoresis in 2.0% standard agarose gels.

Statistical Analyses. Statistical analyses were carried out using SPSS software, version 16.0. Categorical variables were compared by the χ^2 test. $P < 0.05$ was accepted to be significant. Odds ratios (ORs) with 95% confidence intervals (95% CI) were also calculated for these comparisons. $P < 0.05$ was considered statistically significant. The frequencies of P2X7 1513A→C and −762T→C alleles and genotypes in patients with tuberculosis and controls were obtained by direct count, and the departure from the Hardy-Weinberg equilibrium was assessed using Haldane exact test.

RESULTS

The mean (±SD) age was 91.7 (±59.5) months for patients with extrapulmonary tuberculosis and 93 (±59) months for controls. The proportion of female patients was 55% in the patient group and 55% in the control group. Abdominal tuberculosis was diagnosed in 16 (21.6%) cases, central nervous system tuberculosis in 13 (17.6%), tuberculosis lympadenitis in 13 (17.6%), miliary tuberculosis in 8 (10.8%), bone tuberculosis in 8 (10.8%), renal tuberculosis in 6 (8.1%), tuberculosis pericarditis in 5 (6.8%), and pleural tuberculosis in 5 (6.8%). Analysis of the polymorphism at position 1513 revealed a statistically significant difference in the genotype distribution for the 2 groups ($P < 0.05$). A closer look at the genotype distribution showed that the frequency of the CC homozygous and AC heterozygous genotype for the 1513A→C SNP in the patient with extrapulmonary tuberculosis was significantly higher than in healthy controls ($P = 0.035$, $P = 0.041$). The wild-type AA homozygotes were significantly more prevalent in healthy controls ($P = 0.002$). In addition 1513C allele frequency was significantly higher (26.4% versus 14.6%, OR 2.096, 95% CI 1.319–3.29, $P = 0.001$) in patients with extrapulmonary tuberculosis when compared with healthy controls (Table 1). In contrast, no significant association of the P2X7 –762T→C polymorphism and extrapulmonary tuberculosis was detected.

DISCUSSION

Here we describe a genetic association study aiming to obtain insights into the role of the human P2X7 receptor gene in host susceptibility to extrapulmonary tuberculosis. Our re-

TABLE 1. Distribution of P2X7 1513 Adenin→Cytosine Polymorphism and Odds Ratios in Patients With Tuberculosis and the Control Group

	Patients With Tuberculosis Subjects, n (%)	Control Group Subjects, n (%)	*P*	Odds Ratio (95% CI)
Genotype distributions				
AA	39 (52.7)	141 (73.4)	0.002	0.403 (0.231–0.704)
AC	28 (37.8)	46 (24)	0.035	1.932 (1.087–3.433)
CC	7 (9.5)	5 (2.6)	0.041	3.907 (1.199–12.730)
Total	74 (100)	192 (100)	—	—
Allele frequencies				
A	109 (73.6)	328 (85.4)	0.001	0.477 (0.300–0.758)
C	39 (26.4)	56 (14.6)	0.001	2.096 (1.319–3.329)
Total	148 (100)	384 (100)	—	—

n indicates number of individuals; A, adenin; C, cytosine; CI, confidence interval.

