OPTIMIZING INTERPRETATION OF THE TUBERCULIN TEST USING AN INTERFERON-GAMMA RELEASE ASSAY AS A REFERENCE STANDARD

*Ana Méndez-Echevarría, MD, PhD,**
Miguel González-Muñoz, MD, PhD,†
Maria Jose Mellado, MD, PhD,‡
Fernando Baquero-Artigao, MD, Raquel Vecino, MD,**
Elia Pérez, PhD,§ and the Spanish Collaborative Group for the Study of QuantiFERON-TB GOLD Test in Children

Abstract: The interferon-gamma release assays have greater specificity than the tuberculin skin test (TST), and at least equal sensitivity. We analyzed the sensitivity and specificity of the TST in immunocompetent children considering QuantiFERON as the referent standard. A TST cut-off point of ≥5 mm indicates excellent sensitivity (100%) and specificity (93%) in children without Bacillus Calmette-Guérin. In Bacillus Calmette-Guérin-vaccinated children, the TST cut-off point of ≥10 mm had poorer specificity (86%), and a cut-off point of ≥15 mm resulted in reduced sensitivity (60%).

Key Words: tuberculosis, sensitivity, specificity, tuberculin skin test, interferon-gamma release assays, QuantiFERON-TB Gold Test In Tube

Accepted for publication September 30, 2010.
From the *Paediatric Department, La Paz Hospital, Madrid, Spain; Departments of †Immunology and ‡Paediatrics, Hospital Carlos III, Madrid. Spain; and §Department of Biostatistics, La Paz Hospital, Madrid, Spain.
Supported by the 19th Dodot-Ausonia 2007 Grant for Paediatric Research awarded by the Spanish Paediatric Association.
Address for correspondence: Ana Méndez-Echevarría, MD, PhD, Paediatric Department, La Paz Hospital, Paseo de la Castellana 261, 28046, Madrid, Spain. E-mail: amendezes@yahoo.es.
Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3182001294

The Spanish Society for Pediatric Infectious Diseases (SEIP) recommends performance of a tuberculin skin test (TST) as part of the immigrant child's first health check in our country.[1] Most of these children have been vaccinated with Bacillus Calmette-Guérin (BCG), and the interpretation of TST for them is a controversial question.[2] The degree of induration in vaccinated children must be taken into account at the time of the diagnosis of latent tuberculosis infection (LTBI). Some countries adjust TST cut-off points for vaccinated children, even though there is no consensus as to whether, or how much, the cutoff point should be increased.

The new interferon-gamma release assays (IGRAS) have greater specificity than the TST in detection of LTBI.[3] Studies in children show excellent sensitivity of IGRAS in the diagnosis of tuberculosis disease (TBD).[4] Thus, we consider that the QuantiFERON-TB Gold Test In Tube (QTF) could be used as a diagnostic referent to analyze the sensitivity and specificity of the TST in BCG vaccinated and nonvaccinated children.

PATIENTS AND METHODS

A transversal multicenter study was performed in 9 hospitals in Madrid, Spain. The inclusion of patients began in March 2007, and the study finished in February 2009. Immunocompetent children less than 15 years of age who immigrated from TB endemic areas and who arrived in Spain during the prior 2 years,[4] children who were in contact with a person with active tuberculosis and children admitted with symptoms suggestive of TBD were included.

Human immunodeficiency virus-infected cases, children with congenital immunodeficiencies, being treated with immunosuppressants, with autoimmune diseases, malignant proliferative processes, and solid organ or bone marrow transplants were excluded. Likewise, patients were excluded if they had previously received antituberculosis treatment for LTBI or TBD, or if they had received treatment for more than 3 days at the time of inclusion.

Both tests (TST and QTF) were performed on all children. Considered to be BCG vaccinated, were those children with postvaccinal scarring on the deltoid area and who come from countries in which BCG is included in the vaccination schedule.[2]

LTBI was diagnosed in asymptomatic children with normal chest x-rays and/or chest computed tomography scan and with a positive TST under the reading criteria of the SEIP (≥5 mm for children with close contact to TBD and ≥10 mm in screening of immigrant children).[1]

TBD was diagnosed thus (1) Children with microbiological confirmation by growth of *Mycobacterium tuberculosis* in solid or liquid medium or with positive molecular polymerase chain reaction studies for *M. tuberculosis*. (2) Children with symptoms compatible with TBD and without microbiological confirmation, but that presented a TST ≥5 mm and radiologic studies compatible with TBD or the presence of caseous granulomas in histhological specimens (excluding other causes of granulomatous disease).

The TST was carried out according to the recommendations of the SEIP.[1] The TST reading was performed after 72 hours by a single professional in each center. All the QTF assays were performed at the Immunology Department of Carlos III Hospital (Madrid) by a single immunologist following Centers for Disease Control and Prevention recommendations.[5] All the samples were processed within 3 hours of its extraction. The signed informed consent was obtained from parents or guardians beforehand. Reading of results from the 2 tests was blind, the laboratory being without access to the clinical data and the doctors of each hospital not knowing the QTF results prior to TST interpretation.

The data were analyzed at the Biostatistics Department of La Paz Hospital (Madrid) using the SPSS 9.0 program (SPSS Inc.). The data description was expressed in absolute frequencies, using mean and standard deviation, or median and range. The Spearman correlation coefficient was used to analyze the association between quantitative variables. To evaluate the TST sensitivity and specificity of the different reading points recommended in Spain by the SEIP, the area under the receiver operating characteristic curve (ROC curve) was calculated. The study was approved by the Clinical Testing and Ethics Committees of the participating hospitals.

RESULTS

A total of 459 children were included, 264 girls (57.5%) and 195 boys (42.4%), with an average age of 4.73 ± 3.68 years (1 month–15 years). Of total, –318 children were included by screening of immigrants proceeding from TB endemic areas (69.2%), 83 children for contact with TBD (18%), and 58 for suspicion of active TBD (12.6%). In all, 86% of the patients (396/459) came from TB endemic areas (131 Latin America [28.5%], 115 Sub-Saharan Africa [25%]; 67 China [14.5%], 65 Eastern European countries [14.1%], 52 India [11.3%], and 29 Morocco [6.3%]). Scar of the deltoid area compatible with prior BCG vaccination was observed in 46.4% (213/459) of the children. There were no statistically significant differences between the ages of the vaccinated population (4.72 ± 3.89 years) and that of the nonvaccinated population (4.73 ± 3.44 years) ($P = 0.5$). In 318 children (69.2%)

LTBI and TBD were excluded, 73 cases (15.9%) were diagnosed with LTBI and 68 (14.8%) with TBD.

Performance of QTF obtained 343 negative, 96 positive, and 20 indeterminate results due to lack of lymphocyte activation after stimulation with mitogen (4.3%). QTF sensitivity was calculated by extrapolating the results obtained in TBD patients.[5] The QTF was indeterminate in 6 of the 68 children with TBD (8.8%) and was positive in 61 of the 62 TBD children from whom we obtained valid results (sensitivity, 98.3%; 95% confidence interval [CI], 94%–100%). A patient with microbiologically confirmed tuberculosis meningitis presented negative QTF and TST. Specificity was calculated by observing the percentage of negative results among asymptomatic children with negative TST.[3,5] QTF result was indeterminate in 11 of the 318 healthy children (3.4%) and was negative in 304 of the 307 healthy children from whom we obtained valid results (specificity, 99%; 95% CI, 97%–100%).

QTF was positive in 0.9% of children with TST indurations $<$5 mm (3/313), 22.2% of those with indurations between 5 and 9 mm (4/18), in 50.9% of those with indurations between 10 and 14 mm (26/51), and in 81.8% of cases with indurations $\geq$15 mm (63/77). Analyzing the concordant results from both tests, a light-moderate correlation was observed between millimetres of TST indurations and amount of interferon-γ produced after stimulation with ESAT-6 and CFP-10 ($r = 0.743$) (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A649).

As the QTF in our sample demonstrated excellent sensibility and specificity, we consider the test as a diagnostic reference to analyze TST sensitivity and specificity in BCG vaccinated and nonvaccinated populations.[1]

Two hundred forty-six children without BCG scar were included (53.5%). The ROC curve obtained allowed adequate discrimination of points of sensitivity and specificity (Area under curve, 0.977; 95% CI, 0.95–0.99). In our series, a TST induration equal to or greater than 10 mm presented a sensitivity of 95.9% (95% CI, 88.6%–98.6%) and a specificity of 95.5% (95% CI, 91.1%–97.8%). On reducing the reading point to $\geq$5 mm, the TST presented excellent sensitivity (100%; 95% CI, 95%–100%) and specificity (93.6%; 95% CI, 88.7%–96.5%).

Two hundred thirteen children were included with scarring on the deltoid area compatible with prior BCG vaccination. The ROC curve obtained allowed acceptable discrimination of appropriate points of sensitivity and specificity (0.865; 95% CI, 0.76–0.96). In this population, considering as positive TST indurations equal to or greater than 10 mm, TST specificity was only 86% (95% CI, 80%–90%) and sensitivity 82.6% (95% CI, 63%–93%). If we take those indurations of $\geq$15 mm as positive, TST sensitivity reduces to 60.9% (95% CI, 40.8%–77.8%) although specificity improves to 97% (95% CI, 92.4%–98.2%).

DISCUSSION

Interpretation of the TST in vaccinated children continues to be a controversial question. Some meta-analyses have shown that the effect of BCG can endure for up to 15 years.[6,7] However, some authors consider that one should not take previous BCG vaccination history into account when interpreting the TST due to the risk of not treating real LTBI.[8]

In Spain, the first consensus document from the SEIP in 2003 established positively those indurations equal to or greater than 15 mm in BCG-vaccinated children during the last 3 years, and negatively those of less than 10 mm.[1] Individualized reading was recommended in the evaluation of indurations between 11 and 14 mm. However, when there is a TST reaction of $\geq$10 mm in a vaccinated child, given the high risk of developing TBD after the infection, the effect of BCG is usually ignored.

The development of more specific tests such as QTF can help us to differentiate false positive TST results due to prior BCG vaccination. In BCG vaccinated children diagnosed with LTBI, the appearance of discordant positive TST results with negative IGRAS is frequent,[4,9] but this phenomenon does not seem to be observed in children with TBD.[4] Also, it is well known that TST indurations produced after BCG vaccination or by infection by other mycobacterias normally do not exceed 15 mm. However, it is difficult to differentiate whether an indurations between 11 and 14 mm is or is not a false positive.

In our study, excluding indeterminate results, QTF had sensitivity of 98% and specificity of 99%, thus we consider it could be used as a diagnostic reference to analyze TST sensitivity and specificity. We observed how the increase in TST reading point to 15 mm in BCG-vaccinated children could give rise to an important reduction in test sensitivity. As a result, the new consensus document published in 2010 in Spain definitively dismissed the 15 mm cut-off point for those children already vaccinated.[10] The American Academy of Pediatrics and the American Thoracic Society also recognize a cut-off point of 10 mm for these same cases.[11,12]

On the other hand, according to our data, in children without BCG scar and with TB risk factors or suspicion of TBD, a TST $\geq$5 mm presents excellent specificity and sensitivity. In an analysis similar to our own, Bakir et al studied the TST sensitivity and specificity in 979 children in contact with adults diagnosed of pulmonary tuberculosis using an IGRA as a "reference standard." The author found that the sensitivity of TST in infants under 2 years of age using a cut-off point equal to or greater than 10 mm was only 66%. Reducing the cut-off point to 5 mm increased sensitivity to 78%.[13] This fact supports the hypothesis of considering positive indurations above 5 mm in nonvaccinated infants belonging to risk populations.

The current epidemiological situation in Spain, with increase in immigration from developing countries, has led to high rates of TB in our community. TST sensitivity observed using QTF as a reference in immigrant children without BCG scar raises the question of whether in this population a cut-off point of 5 mm is correct. The performance of an IGRA in these children with indurations between 5 and 10 mm would confirmed tuberculosis infection and would rule out the presence of the majority of environmental mycobacterias.

APPENDIX

The Spanish Collaborative Group for the study of QuantiFERON-TB GOLD Test in children Collaborators are Daniel Blázquez, MD; María Penín, MD; Maria Luisa Navarro, MD, PhD; Jesús Saavedra-Lozano, MD, PhD; Maria Isabel González-Tomé, MD, PhD; Cristina Calvo, MD, PhD; Marta Ruiz, MD; Sara Guillén, MD, PhD; Jose Tomás Ramos, MD, PhD; Teresa Hernandez-Sampelayo, MD, PhD; Ramón Velázquez, MD; Beatriz Pérez-Gorricho, MD, PhD; and Jorge Martinez, MD.

REFERENCES

1. Grupo de trabajo de Tuberculosis de la Sociedad Espanola de Infectologia Pediatrica. Interpretación de la prueba de tuberculina en niños. *An Pediatr (Barc).* 2003;59:582–585.
2. Araujo Z, de Waard JH, de Larrea CF, et al. The effect of Bacille Calmette-Guerin vaccine on tuberculin reactivity in indigenous children from communities with high prevalence of tuberculosis. *Vaccine*. 2008;26: 5575–5581.
3. Menzies D, Pai M, Comstock G. Meta-analysis: new tests for the diagnosis of latent tuberculosis infection: areas of uncertainty and recommendations for research. *Ann Intern Med*. 2007;146:340–354.
4. Bianchi L, Galli L, Moriondo M, et al. Interferon-gamma release assay improves the diagnosis of tuberculosis in children. *Pediatr Infect Dis J*. 2009;28:510–514.

5. Mazurek GH, Jereb J, Lobue P, et al. Guidelines for using the QuantiFERON-TB Gold test for detecting *Mycobacterium tuberculosis* infection, United States. *MMWR Recomm Rep*. 2005;54(RR-15):49–55.
6. Pai M, Zwerling A, Menzies D. Systematic review: T-cell-based assays for the diagnosis of latent tuberculosis infection: an update. *Ann Intern Med*. 2008;149:177–184.
7. Wang L, Turner MO, Elwood RK, et al. A meta-analysis of the effect of Bacille Calmette Guerin vaccination on tuberculin skin test measurements. *Thorax*. 2002;57:804–809.
8. Sleiman R, Al-Tannir M, Dakdouki G, et al. Interpretation of the tuberculin skin test in Bacille Calmette-Guerin vaccinated and nonvaccinated school children. *Pediatr Infect Dis J*. 2007;26:134–138.
9. Lighter J, Rigaud M, Eduardo R, et al. Latent tuberculosis diagnosis in children by using the QuantiFERON-TB Gold In-Tube test. *Pediatrics*. 2009;123:30–37.
10. Moreno-Pérez D, Andrés-Martín A, Altet Gómez MN, et al. Diagnosis of tuberculosis in paediatrics. *An Pediatr (Barc)*. 2010;72:283.e1–283.e14. doi: 10.1016/j.anpedi.2010.01.002.
11. Targeted tuberculin testing and treatment of latent tuberculosis infection. This official statement of the American Thoracic Society was adopted by the ATS Board of Directors, July 1999. This is a Joint Statement of the American Thoracic Society (ATS) and the Centers for Disease Control and Prevention (CDC). This statement was endorsed by the Council of the Infectious Diseases Society of America. (IDSA), September 1999, and the sections of this statement. *Am J Respir Crit Care Med*. 2000;161(4 pt 2):S221–S247.
12. American Academy of Paediatrics. Tuberculosis. In: Pickering L, Baker C, Long SS, eds. *Red Book: 2006 Report of the Committee on Infectious Diseases. 25th ed*. Elk Grove Village, IL: American Academy of Paediatrics; 2006:678–698.
13. Bakir M, Dosanjh DP, Deeks JJ, et al. Use of T cell-based diagnosis of tuberculosis infection to optimize interpretation of tuberculin skin testing for child tuberculosis contacts. *Clin Infect Dis*. 2009;48:302–312.

EPIDEMIOLOGY OF CHILDHOOD TUBERCULOSIS

USE AND EVALUATION OF THE PEDIATRIC HEALTH INFORMATION SYSTEM TO ASSESS LOCAL AND NATIONAL INCIDENCE

David Siegel, MD, MPH, Xiaoyan Song, PhD, MB,†‡ Karl Klontz, MD, MPH,* William Pastor, MA, MPH,§ and Nalini Singh, MD, MPH*†*

Abstract: We used the Pediatric Health Information System (PHIS) administrative database to compare the incidence of childhood tuberculosis at Children's National Medical Center (CNMC) in Washington, DC, with national rates and evaluated PHIS as an epidemiologic tool. The incidence rate of tuberculosis at CNMC was approximately twice that of the national PHIS rate (incidence rate ratio, 1.9; 95% confidence interval 1.4–2.5). Of the 51 cases listed by PHIS as tuberculosis at CNMC between 2003 and 2007, 41 represented active tuberculosis validated by internal chart review, with a positive predictive value of 80%.

Key Words: tuberculosis, pediatrics, epidemiology, children

Accepted for publication October 5, 2010.

From the *Department of Epidemiology and Biostatistics, School of Public Health, The George Washington University, Washington, DC; †Division of Infectious Diseases, Children's National Medical Center, Washington, DC; ‡Department of Pediatrics, School of Medicine, The George Washington University, Washington, DC; and §Performance Improvement, Children's National Medical Center, Washington, DC.

Address for correspondence: Nalini Singh, MD, MPH, Pediatrics, Children's National Medical Center, 111 Michigan Ave. NW, Washington, DC 20010. E-mail: nsingh@cnmc.org.

DOI: 10.1097/INF.0b013e318211581e

Although the incidence of childhood tuberculosis (C-TB) in the United States decreased from 2.9 cases per 100,000 in 1993 to 1.5 cases per 100,000 in 2003, the incidence has risen in specific subpopulations such as immigrants, Hispanics, African Americans, and children less than 5 years of age.[1–3] Previous studies have stated that children who live in major metropolitan areas may be at high risk for TB infection as a result of being foreign-born or having TB sick contacts.[1] There is an increasing need to identify trends of TB incidence, especially in these high-risk metropolitan areas. The Pediatric Health Information System (PHIS), an administrative database, has not yet been evaluated in the literature in estimating C-TB incidence. The goals of this study were (1) to compare the incidence of C-TB at Children's National Medical Center (CNMC) with the national incidence using data from PHIS and then (2) to evaluate PHIS as a tool for conducting epidemiologic studies of C-TB.

MATERIALS AND METHODS

Study Setting and Population. This study was conducted at CNMC in Washington, DC. As the only exclusive provider of pediatric care in the metropolitan Washington, DC area, CNMC serves as the regional referral center for pediatric emergency, trauma, cancer, cardiac, and critical care as well as neonatology, orthopedic surgery, neurology, and neurosurgery.

Data Source. The PHIS database was used to calculate the number and incidence of inpatient C-TB cases at CNMC and nationally. PHIS was developed by the Child Health Corporation of America (Shawnee Mission, Kansas) and includes pooled demographic and diagnostic data from 41 freestanding, noncompeting children's hospitals across the nation. For every hospital encounter, the PHIS database records variables such as patient demographics, admission and discharge date, locations in the hospital (inpatient, outpatient, emergency department or operating room), itemized hospital charges, medications, and 20 diagnosis and procedure codes assigned to the encounter by the billing provider. Data are subjected to 175 reliability and validity checks and are only accepted into the PHIS database when classified errors occur in $<2\%$ of a hospital's quarterly data.[4,5] Diagnosis codes contained in the database are expressed in International Classification of Diseases, Ninth Revision (ICD-9) codes.

Case Finding and Statistical Analysis. For the study period 2003 through 2007, we queried PHIS for all cases and ages with one or more TB-related ICD-9 codes (01000 to 01896), either as primary or secondary diagnosis in any one of the 20 entry spaces PHIS allows for diagnosis codes. To calculate incidence rates of inpatient C-TB, we divided the number of PHIS inpatient cases of TB by the total number of inpatient admissions for all causes to PHIS hospitals during the years 2003 through 2007. Age-specific rates were calculated in like fashion using the specific age strata used by the Centers for Disease Control and Prevention (CDC) to study TB. We limited our analysis to inpatient cases of TB so as to exclude asymptomatic patients without tuberculosis who were attended to in emergency rooms and who received TB-related ICD-9 codes for "rule out tuberculosis" in conjunction with chest x-ray screening. Cumulative incidence ratio (CIR) defined as the cumulative incidence of TB at CNMC per PHIS compared with the cumulative incidence of TB at all PHIS hospitals was calculated. Analysis was done by Statistical Analysis Software (SAS Institute, NC).