sults suggest that the 1513A→C polymorphism of P2X7 was significantly associated with extrapulmonary tuberculosis in children. To our knowledge, this is the first study demonstrating that 1513A→C polymorphism has been associated with extrapulmonary tuberculosis in children. Although previous studies have revealed genetic associations with pulmonary tuberculosis, only a small number of genes have been associated with an increase in susceptibility to extrapulmonary tuberculosis, including mannose binding lectin, TLR2 and TIRAP with tuberculous meningitis, SLC11A1 with pleurisy, and IL-1 Ra with extrapulmonary tuberculosis.[5–9] Researchers from Australia found an association between the 1513C allele and extrapulmonary tuberculosis in adults.[10] Children are particularly vulnerable to extrapulmonary tuberculosis following an infection, which is a major cause of morbidity and mortality. The mechanism of predisposition to extrapulmonary disease in individuals with the 1513C allele probably lies at the macrophage level.[10] The alveolar macrophage is the first line of defense in the innate immune response to tuberculosis. Stimulation of infected macrophages through P2X7 receptor induces a range of cellular changes, which results in phagosome-lysosome fusion and the killing of *M. tuberculosis*. The 1513A→C polymorphism leads to reduced receptor function on the macrophage surface, which reduces the response of P2X7 to ATP, and reduces ATP-mediated mycobacterial killing.[1] After inhalation of *M. tuberculosis*, initial control of infection in alveolar macrophages may rely in part on the activation of P2X7 receptors.[10] A deficiency of P2X7-mediated control of mycobacterial infection within macrophages in the lung may cause dissemination to extrapulmonary sites in children. While many cases of primary tuberculosis infection in children are asymptomatic, an alternative explanation is that signaling through P2X7 plays a more important role in the control of reactivated tuberculosis infection than in the initial containment of *M. tuberculosis* within the lung. After reactivation of dormant bacilli, the absence of P2X7-mediated killing of mycobacteria in pulmonary macrophages could then result in spread to extrapulmonary sites. In addition, Fernando et al. showed that patients with latent infection who harbor the 1513 SNP developed extrapulmonary tuberculosis.[10] They examined the association of the 1513C allele with tuberculosis in 2 independent case-control studies. In the Liverpool cohort, univariate analysis of genotyping data revealed a significant association between the 1513C allele and extrapulmonary disease with an OR of 4.0 (95% CI, 1.7–9.3; $P \leq 0.001$). In the Sydney cohort, as in the Liverpool cohort, the association was only evident for extrapulmonary disease with an OR of 3 (95% CI, 1.5–6.1; $P < 0.01$). Our study showed that the frequency of the CC homozygous and AC heterozygous genotype for the 1513A→C SNP and C allele was significantly higher in patients with extrapulmonary tuberculosis than in healthy controls ($P = 0.035$, $P = 0.041$ and $P = 0.001$). We also found that the CC homozygous genotype for the 1513A→C SNP increases susceptibility to extrapulmonary tuberculosis in children with an OR of 3.907 (95% CI, 1.199–12.730; $P = 0.041$). The wild-type AA homozygotes were significantly more prevalent in healthy controls ($P = 0.002$). Although no data are available regarding the frequency of 1513C allele in the healthy Turkish population, 1513C allele is a risk factor for extrapulmonary tuberculosis ($P = 0.035$, 95% CI, OR = 1.932, 1.087–3.433) with deleterious action manifested even in the heterozygous state ($P = 0.035$, 95% CI, OR = 1.932, 1.087–3.433).

In summary, we found that 1513A→C SNP in the P2X7 gene showed a significant association with extrapulmonary tuberculosis, further supporting evidence that this receptor plays a relevant role in the pathogenesis of childhood tuberculosis. In contrast, no significant association of the P2X7 −762 gene polymorphism with extrapulmonary tuberculosis was detected.

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PERICARDIAL EFFUSION COMPLICATING NOVEL INFLUENZA A (H1N1) INFECTION IN AN INFANT

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Abstract: We report the case of a 3-month-old boy with novel influenza A (H1N1) infection complicated by pericardial effusion. The patient was treated with pericardial drainage, oseltamivir, and ibuprofen and improved. Pericarditis and pericardial effusion have been occasionally associated with influenza A infections. To our knowledge, this is the first case of pericardial effusion reported during the current novel influenza A (H1N1) pandemic.

Key Words: pericardial effusion, H1N1 influenza, infant

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A novel recombinant influenza A (H1N1) infection was first identified in Mexico in April 2009 and rapidly spread globally.[1] The full spectrum of clinical manifestations of this novel virus is not yet fully known but it seems to be similar to seasonal influenza. Although the infection is generally mild, severe complications and mortality have been reported in children, young adults, pregnant women, and those with underlying medical conditions. Common complications of seasonal influenza and presumably of novel influenza A (H1N1) include viral pneumonia, exacerbation of asthma, otitis media, and bacterial pneumonia. Rarely, cardiac complications including pericarditis and pericardial effusion have also been recognized to complicate seasonal influenza infections. Pericarditis has long been associated with several viral infections, and it is assumed that 90% of cases of "idiopathic" pericarditis are of viral origin.[2] We report the first case of pericardial effusion in a young infant, resulting from novel influenza A (H1N1) infection.

CASE HISTORY

A 3-month-old boy was admitted to the hospital because of fever, cough, and cardiomegaly. He was well until 4 days before admission when he developed nasal congestion and dry cough. He fed less than usual and was irritable. On the day of admission in October 2009, his temperature rose to 38°C. He was taken to an urgent care facility where a chest radiograph revealed moderate cardiomegaly, and he was admitted to the hospital.

He was born full term and delivered by cesarean section because of breech presentation. His father had cough and nasal congestion beginning 1 week before the illness of the patient.