Validation of PHIS. To evaluate the ability of PHIS to estimate cases of active TB, we reviewed the CNMC internal chart of any patient PHIS reported to have been treated for TB at CNMC. Patients were determined to have active TB by chart review as defined by CDC, which defines a case as having a positive purified protein derivative test, signs and symptoms consistent with TB (including radiography), treatment with 2 or more antituberculosis medications, and having a completed diagnostic evaluation.[6] Additionally, we compared the list of TB cases at CNMC according

to PHIS with the CNMC Office of Epidemiology/Infection Control's internal list of suspected or confirmed TB cases generated from hospital communicable disease report forms. Cases were kept on this list only if they met the CDC definition.

This study was approved by the CNMC Institutional Review Board and by Child Health Corporation of America.

RESULTS

Comparing Incidence. Between 2003 and 2007, PHIS identified 1089 distinct inpatient C-TB cases at all PHIS hospitals, 51 of which were at CNMC (Table 1). The oldest patient identified in the PHIS database at CNMC was 19 years old. The demographics of the C-TB patients at CNMC identified by PHIS were similar to those reported in both local and national studies in terms of age and gender distribution, percentage foreign born (32%), percentage with sick contacts (44%), and percentage pulmonary (85%).[1,3,7]

The incidence rate at CNMC was 8.69 per 10,000 hospital admissions compared with 4.66 per 10,000 hospital admissions at all PHIS hospitals (incidence rate ratio: 1.9, 95% confidence interval [CI] = 1.4–2.5). We observed a significantly higher incidence of C-TB at CNMC compared with all PHIS hospitals among patients less than 5 years of age (CIR = 1.89, 95% CI = 1.26– 2.81) and in the 10 to 14 years (CIR = 2.45, 95% CI = 1.39–4.32) and 15–24 years (CIR = 2.07, 95% CI = 1.13–3.80) age groups. Although the rate of inpatient C-TB remained unchanged at CNMC during the study period, there was a significant increase in the rate for PHIS hospitals collectively (annual incidence ratio = 1.05, CI = 1.01–1.10).

Validation of PHIS. Of the 51 cases identified by PHIS at CNMC, 41 met the CDC definition of active tuberculosis, a positive predictive value of 80%. Of the other 10 who were given TB ICD-9 codes in the PHIS database but failed to meet the CDC definition, 6 (12%) represented latent TB and 4 (8%) represented asymptomatic persons or persons who had a TB sick contact and were tested in the hospital but did not have either active or latent TB.

We further evaluated PHIS by reviewing the CNMC hospital communicable disease report forms, which included 46 active cases of TB by the CDC definition. This list included the 41 cases identified by PHIS and 5 others. Using the communicable disease report list as a gold standard, we estimate that PHIS has a sensitivity of 89% for identifying clinically defined TB cases.

TABLE 1. Selected Demographic Features of Childhood Case-patients With Active Tuberculosis Admitted to Children's National Medical Center (CNMC) Compared With Those Identified at All Hospitals Participating in the Pediatric Health Information System (PHIS), 2003 to 2007

	PHIS Database-identified TB Cases at CNMC Number (%)	PHIS Database-identified TB Cases at All 41 PHIS Hospitals Number (%)
Cases of TB	51	1089
CNMC TB cases correctly identified as TB	41 (80)	
CNMC TB cases falsely identified as TB	10 (20)	
Male	28 (55)	522 (48)
Age (yr)		
<5	25 (49)	548 (50)
5–9	4 (8)	156 (14)
10–14	13 (25)	180 (17)
15–24	9 (18)	198 (18)
24+	0 (0)	7 (1)

TB indicates tuberculosis.

DISCUSSION

PHIS has previously been used to assess the incidence of disease and response to therapy in hospital-defined communities, but investigators have raised concerns about misclassification of PHIS data.[4,5,8] Patients are often assigned ICD-9 codes for billing purposes that may or may not conform to the clinical definition of a given disease. While studying response to osteomyelitis treatment in 2009, Zaoutis et al created a validation study where 10% of charts were reviewed from 19 of the 29 PHIS hospitals studied and the authors excluded 1056 of 6348 patients due to incomplete or low-quality data. Of the 19 audited PHIS hospitals, 13 had no evidence of misclassification, but 6 had misclassification of assignment to a treatment group in 11% to 50% of all cases.[4] In a similar study using PHIS, Fisher et al in 2010 used ICD-9 codes to estimate coccidioidomycosis hospitalization rates. They recognized that misclassification of discharge diagnosis could influence their results, but their study did not quantify rate of misclassification with a validation study.[5] One obstacle to auditing the PHIS database at external hospitals is that chart review of any PHIS hospital aside from the researcher's hospital requires special permission.

Misclassification is a known limitation of using national administrative databases such as PHIS. Other studies have suggested that ICD-9 codes are more specific than sensitive and underestimate the true frequency of the condition in question.[9] However, even for inpatients, a tuberculosis ICD-9 code may have poor specificity because a high number of false positives were generated when cases were given the ICD-9 code of "unspecified pulmonary tuberculosis, unspecified examination," but were actually "rule out" tuberculosis cases that did not meet the CDC case definition. Although our study did not use PHIS to detect "true negatives" for tuberculosis, a future study could use PHIS in this manner and could further calculate a clinically significant value for specificity or negative predictive value.

Notwithstanding the limitation of misclassifications in the PHIS database, we were able to use PHIS data to demonstrate that children in metropolitan Washington, DC seeking care at CNMC had significantly higher rates of tuberculosis cases identified by TB-related ICD-9 codes than the other PHIS hospitals. However, we draw our conclusions assuming that other PHIS hospitals had a similar type and rate of coding misclassifications with respect to tuberculosis. Future studies may be needed to examine the consistency of ICD-9 coding at PHIS hospitals to validate studies that use PHIS as an epidemiologic tool for measuring incidence of infectious diseases in children. Nevertheless, PHIS can be an effective epidemiologic tool when measures are taken to minimize misclassification and to choose variables that are not frequently miscoded in the diagnosis and billing process.

REFERENCES

1. Nelson L, Schneider E, Wells C, et al. Epidemiology of childhood tuberculosis in the United States, 1993–2001: the need for continued vigilance. *Pediatrics*. 2004;114:333–341.
2. Kellerman S, Simonds D, Banerjee S, et al. APIC and CDC survey of *Mycobacterium tuberculosis* isolation and control practices in hospitals caring for children. Part 1: patient and family isolation policies and procedures. *Am J Infect Control*. 1998;26:478–482.
3. Randle V, Walsh T. Pediatric tuberculosis case detection, Montgomery County, Maryland, 1997–2006. Presented at the 12th Annual Conference, International Union Against Tuberculosis and Lung Disease – North American Region, San Diego, CA, February 28, 2008.

4. Zaoutis T, Localio A, Leckerman K, et al. Therapy for acute osteomyelitis in children prolonged intravenous therapy versus early transition to oral antimicrobial. *Pediatrics*. 2009;123:636–642.
5. Fisher T, Chiller T, Prasad P, et al. Hospitalizations for coccidioidomycosis at forty-one children's hospitals in the United States. *Pediatr Infect Dis J*. 2010;29:243–247.
6. Centers of Disease Control and Prevention. Tuberculosis 1996 Case Definition. Available at: http://www.cdc.gov/ncphi/disss/nndss/casedef/tuberculosis_1996.htm. Accessed August 15, 2010.
7. Pediatric Tuberculosis Collaborative Group. Targeted tuberculin skin testing and treatment of latent tuberculosis infection in children and adolescents. *Pediatrics*. 2004;114:1175–1201.
8. Todd J, Bertoch D, Dolan S. Use of a large national database for comparative evaluation of the effect of a bronchiolitis/viral pneumonia clinical care guideline on patient outcome and resource utilization. *Arch Pediatr Adolesc Med*. 2002;156:1086–1090.
9. Zaoutis T, Heydon K, Chu J, et al. Epidemiology, outcomes, and costs of invasive aspergillosis in immunocompromised children in the United States, 2000. *Pediatrics*. 2006;117:e711–e716.

EFFECTIVENESS OF *HAEMOPHILUS INFLUENZAE* TYPE B CONJUGATE VACCINE FOR PREVENTION OF MENINGITIS IN SENEGAL

Jessica A. Fleming, PhD, MSc, Yakou Dieye, MD,† Ousseynou Ba, MD,† Boniface Mutombo wa Mutombo, MD, MPH,† Ndiouga Diallo, PharmD,† Pape Coumba Faye, MD, MPH,‡ Mamadou Ba, MD, PhD,§ Moussa Fafa Cisse, MD, PhD,¶ Aissatou Gaye Diallo, MD, PhD,‖ Mady Ba, MD, MPH,‡ Mary P. E. Slack, MB, FRCPath,** and Noel S. Weiss, MD, DrPH††*

Abstract: A total of 24 cases of hospitalized, laboratory-confirmed *Haemophilus influenzae* type b (Hib) meningitis were identified through a regional pediatric bacterial meningitis surveillance system. Each case was matched by age and residence to 4 neighborhood controls. The adjusted vaccine effectiveness for ≥2 doses was 95.8% (95% confidence interval, 67.9%–99.4%). Hib vaccine appears to be highly effective in preventing Hib meningitis in Senegal.

Key Words: *Haemophilus influenzae* type b, *Haemophilus influenzae* type b conjugate vaccine, vaccine effectiveness, bacterial meningitis, Senegal

Accepted for publication October 26, 2010.

From the *Vaccine Access and Delivery, PATH, Seattle, WA; †PATH, Dakar, Senegal, Africa; ‡Medical Prevention Department, Senegal Ministry of Health, Dakar, Senegal, Africa; §Pediatrics, Albert Royer Hospital, Dakar, Senegal, Africa; ¶Bacteriology, Albert Royer Hospital, Dakar, Senegal, Africa; ‖Bacteriology, LeDantec Hospital, Dakar, Senegal, Africa; ***Haemophilus* Reference Unit, UK Health Protection Agency Centre for Infections, London, United Kingdom; and ††Department of Epidemiology, ‖University of Washington, Seattle, WA.

Supported by The Bill and Melinda Gates Foundation.

Address for correspondence: Jessica Fleming, PhD, MSc, PATH, PO Box 900922, Seattle, WA 98109. E-mail: jfleming@path.org.

DOI: 10.1097/INF.0b013e318203aaa0

Infection with *Haemophilus influenzae* type b (Hib) is responsible for at least 3 million severe illnesses and nearly 400,000 deaths worldwide each year, mainly in the developing world.[1] On the basis of numerous studies from the African continent documenting the effectiveness of Hib conjugate vaccine on meningitis and pneumonia incidence, the majority of countries on the continent have now introduced or are committed to introducing vaccine.[2–5]

With funding from the GAVI Alliance, the Senegal Ministry of Health (MOH) introduced Hib conjugate vaccine into their routine immunization program in July 2005. The case–control study described in this study is part of the Hib Impact Project, a collaborative evaluation of the burden of Hib disease in the country, conducted by the MOH and PATH, an international, nonprofit organization. It includes cases identified through a regional pediatric bacterial meningitis (PBM) surveillance system and provides additional evidence for ongoing support of Hib vaccine by the MOH, when Senegal's GAVI Alliance vaccine donation ends in 2010.

METHODS

The MOH began PBM surveillance in January 2002 at the national pediatric reference hospital in Dakar as part of the World Health Organization African Pediatric Bacterial Meningitis Surveillance Network (AFRO PBM Network). Hib vaccine was introduced into routine immunization in all regions in July 2005. In February 2006, the PBM surveillance system was expanded to 6 regional hospitals in Diourbel, Kaolack, Saint Louis, Tambacounda, Thiès, and Ziguinchor regions.

Two Hib vaccines were used during the study period. Lyophilized Hiberix (GlaxoSmithKline Biologicals, Rixensart, Belgium) reconstituted with Tritanrix (GlaxoSmithKline Biologicals) (containing diphtheria, tetanus, whole cell pertussis, and hepatitis B) was replaced in January 2007 by Quinvaxem (Crucell, Berna Biotech Korea Corp., Incheon, Korea), a fully liquid diphtheria, tetanus, whole cell pertussis, hepatitis B, Hib vaccine.

The Hib vaccine schedule includes 3 doses given at 6, 10, and 14 weeks, administered concurrently with oral polio vaccine. No catch-up campaign was held. Case definitions for PBM were based on World Health Organization guidelines[6] and included children aged 6 weeks to <2 years with laboratory-confirmed Hib meningitis, hospitalized on or after July 22, 2005 (21 days after vaccine introduction). An exclusion criterion was residence outside of Senegal. Children aged up to 18 weeks at vaccine introduction were included to accommodate for late vaccination; case accrual ended on December 31, 2008. Lumbar puncture was routine practice for suspected PBM in all participating hospitals; confirmed cases were identified through cerebrospinal fluid (CSF) logbooks.

Suspected PBM was defined as clinical illness consistent with meningitis, including sudden fever onset and one or more of the following: stiff neck, bulging fontanelle, poor sucking, altered consciousness, irritability, seizures, other meningeal signs, toxic appearance, and petechial or purpural rash. Laboratory confirmation of Hib was determined by either Hib isolation by culture or Hib antigen detection in CSF by latex agglutination, as described previously.[7] Meningitis was reported as the cause of death if indicated on the surveillance form or hospital discharge records or by parents during study interview, and occurred within 90 days of hospital discharge.

Four neighborhood controls were identified for each case. Controls were born within 28 days of the birth of the case and were living in the same locality at the time of case illness. One ineligible control was dropped during analysis. Parents of all cases and controls agreed to participation. Date of vaccination was transcribed from vaccination cards; if unavailable, verbal vaccination history was recorded and verified when possible through health facility records. Verbal report was used if written confirmation was unavailable. The reference date was the date of illness onset in the case. A child was reported to have received 1 dose of Hib vaccine if at least 14 days elapsed between the administration of the only dose of Hib vaccine and the reference date. Second and third doses were counted if the dose was received at least 21 days after the

TABLE 1. Selected Characteristics of *Haemophilus influenzae* Type b Meningitis Cases and Controls

Characteristics	Cases (N = 24) n (%)	Controls (N = 95) n (%)
Sex		
Female	9 (37.5)	39 (41.1)
Age at illness onset or reference date for controls (mo)		
<6	14 (58.3)	60 (63.2)
≥6, <12	10 (41.7)	35 (36.8)
Formal education of mother		
None	10 (41.7)	29 (30.5)
Nonformal education*	11 (44.8)	32 (33.7)
Primary school	3 (12.5)	26 (27.4)
Secondary school	0 (0)	8 (8.4)
Source of family income		
Mandate	3 (12.5)	11 (11.6)
Salary	3 (12.5)	22 (23.1)
Income generating activities	15 (62.5)	43 (45.3)
Other	3 (12.5)	19 (20.0)
Children under 5 yr living in household†		
<3	9 (37.5)	45 (47.4)
≥3	15 (62.5)	50 (52.6)

*Includes skills for reading the Koran or reading, writing, and calculating in a national language.

†Including study participant.

prior dose and if, after administration of the dose, at least 7 days elapsed before the reference date.

Potential confounding factors including maternal education and number of children aged <5 years living in the home were adjusted for in the analysis; maternal education alone was included in the analysis for death. Vaccine effectiveness was calculated as (1 − odds ratio) × 100%.[8] Analyses were performed using STATA, version 9.0 (StataCorp LP, College Station, TX).

RESULTS

Hospital records of 24 of 34 (70.6%) eligible cases included sufficient contact information to locate children for interview. The children not located were slightly more likely to be female (60.0%) and under 6 months of age (70.0%) compared with study children (37.5% and 58.3%, respectively).

All study cases were less than 12 months of age. Of 24 cases, 13 (54.2%) were CSF culture positive; all 22 cases with latex results were latex positive. Case fatality was 41.7% (10/24); 80.0% (8/10) of deaths occurred in children aged 6 months or younger. Most baseline characteristics were similar between cases and controls, though mothers of cases were more likely to have received no or nonformal education compared with mothers of controls (Table 1).

Written documentation for vaccination (either vaccination card or health facility records) was available for 75.0% (18/24) of cases and 95.8% (91/95) of controls. Of all cases, 66.7% (16/24) received no Hib vaccine compared with 21.1% (20/95) of controls; 87.5% (21/24) of cases and 46.3% (44/95) of controls received <2 doses. No Hib vaccine was received by 50% (5/10) of fatal cases. Two cases were fully immunized (both had written verification), and received their first Hib vaccine dose between 6 and 7 weeks and their second and third doses at 12 and 17 weeks, respectively. One case had 2 Hib vaccine doses (verified in writing), the first between 7 and 8 weeks and the second at 12 weeks.

Vaccine effectiveness against Hib meningitis was high (≥2 doses, 95.8% [67.9%–99.4%]) (Table 2). Effectiveness against death from Hib meningitis was also high (≥2 doses, 98.8% [−88.9% to 100.0%]), but with only 10 fatalities, our findings were not statistically precise.

DISCUSSION

We observed high vaccine effectiveness against Hib meningitis through 2 years of age. Similar results from other African countries incorporating Hib vaccine into their routine immunization systems include The Gambia (93% [42%–99%]), Uganda (99% [92%–100%]), and Malawi (91% [68%–97%]).[3,9,10]

There are several limitations of our study, including the potential for misclassification of both exposure and outcome. Verbal record of vaccination was used when written records were unavailable (6 cases [25.0%] and 4 controls [4.2%]). However, parents of all cases and 3 of the controls reported no vaccine given, providing reassurance that doses reported were not exaggerated.

It is possible that antibiotic use before CSF collection could have led to an underestimate of true case numbers, as their use can cause Hib to be unviable through culture.[11] Compared with 56.7% of all suspected PBM cases <2 years, 38.9% of cases (7/18) with information on antibiotic use received the drug before their CSF draw. Supplementing culture with latex agglutination improved our laboratory sensitivity; 100% of cases with latex results were positive, whereas 54.2% showed culture positivity.

Case fatality for children <2 years calculated in this study (41.7%) is higher than averages cited for children <5 years by the World Health Organization for developing countries (17.3%) and the AFRO region (27.6%).[12] Estimates may be exaggerated if less-severe cases did not present to hospital or were less likely to receive a lumbar puncture, or if non-Hib deaths were misclassified as Hib. We prioritized hospital dis-

TABLE 2. Vaccine Effectiveness (VE) for *Haemophilus influenzae* Type b (Hib) Meningitis and Death From Hib Meningitis in Children <2 Years of Age

No. Doses Received	Odds Ratio	95% CI	Adjusted OR*†	95% CI	Adjusted VE (%)*†	95% CI
Hib meningitis						
Any vs. 0	0.08	0.02 to 0.29	0.09	0.02 to 0.34	91.45	65.85 to 97.86
≥2 vs. 0	0.07	0.02 to 0.33	0.04	0.01 to 0.32	95.77	67.94 to 99.44
Death						
Any vs. 0	0.08	0.01 to 0.77	0.11	0.01 to 1.17	89.46	−16.69 to 99.05
≥2 vs. 0	0.10	0.01 to 1.01	0.01	0.00 to 1.89	98.83	−88.93 to 99.99

*Hib meningitis outcome adjusted for child's age, region of illness onset, number of children <5 years living in the home and maternal education.

†Death outcome adjusted for child's age, region of illness onset, and maternal education.

OR indicates odds ratio; CI, confidence interval.

charge records for categorizing outcomes and restricted deaths to within 90 days of hospital discharge to limit misclassification. Although it is probable that children in more rural areas have less access to care,[11] clinical signs of meningitis are often severe, and the likelihood of seeking medical attention and referral to tertiary care is high. Four of our cases came from outside surveillance regions.