On presentation to the hospital, his temperature was 37.2°C, blood pressure 92/71 mm Hg, and respiratory rate of 56 beats per minute, and heart rate 166 beats per minute. He was pale, irritable, and had grunting respirations. He had subcostal and intercostal retractions, but the lungs were clear to auscultation. Heart tones were normal, and he did not have heart murmur or gallop rhythm. His abdomen was slightly distended and tender. Liver edge was palpable at 5 cm below the right costal margin. Cardiac echo/Doppler study on the day of admission demonstrated moderate to severe pericardial effusion. Ultrasound-guided pericardiocentesis performed about 16 hours after admission and yielded 70 mL of light yellow fluid. The same day influenza A test from nasal pharyngeal swab was reported as positive, and 5 day course of oseltamivir and 28 day course of ibuprofen were started. Additional 15 mL of fluid was obtained by a pericardial drain, which was removed 2 days later. Tachycardia and tachypnea gradually improved, and he was discharged after 5 days of hospitalization.

On admission, the white blood cell count in blood was 13,400 cell/mm^3 with 58% segmented neutrophils, 33% lymphocytes, and 9% monocytes. The hemoglobin was 13.4 g/dL. Pericardial fluid had 6410 red blood cells/mm^3, 2090 white blood cells/ mm^3, protein of 3.2 g/dL, albumin of 1.6 g/dL, and lactate of 654 U/L. Nasopharyngeal swab tested positive for influenza A by in-house direct fluorescent antibody testing and influenza A subtype 2009 H1N1 (Focus Diagnostics). In-house polymerase chain reaction testing from throat, adenovirus from eye, and rhinovirus, adenovirus, enterovirus, human metapneumovirus, and parainfluenza virus from the pericardial fluid were all negative. Bacterial, acid fast bacteria, fungal, and viral cultures in the pericardial fluid were sterile. Influenza A by in-house direct fluorescent antibody and 2009 H1N1 influenza A by real-time polymerase chain reaction (Focus Diagnostics) tests in the pericardial fluid were negative.

On follow-up visit 11 days later, the parents reported that he was feeding normally and acting well. Examination revealed a well appearing baby with heart rate of 130 beats per minute and respiratory rate of 44 breaths per minute. At the apex of his heart, a grade II/VI pericardial friction rub was heard. The liver edge was not palpable. Cardiac echo/Doppler study showed a small rim of pericardial effusion with normal wall motion of all 4 heart chambers.

DISCUSSION

Acute pericarditis has infectious and noninfectious causes. It is often asymptomatic but may present with chest pain or life-threatening pericardiac tamponade. Purulent pericarditis is generally caused by *Staphylococcus aureus*. In the postvaccine-era pericarditis caused by *Haemophilus influenzae* type b is very rare in vaccinating countries. Idiopathic or "benign" pericarditis is most often of viral etiology. Among the viruses, enterovirus (coxsackie B), adenovirus, cytomegalovirus, herpes simplex virus, Epstein-Barr virus, and more recently human immunodeficiency virus have been implicated. Although influenza virus, especially influenza A virus, is mentioned in textbooks as a cause of myopericarditis, pericarditis, and pericardial effusion, few cases have been reported in the medical literature.[2,3] An adult suspected of acute myocardial infarction was found to have pericardial effusion and perimyocarditis. Rise of influenza A antibody supported the diagnosis of viral cause.[4] Asian influenza virus was isolated from the pericardial fluid of a 5-year-old child with pericardial tamponade. The child underwent pericardiocentesis twice and recovered.[5] An adult with acute effusive-constrictive pericarditis because of influenza A infection was reported from Israel in 1997. This patient was treated with corticosteroids and pericardiectomy.[6]

The pathogenesis of influenza pericarditis is not clear. Direct viral invasion with growth of influenza virus from the pericardial fluid has been described.[5] Immune-mediated pathology in case of infectious pericarditis has been suspected, and antimyolemmal and antisarcolemmal antibodies have been identified.[7] Collection of large pericardial fluid or rapid accumulation of pericardial fluid may lead to pericardial tamponade. Hypotension and muffled heart tones, present in cases of large pericardial effusion or tamponade were absent in our patient. Pulsus paradoxicus and jugular venous distention in young

children may be difficult to detect by physical examination and were not found in our patient. Based on the presence of cough, grunting respirations, and tachycardia, the diagnosis of bronchiolitis was made on admission. Because his chest radiograph showed cardiomegaly, diagnosis of viral myocarditis or pericardial effusion were also considered. Cardiac echo/Doppler obtained shortly after admission revealed the presence of significant pericardial effusion.

The current pandemic caused by novel influenza A (H1N1) first reported in April 2009 in Mexico spread rapidly and has been reported worldwide. Although the illness is generally self-limited and mild, cases of severe disease and death have been reported in previously healthy individuals, especially in young adults and children possibly because of lack of cross-reacting antibodies.[8] Here, we report from the United States the first case of pericardial effusion in a healthy young infant infected with novel influenza A (H1N1) virus. After pericardiocentesis and drainage of the pericardial fluid, the infant made a good recovery. Although a very rare complication, pericardial effusion needs to be considered when assessing an individual with "flu-like" symptoms and cardiomegaly.

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