Although estimates of vaccine effectiveness are high, the presence of 2 fully vaccinated cases and 1 receiving 2 doses raises concerns of vaccine failure. All failed doses were received in 2005 or 2006. Although specific information for each of these doses is unavailable, it is known that vaccines can lose potency through exposure to prolonged heat or freezing.[13] An evaluation by the Hib Impact Project in 2006 found that some vaccines at each level of the cold chain were exposed—at least transiently—to temperatures outside the recommended 2 to 8°C range (Ndiouga Diallo, personal communication, 2008) (Static cold chain monitoring was conducted from 2006–2008 at 6 regional, 12 district and 24 health facility vaccine stores). Although accumulated heat exposure can be visually tracked through the use of vaccine vial monitors, the "shake test," which compares vaccine sedimentation rates with a known unfrozen vaccine from the same manufacturer[14] is less reliable for indications of vaccine freezing,[15] as the test is highly subjective to user interpretation. These issues highlight the importance of sufficient capacity and proper cold chain maintenance for vaccines throughout the country.

Regional surveillance provides critical information on disease burden and allows the MOH to monitor the long-term effect of Hib vaccine and identify potential vaccine failures—a point of particular interest, as there are presently no plans to provide a booster dose, despite documented cases of Hib disease in older children in neighboring Gambia.[16] Sustaining surveillance efforts is also important as Senegal begins to self finance the Hib vaccine after support from the GAVI Alliance ends and the country considers inclusion of other new vaccines in their routine immunization schedule.

REFERENCES

1. World Health Organization. WHO position paper on *Haemophilus influenzae* type b conjugate vaccines. *Wkly Epidemiol Rec*. 2006;81:445–452.
2. Mulholland K, Hilton S, Adegbola R, et al. Randomised trial of *Haemophilus influenzae* type-b tetanus protein conjugate vaccine [corrected] for prevention of pneumonia and meningitis in Gambian infants. *Lancet*. 1997;349:1191–1197.
3. Adegbola RA, Secka O, Lahai G, et al. Elimination of *Haemophilus influenzae* type b disease from The Gambia after the introduction of routine immunisation with a Hib conjugate vaccine: a prospective study. *Lancet*. 2005;366:144–150.
4. Cowgill KD, Ndiritu M, Nyiro J, et al. Effectiveness of *Haemophilus influenzae* type b conjugate vaccine introduction into routine childhood immunization in Kenya. *JAMA*. 2006;296:671–678.
5. von Gottberg A, de Gouveia L, Madhi SA, et al. Impact of conjugate *Haemophilus influenzae* type b (Hib) vaccine introduction in South Africa. *Bull World Health Organ*. 2006;84:811–818.
6. Otten MW, Soriano-Gabarró M, Shaba K, et al. *Hib-Paediatric Bacterial Meningitis (Hib-PBM) Surveillance Network: Surveillance Manual, Field Test Version, July 2001.* Harare, Zimbabwe: World Health Organization Regional Office for Africa; 2001.
7. Ba O, Fleming J, Dieye Y, et al. Hospital surveillance of childhood bacterial meningitis in Senegal and the introduction of *Haemophilus influenzae* type b conjugate vaccine. *Am J Trop Med Hyg.* In press.
8. Orenstein WA, Bernier RH, Dondero TJ, et al. Field evaluation of vaccine efficacy. *Bull Word Health Organ*. 1985;63:1055–1068.
9. Lee EH, Lewis RF, Makumbi I, et al. *Haemophilus influenzae* type b conjugate vaccine is highly effective in the Ugandan routine immunization program: a case-control study. *Trop Med Int Health*. 2008;13:495–502.
10. Daza P, Banda R, Misoya K, et al. The impact of routine infant immunization with *Haemophilus influenzae* type b conjugate vaccine in Malawi, a country with high human immunodeficiency virus prevalence. *Vaccine*. 2006;24:6232–6239.
11. Peltola H. Worldwide *Haemophilus influenzae* type b disease at the beginning of the 21st century: global analysis of the disease burden 25 years after the use of the polysaccharide vaccine and a decade after the advent of conjugates. *Clin Microbiol Rev*. 2000;13:302–317.
12. World Health Organization. *Haemophilus influenzae* type b (Hib) meningitis in the pre-vaccine era: a global review of incidence, age distributions, and case-fatality rates. WHO/V&B/02.18. Geneva: World Health Organization Department of Vaccines and Biologicals; 2002.
13. World Health Organization. Aide-memoire for prevention of freeze damage to vaccines. WHO/IVB/07.09. Geneva: World Health Organization Department of Immunization, Vaccines and Biologicals; 2007.
14. World Health Organization. *Training for Mid-Level Managers (MLM); Module 1: Cold chain, Vaccines and Safe Injection Equipment Management.* WHO/IVB/08.01. Geneva: World Health Organization Department of Immunization, Vaccines and Biologicals; 2008.
15. Grover A, Singh A. Validity of the shake test to identify the frozen damaged vaccine vials. *Indian Pediatr*. 2005;42:724–725.
16. Howie SR, Antonio M, Akisanya A, et al. Re-emergence of *Haemophilus influenzae* type b (Hib) disease in The Gambia following successful elimination with conjugate Hib vaccine. *Vaccine*. 2007;25:6305–6309.

EFFECTS OF PUBLIC HEALTH INTERVENTIONS IN REDUCING TRANSMISSION OF HAND, FOOT, AND MOUTH DISEASE

Edmond Ma, MB ChB, MMedSc, PdipID, PDip (Epidemiology and Biostatistics), Pdip (Community Geriatric), FFPH, FHKCCM, FHKAM, Simon Wong, BA, MECON, Christine Wong, FHKCCM, FHKAM, Shuk Kwan Chuang, FHKCCM, FHKAM, and Thomas Tsang, FHKCCM, FHKAM

Abstract: We evaluated the effect of public health interventions including territory-wide school closure and hygiene campaigns during 2003 and 2009 in reducing hand, foot, and mouth disease (HFMD). We compared the observed and projected HFMD consultation rates among sentinel general practitioners using a decomposition regression model. Public health measures were effective in reducing HFMD by 57.2% (95% CI: 53.0%–60.7%) and 26.7% (95% CI: 19.5%–32.7%) in 2003 and 2009, respectively.

Key Words: hand, foot, mouth disease, communicable diseases, public health

Accepted for publication October 24, 2010.

From the Surveillance and Epidemiology Branch, Centre for Health Protection, Department of Health, Hong Kong Special Administrative Region, Hong Kong, China.

Address for correspondence: Edmond Ma, MB ChB, MMedSc, PdipID, PDip (Epidemiology and Biostatistics), Pdip (Community Geriatric), FFPH, FHCCM, FHKAM, 4/F Surveillance and Epidemiology Branch, Centre for Health Protection, 147C Argyle St., Hong Kong Special Administrative Region, China. E-mail: edmond_ma@dh.gov.hk.

DOI: 10.1097/INF.0b013e3182127782

Hand, foot, and mouth disease (HFMD) related to infection caused by different enteroviruses has caused cyclical epidemics in Southeast Asia Region in the past decades. The recent epidemic in 2010 also prompted the World Health Organization to issue warning against the disease.[1] Although the infection often needs a mild clinical course, severe infections can result in aseptic meningitis, encephalitis, acute flaccid paralysis, myocariditis, pulmonary edema, or even death, especially those caused by enterovirus 71.[2,3] In a recent outbreak in Anhui Province of China, among the 6049 cases reported from March to early May, 3023 (50%) were hospitalized, 353 (5.8%) were severe, and 22 (0.36%) were fatal.[4] Apart from fecal-oral route and direct contact with

open skin vesicles, enteroviruses can also be transmitted through droplets because it is not uncommon for patients with HFMD to have respiratory symptoms. Besides, indirect transmission through contaminated objects can also occur as enteroviruses can survive as fomites at room temperature.

Because there is no effective vaccine or chemoprophylaxis presently for controlling HFMD, public health interventions rest almost solely on nonpharmacologic measures. These include increasing awareness of the disease, promoting personal hygiene such as covering nose and mouth while coughing or sneezing, hand washing, cleansing and disinfection, social distancing, and school closure. Such public health measures are often implemented before or during the peak season of HFMD. Nevertheless, there have been few studies to evaluate them.

In Hong Kong, sustained and intensive public health interventions were implemented during the severe acute respiratory syndrome (SARS) outbreak in 2003 and pandemic influenza H1N1 in 2009, featuring territory-wide school closure and massive hygiene campaign on hand washing, wearing mask, and disinfection. In this study, we evaluated the impact of these measures on HFMD transmission in the community.

MATERIALS AND METHODS

We used the sentinel surveillance data collected from a network of 40 general practitioners to assess the community activity of HFMD from January 2001 to June 2010. The general practitioners who participated in the surveillance system reported the weekly number of HFMD cases to the Department of Health. The consultation rate of HFMD was calculated by dividing the total number of HFMD by the total number of consultations conducted by the sentinel doctors. We assessed the effectiveness of the public health measures by comparing the observed HFMD consultation rates and the projected rates in defined periods of 2003 and 2009 during which territory-wide public health interventions against SARS and pandemic influenza H1N1 (2009) were implemented. The period in 2003 was defined to span from week 7 to week 31 during which Hong Kong experienced the SARS epidemic. In 2009, the period was defined to span from week 17, when outbreaks of the pandemic influenza H1N1 was first reported in Mexico, to week 40 when schools in Hong Kong reopened after the summer break.

We first found the best-fitted model in projecting weekly HFMD rates by decomposition regression analysis using data collected from January 2001 to June 2010, excluding the data collected in the 2 defined periods in 2003 and 2009. We took into account any long-term trend, cyclical and seasonal patterns of HFMD in projecting the HFMD consultation rates. We included variables into the regression model to test whether there was any linear trend from January 2001 to June 2010, 2-year or 3-year cycles, and seasonal pattern. Statistics Analysis System software was used to determine the statistical significance of all parameters inputted and stepwise regression was adopted in the selection of parameters. The percentage reduction in HFMD rates was then computed by calculating the percentage difference in the areas under curve of the observed rates and the projected rates.

RESULTS

The best-fitted model was identified by the regression analysis with adjusted R^2 value of 0.22. The observed and the projected HFMD rates from January 2001 to June 2010 were illustrated in Figure 1A, which showed that the 2 rates matched quite well with each other. HFMD consultations rates showed a statistically significant 2-year cyclical pattern (but not a 3-year cyclical pattern). Seasonal effect was also found to be statistically significant for explaining the HFMD rates with higher activity detected in summer months. There was no significant linear trend in the study period. The final regression model constructed was:

$$R(t) = 1.11549 - 0.42384 \cos(2\pi * m/12) - 0.10447 \sin(2\pi * m/24),$$

where $R(t)$: projected HFMD rate at week t.

t: the number of week counting from week 1 of 2001.

m: the number of month counting from January of 2001 covering week t.

There was a reduction of 57.2% (95% CI: 53.0%–60.7%) in HFMD consultation rates during the SARS period in 2003 and a reduction of 26.7% (95% CI: 19.5%–32.7%) during the pandemic influenza H1N1 period in 2009. In both 2003 and 2009, the projected HFMD rates were lower than the observed rates during the whole period of school closure (Fig. 1B, C).

When we compared the projected and the observed HFMD rates for these 2 years, it was noted that the projected rates were still lower than the observed rates in 2003 beyond week 31 until almost the end of the year. On the contrary, in 2009, the observed HFMD consultation rates became comparable to that of the projected rates in August, before the end of the defined intervention period (ie, reopening of schools).

DISCUSSION

School closure was often considered a key component in controlling outbreaks of HFMD but there was limited scientific evidence from the literature on its sole impact on HFMD. Whether schools should be closed for HFMD outbreaks probably depends on a number of factors including clinical severity of the patients, extent of the outbreak (number of school outbreaks and number of children affected in each school), and occurrence of a novel virulent strain. On the other hand, school closure has been investigated as a nonpharmacologic intervention to halt influenza pandemic. Using sentinel data of influenza, the effect of school closure has been quantified to reduce the cumulative number of cases by 13% to 17% during a pandemic.[5] In another study, school closure was estimated to reduce 28% of physicians' visits for respiratory infection among children.[6] Although our study cannot segregate the effect of school closure from other public health measures, the results did provide supportive evidence since the projected HFMD rates were lower than the observed rates during the whole period of school closure in both the years.

Using a statistical model and taking into account long-term trend, cyclical and seasonal effects, our study found a substantial and statistically significant reduction in HFMD consultation rates among sentinel doctors during the 2 defined periods of public health interventions against SARS and pandemic influenza H1N1 in 2003 and 2009, respectively. There are few comparable studies in the literature. A reduction in the number of HFMD cases was reported in Singapore during an HFMD epidemic in 2000 after implementation of similar public health measures, including nation-wide closure of all preschools from October 1 to 15.[7] However, no measurement was made to quantify the effect of these interventions. The percentage reduction (57.2%) in HFMD measured in SARS period in 2003 was nearly double compared to that measured during pandemic influenza H1N1 in 2009 (26.7%). In addition, it is worth noting that a sustained effect of reduced transmission was observed in 2003 until the end of the year, whereas the effectiveness gradually declined around August in 2009 despite persistence of public health interventions. This might be explained by the different risk perception toward the 2 diseases which in turn affected people's preventive behavior.[8–12] It would

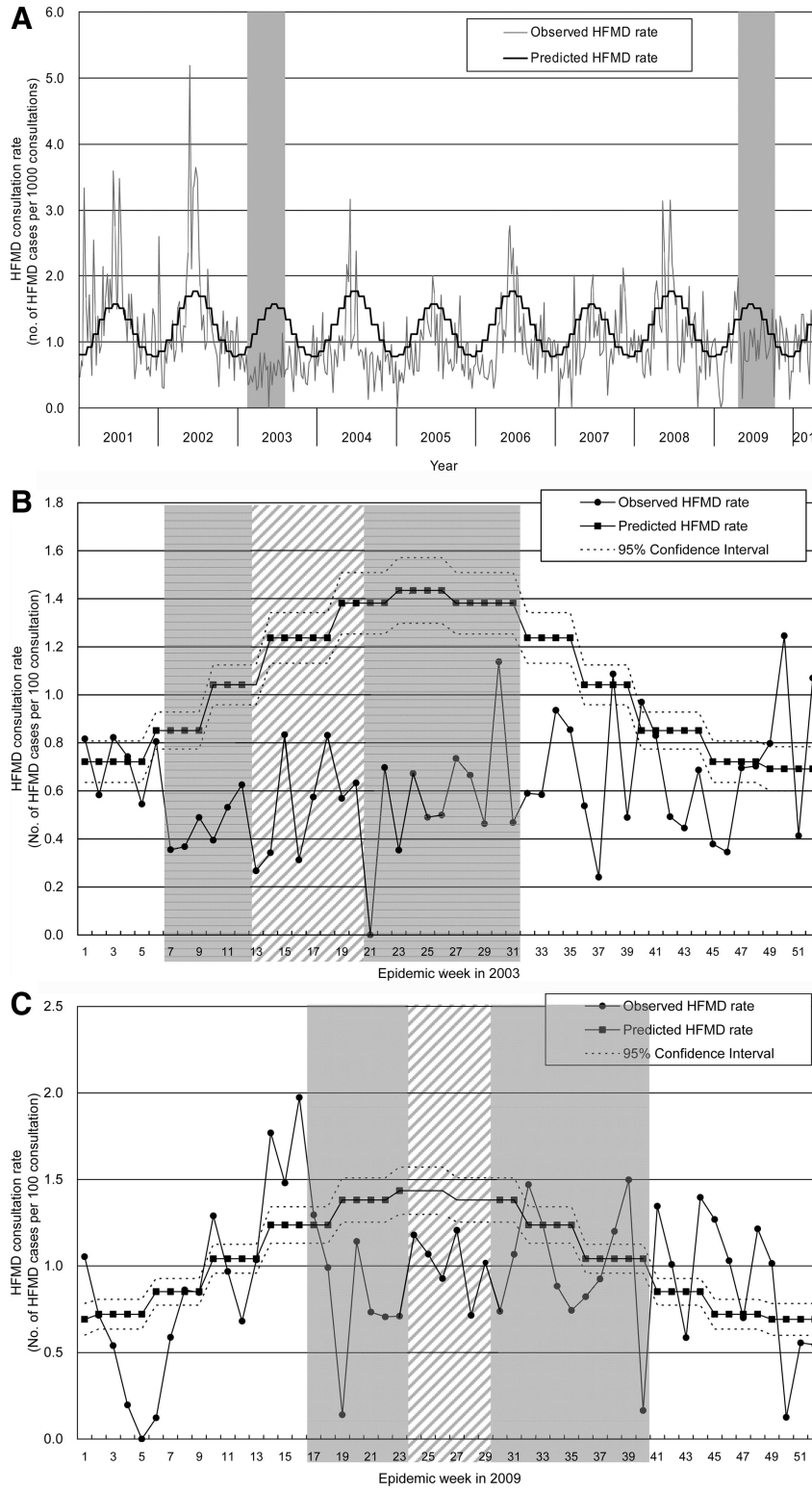

FIGURE 1. Projected and observed hand, foot, and mouth disease (HFMD) consultation rates reported by sentinel surveillance system. A, showed projected and observed HFMD consultation rates from January 2001 to June 2010. The projected rates match well with the observed ones except in the shaded periods, that is, the periods when enhanced public health measures were implemented. B, C, Illustrated the projected and observed HFMD consultation rates in 2003 and 2009, respectively. The shaded area indicated the period when territory-wide public health measures were enhanced during week 7 to week 31 in 2003, and week 17 to week 40 in 2009. Schools were closed during week 13 to week 20 in 2003, and week 24 to week 29 in 2009.

be useful to track how these perceptions changed as the pandemic evolved so as to better formulate sustained preventive strategies.

ACKNOWLEDGMENTS

The authors thank all doctors participating in the sentinel surveillance system to monitor the trend of HFMD in Hong Kong.

REFERENCES

1. World Health Organization. Hand, foot and mouth disease: a rising menace in Asia. Available at: http://www.wpro.who.int/media_centre/news/news_20090713.htm. Accessed August 2, 2010.
2. AbuBakar S, Chee HY, Al-Kobaisi MF, et al. Identification of enterovirus 71 isolates from an outbreak of hand, foot and mouth disease (HFMD) with fatal cases of encephalomyelitis in Malaysia. *Virus Res*. 1999;61:1–9.
3. Gilbert GL, Dickson KE, Waters MJ, et al. Outbreak of enterovirus 71 infection in Victoria, Australia, with a high incidence of neurologic involvement. *Pediatr Infect Dis J*. 1988;7:484–488.
4. Zhang Y, Zhu Z, Yang W, et al. An emerging recombinant human enterovirus 71 responsible for the 2008 outbreak of hand foot and mouth disease in Fuyang city of China. *Virol J*. 2010;7:94.
5. Cauchemez S, Valleron AJ, Boëlle PY, et al. Estimating the impact of school closure on influenza transmission from Sentinel data. *Nature*. 2008; 452:750–754.
6. Heymann A, Chodick G, Reichman B, et al. Influence of school closure on the incidence of viral respiratory diseases among children and on health care utilization. *Pediatr Infect Dis J*. 2004;23:675–677.
7. Chan KP, Goh KT, Chong CY, et al. Epidemic hand, foot and mouth disease caused by human enterovirus 71, Singapore. *Emerg Infect Dis*. 2003;9:78–85.
8. de Zwart O, Veldhuijzen IK, Elam G, et al. Perceived threat, risk perception, and efficacy beliefs related to SARS and other (emerging) infectious diseases: results of an international survey. *Int J Behav Med*. 2009;16:30–40.
9. Wilson N, Baker MG. The emerging influenza pandemic: estimating the case fatality ratio. *Euro Surveill*. 2009;14.pii:19255.
10. World Health Organization. Strategic Advisory Group of Experts on Immunization—report of the extraordinary meeting on the influenza A (H1N1) 2009 pandemic, 7 July 2009. *Wkly Epidemiol Rec*. 2009; 84:301–308. Available at: http://www.who.int/wer/2009/wer8430.pdf. Accessed August 2, 2010.
11. Centers for Disease Control and Prevention (CDC). Update: influenza activity—United States, April–August 2009. *MMWR Morb Mortal Wkly Rep*. 2009;58:1009–1012.
12. Lau JT, Griffiths S, Choi KC, et al. Prevalence of preventive behaviors and associated factors during early phase of the H1N1 influenza epidemic. *Am J Infect Control*. 2010;38:374–380.

IMPACT OF CD4 T CELL COUNT ON THE OUTCOME OF PLANNED TREATMENT INTERRUPTIONS IN EARLY-TREATED HUMAN IMMUNODEFICIENCY VIRUS-INFECTED CHILDREN

Clàudia Fortuny, MD, PhD, Antoni Noguera-Julian, MD, PhD,* Laia Alsina, MD, PhD,* Rocío Bellido, MSc,† Emília Sánchez, MD, PhD,‡ Carmen Muñoz-Almagro, MD, PhD,§ Lidia Ruiz, PhD,† and Rafael Jiménez, MD, PhD**

Abstract: Early highly active antiretroviral therapy is recommended in all vertically human immunodeficiency virus (HIV)-infected infants. We describe the long-term immunologic outcome after planned treatment interruption (PTI) in 7 children diagnosed and treated during acute HIV infection (age <12 weeks). Children had remained a median of 57 months off treatment, 3 of them indefinitely. The 2 patients with the lowest nadir CD4% reinitiated highly active antiretroviral therapy because of a CD4 cell decline of <20%; 2 children resumed treatment because of clinical progression and parents' wishes. All patients experienced a decrease in CD4% after PTI, which particularly affected the naive subpopulation. The interferon-γ response against HIV-p24 antigen directly correlated with nadir CD4%. Our results suggest that early treatment in HIV-infected infants increases their potential to safely control viral replication after PTI for long periods.

Key Words: acute HIV infection, vertically transmitted HIV infection, highly active antiretroviral therapy, immunologic evolution, treatment interruption

Accepted for publication September 30, 2010.

From the *Infectious Diseases Unit, Department of Pediatrics, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain; †Irsi Caixa Foundation, Hospital Germans Trias i Pujol, Universitat Autònoma de Barcelona, Badalona, Spain; ‡Blanquerna School of Health Science, Universitat Ramon Llull, Barcelona, Spain; and §Department of Microbiology, Hospital Sant Joan de Déu, Universitat de Barcelona, Barcelona, Spain.

Supported by Fondo de Investigación Sanitaria (FIS 99/0051–01). Supported by Pedro Pons Grant, Universitat de Barcelona (to L.A.).

Antoni Noguera-Julian is the recipient of an award from the European Society for Pediatric Infectious Diseases (ESPID).

Presented at the 15th American Conference on Retroviruses and Opportunistic Infections (CROI), Boston, MA, on February 2008. Abstract 619.

Address for correspondence: Antoni Noguera-Julian, MD, PhD, Infectious Diseases Unit, Pediatrics Department, Hospital Sant Joan de Déu, Passeig Sant Joan de Déu 2, 08950 Esplugues, Barcelona, Spain. E-mail: ton@hsjdbcn.org.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3181ff8661

Mother-to-child transmission of human immunodeficiency virus (HIV) remains a major health problem, with a yearly estimate of 370,000 new pediatric infections. In infants, diagnosis is often established during acute infection (ie, the first 12 weeks of life), offering an opportunity for early treatment, in contrast to adult patients. In 2008, the CHER trial[1] provided definite evidence supporting early highly active antiretroviral therapy (HAART) in vertically HIV-infected infants. The potential advantages of early HAART include preservation of HIV-specific cellular immune function, decreased B cell activation, limitation of viral mutation, and establishment of a lower viral set point.[2,3] Because of a robust thymopoietic response, such immunologic recovery may be even better in infants.[4]

Initiating HAART has now become mandatory in all HIV-infected infants who are <12 months. As these patients remain exposed to HAART for life, difficulties for long-term adherence, drug toxicities, and development of drug resistance are all major concerns. Thus, well-designed and clinically verified treatment interruptions could be of benefit for this population, not least because of often limited resources to sustain lifelong HAART treatment. We describe the long-term immunologic evolution after a planned treatment interruption (PTI) in a series of vertically HIV-infected children treated with HAART during acute infection.

MATERIALS AND METHODS

Single-center case series of 7 vertically HIV-infected children who were diagnosed and received HAART within the first 12 weeks of life and who later underwent PTI. At the start of PTI, all patients were 2 years of age or older, were free of any active HIV-related clinical condition, and had shown a maintained CD4% (flow cytometry, FACSCalibur; BD Biosciences, San Jose, CA) within CDC Immunologic Category 1 (>25% for children aged ≤12 years) for at least the previous 2 years. When the possibility of PTI was raised, reasons, risks, and plans were discussed with the

parents, and timelines and criteria for restarting therapy were established. The study was approved by the local ethics committee and informed consent was obtained. HAART resumption was planned in case any clinical progression of HIV infection or a decrease in CD4 below 20% was observed.

A close clinical and biologic follow-up after PTI was performed (monthly controls during the first 6 months and every 2–3 months thereafter), which included the assessment of CD4 lymphocyte subsets, naive (CD45RA) and memory (CD45RO) CD4 cells (measured at PTI and 3, 6, 12, 24, and 36 months later), and antigen-specific CD4 cell-mediated immune responses (measured twice, while on HAART and 3 years after PTI, median time, range: 12–59 months). CD4 cell-mediated immune responses were assessed for interferon-gamma (IFN-γ) production measured with an enzyme-linked immunosorbent spot assay (ELISPOT). The antigens for the study of IFN-γ release in peripheral blood mononuclear cells (PBMC) were p24 for HIV (Protein Sciences, Meriden, CT) and cytomegalovirus (CMV, Biowhittaker, Walkersville, MD). Results were expressed as spot-forming cells (SFC) per million PBMC. A response was considered positive with values above 50 SFC/10^6 PBMC after subtracting negative control values.

All results were expressed as median values (range). Fisher exact test and Mann-Whitney *U* tests were used to compare proportions and continuous variables, respectively. Other nonparametric tests were used as indicated, and the level of significance was set at $P < 0.05$.

RESULTS

The study included 7 patients (Table 1). At diagnosis, all patients were symptom-free for HIV infection (clinical CDC Category N). Age, CD4%, and RNA-HIV viral load (CA HIV Monitor; Roche, Basel, Switzerland; limit <50 copies/mL) were as follows: 1.0 month (0–1.5), 42% (40%–50%), and 6.1 log copies/mL (5.1–6.5 log), respectively. HAART was implemented in all patients at diagnosis of HIV infection. The patients remained on HAART during 44 months (35–72). Five children (patients 1, 2, 3, 6, and 7) achieved complete suppression of viral replication; patients 1 and 2 indeed cleared HIV-specific maternal antibodies and became undetectable for DNA-HIV in PBMC.[5] Virologic failure occurred in patients 5 and 6 because of adherence problems, requiring changes to the HAART regimens; only patient 6 attained complete viral suppression afterward. While on treatment, all patients remained asymptomatic.

The reasons leading to PTI were treatment fatigue and adherence problems. Children had remained 57 (28–86) months off treatment. Three (patients 3, 4, and 7) remained off HAART indefinitely. Patients 2 and 5 resumed HAART because of a CD4 cell decline to 19% and 15% after 35 and 70 months, respectively. Of note, these 2 patients had the lowest nadir CD4% when initiating PTI. Progression to CDC clinical category A (recurrent upper respiratory tract infections) led to HAART resumption in patient 1 (CD4 cell 23% after 60 months), whereas HAART was resumed in patient 6 because of the parents' wishes (CD4 cell 24% after 28 months). Importantly, all 4 patients resuming therapy showed an optimal virologic response (undetectable viral load within 3–7 months), and a restoration of CD4% to more than 25% within 1 to 7 months after HAART resumption.

For all 7 subjects, HIV plasma viral load increased to 5.6 (3.6–6.0) log copies/mL 1 month after starting PTI, and decreased thereafter to a viral set point of 4.8 (3.3–5.2) log copies/mL. Both peak and viral set point viral loads were lower compared with those at HIV infection diagnosis (Wilcoxon test, $P = 0.042$ and $P = 0.027$, respectively). During PTI, none of the subjects experienced symptoms consistent with an acute retroviral syndrome. However, all patients experienced a decrease in CD4% (from 36.4% to 22.5%, $P = 0.028$; slope: −0.29% per month; Fig. A, Supplemental Digital Content 1, http://links.lww.com/INF/A647). The slope of CD4% decrease during the first 3 months after PTI was faster than the slope after 3 months (median values: −0.7% vs. −0.04% per month; $P = 0.028$). This early depletion of CD4 cells particularly affected the naive subpopulation (reduction from 63% at PTI to 49% 3 months later; Fig. B, Supplemental Digital Content 1, http://links.lww.com/INF/A647), whereas the memory population increased in its contribution to the total CD4%. Both categories recovered normal values for age along the following months, despite ongoing viral replication.

A strong CMV-specific CD4 cell-mediated immune response was observed whereas on HAART in 4 of 6 tested individuals (Table 1). This response was preserved or even boosted upon PTI. In contrast, HIV p24-specific responses were only detectable in one subject while on HAART, but were observed in

TABLE 1. Main Clinical Characteristics and Details on Immunologic Evolution of the 7 Early-treated Children Who Underwent HAART Interruption

Patient/ Gender	HAART Regimen	At PTI					CD4 Cell % Slope During PTI (per mo)	IFN-γ Production (No. SFC/10^6 PBMC)*				Current Situation: Age (yr)/CD4 Cell %/HIV VL (Copies/mL)
		Nadir CD4 Cell %	CD4 Cell %	CD45RA/ CD45RO cell %	HIV VL (Copies/mL)	Age (mo)		On HAART		After PTI		
								p24	CMV	p24	CMV	
1/F	ZDV-ddI-NVP	31	42	63/35	<50	73	−0.31	0	0	610	140	11.8/39/<50
2/F	d4T-ddI-NVP-NFV	14	29	58/42	<50	60	−0.27	0	60	10	1630	10.8/32/<50
3/F	ZDV-3TC-ABC-NVP	41	44	85/14	<50	41	−0.40	0	0	SAT	10	8.0/22/130,000
4/M	d4T-ddI-NVP-NFV	27	31	52/33	<50	37	−0.09	160	500	190	SAT	10.3/23/2800
5/M	d4T-ddI-NVP → d4T-ddI-3TC-NFV → ABC-ZDV-NVP-NFV	13	25	55/30	51,000	37	−0.13	30	130	140	120	11.1/29/<50
6/F	d4T-ddI-NVP → d4T-3TC-NFV	25	49	66/19	<50	50	−0.89	ND	ND	20	360	10.2/30/<50
7/F	ZDV-3TC-NVP	48	51	71/21	<50	27	−0.29	20	90	230	500	4.3/30/320,000
Median		27	42	63/30		41	−0.29					

*Positive response: values above 50 SFC/10^6 PBMC.

F indicates female; M, male; HAART, highly active antiretroviral therapy; VL, viral load; ZDV, zidovudine; ddI, didanosine; NVP, nevirapine; d4T, stavudine; NFV, nelfinavir; 3TC, lamivudine; ABC, abacavir; PTI, planned treatment interruption; SFC, spot-forming cells; PBMC, peripheral blood mononuclear cells; SAT, saturated (>2000 SFC/10^6 PBMC); CMV, Cytomegalovirus; ND, not done.

5 subjects during PTI. The ability to mount a detectable IFN-γ response against HIV p24 antigen after PTI correlated with nadir CD4% at PTI (the higher the nadir CD4%, the higher the IFN-γ production against p24 antigen; Spearman rho, $r = 0.829$; $P = 0.042$), indicating that preserved CD4 cell counts before PTI may contribute to the induction of new p24-specific CD4 cell responses in the pediatric age.

No other differences in clinical, immunologic, or virologic variables were observed between patients who had to resume HAART because of clinical and/or immunologic progression (patients 1, 2, and 5) versus the rest. However, an inverse correlation was observed between CD4% at PTI and the slope of the decrease in CD4 cells after PTI (Spearman rho, $r = -0.733$; $P = 0.039$).

DISCUSSION

In the CHER trial,[1] early HAART reduced infant mortality by 76% and HIV progression by 75%, with this effect being strongest in the youngest infants. Early HAART is safe, effective, and well-tolerated during infancy, and it preserves the development of immune function, despite variable rates of viral load suppression.[1,6] Accordingly, at the time of PTI, all patients in our series showed normal CD4%, CD45RA, and CD45RO cell subsets for age, and a strong CMV-specific response, known to be a marker of a good effector memory CD4 cell function in HIV-infected patients.[7] After early suppression of viral replication with HAART and because of the relative immaturity of the neonatal immune system,[8] HIV p24-specific CD4 cell-mediated immune responses were lacking in most individuals. Only patient 5 showed a positive ELISPOT response against HIV p24 antigen.

In HIV-infected infants, the clinical and immunologic benefits of early HAART have to be balanced against the long-term limitations of continuous therapy. In this setting, planned interruptions of HAART beyond the age of 2 years, when the risk of disease progression decreases, have the potential to reduce some of these long-term limitations. Recently, the first pediatric trial of PTI (PENTA 11) was published[9]: 109 HIV-infected children were randomized to continuous therapy or PTI. After a median of 130 weeks of follow-up, no deaths or new AIDS-defining events were reported. Lower nadir CD4% and older age predicted faster CD4% depletion after PTI, whereas younger age and higher nadir CD4% predicted better CD4% recovery upon HAART resumption. Although our results support some of these findings, the data from the PENTA 11 trial should only be compared with our series with caution, as most of the patients in PENTA 11 first received HAART during chronic infection (median age: 2.2 years). In contrast, treatment was initiated within 12 weeks of life in the children we present. Thus, although in both PENTA 11 and our series CD4% decreased sharply in the first 10 to 12 weeks off HAART, the need to restart HAART due to symptom-free immunosuppression occurred in PENTA 11 mainly in the first 12 weeks off HAART, much earlier than in our series (patients 2 and 5). Moreover, the required CD4% threshold to undergo PTI in young children was higher in PENTA 11 (above 30%) than in these 2 patients (29% and 25%, respectively). These differences may be related to the presence of detectable virus-specific CD4 responses in the subjects described here, and point out the different immunologic features of acute-treated and chronic-treated pediatric HIV infection and the need for specific trials on PTI in the former. In addition, as both PENTA 11 and our study document an association between lower nadir CD4% at PTI and faster CD4 cell depletion, future PTI strategies should be discouraged in children with low nadir CD4% (<20%).

Two interesting immunologic phenomena were observed in our series. First, the early depletion in CD4 cells upon PTI and HIV rebound mostly affected the naive subpopulation of thymic cells. In adult patients undergoing PTI, the CD4 cell depletion has been described as affecting equally the naive and memory subsets.[10] This difference could be because of the almost de novo exposure to HIV after early viral suppression, leading to the prime of naive cells into memory cells, together with a hyperactivation of the immune system that renders CD4 cells more susceptible to direct HIV cythopatic effect.[11,12] Second, 5 of 7 patients developed a strong long-term HIV-specific CD4 cell response after PTI, which has been associated with efficient natural control of primary HIV infection in adults.[13] However, the effect of PTI on HIV-specific immune responses in the adult infection remains unclear,[14] and no data exist for the pediatric setting. Although the ELISPOT results did not correlate with the risk of immunosuppression in our series, they did correlate with nadir CD4% at PTI. It is thus possible that it is not IFN-γ production, but rather other proliferative HIV-specific CD4 cell responses that may be of greater use in evaluating the specific immunity against HIV and predicting the response to PTI strategies in HIV-infected children.[15]

In conclusion, our analyses are in line with the first preliminary data on HAART interruption in children. The differences in natural history and immune pathogenesis between pediatric and adult HIV infection need to be taken into account, and further investigation is warranted.

REFERENCES

1. Violari A, Cotton MF, Gibb DM, et al. Early antiretroviral therapy and mortality among HIV-infected infants. *N Engl J Med*. 2008;359:2233–2244.
2. Hecht FM, Wang L, Collier A, et al. A multicenter observational study of the potential benefits of initiating combination antiretroviral therapy during acute HIV infection. *J Infect Dis*. 2006;194:725–733.
3. Hoen B, Cooper DA, Lampe FC, et al. Predictors of virological outcome and safety in primary HIV type 1-infected patients initiating quadruple antiretroviral therapy: QUEST GW PROB3005. *Clin Infect Dis*. 2007;45:381–390.
4. Resino S, Seoane E, Pérez A, et al. Different profiles of immune reconstitution in children and adults with HIV-infection after highly active antiretroviral therapy. *BMC Infect Dis*. 2006;6:112.
5. Muñoz-Almagro C, Fortuny C, Juncosa T, et al. Absence of detectable HIV-1 provirus DNA after early highly active antiretroviral therapy in children infected by vertical transmission. *J Acquir Immune Defic Syndr*. 2002;31:450–452.
6. Goetghebuer T, Haelterman E, Le Chenadec J, et al. Effect of early antiretroviral therapy on the risk of AIDS/death in HIV-infected infants. *AIDS*. 2009;23:597–604.
7. French M, Keane N, McKinnon E, et al. Susceptibility to opportunistic infections in HIV-infected patients with increased CD4 T-cell counts on antiretroviral therapy may be predicted by markers of dysfunctional effector memory CD4 T cells and B cells. *HIV Med*. 2007;8:148–155.
8. Luzuriaga K, Sullivan JL. Viral and immunopathogenesis of vertical HIV-1 infection. *Pediatr Clin North Am*. 2000;47:65–78.
9. Paediatric European Network for Treatment of AIDS. Response to planned treatment interruptions in HIV infection varies across childhood. *AIDS*. 2010;24:231–241.
10. Nemes E, Lugli E, Nasi M, et al. Immunophenotype of HIV+ patients during CD4 cell-monitored treatment interruption: role of the IL-7/IL-7 receptor system. *AIDS*. 2006;20:2021–2032.
11. Yue FY, Kovacs CM, Dimayuga RC, et al. Preferential apoptosis of HIV-1-specific CD4+ T cells. *J Immunol*. 2005;174:2196–2204.
12. Douek DC, Brenchley JM, Betts MR, et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature*. 2002;417:95–98.
13. Gloster SE, Newton P, Cornforth D, et al. Association of strong virus-specific CD4 T cell responses with efficient natural control of primary HIV-1 infection. *AIDS*. 2004;18:749–755.
14. Kaufmann DE, Rosenberg ES. The value of preserving HIV-specific immune responses. *J HIV Ther*. 2003;8:19–25.

15. Scott ZA, Beaumier CM, Sharkey M, et al. HIV-1 replication increases HIV-specific CD4+ T cell frequencies but limits proliferative capacity in chronically infected children. *J Immunol*. 2003;170:5786–5792.

COMPARISON OF PANDEMIC AND SEASONAL INFLUENZA REVEALS HIGHER MORTALITY AND INCREASED PREVALENCE OF SHOCK IN CHILDREN WITH SEVERE H1N1/09 INFECTION

Jethro A. Herberg, PhD,†‡*
Kelsey D. J. Jones, MB BS, BA, MRCPCH,‡*
Stéphane Paulus, MD, FAAP, Stuart Gormley, MNurSci,*†*
David Muir, PhD,§ Mehrengise Cooper, FRCPCH,‡
and Michael Levin, FMedSci†‡*

Abstract: Comparison of the clinical features of H1N1/09 and previous years' influenza A cases reveals that, in children presenting with severe disease, H1N1/09 influenza is associated with an increased prevalence of shock, duration of admission, and mortality. This was not attributable to demographic differences or underlying disease. H1N1/09 influenza is associated with more severe diseases than those with previous years' influenza A strains.

Key Words: H1N1/09 influenza, children, shock, mortality, comparison with seasonal influenza

Accepted for publication October 5, 2010.

From the *Section of Paediatrics, Division of Infectious Disease, Imperial College, London, United Kingdom; †Centre for Respiratory Infection, Imperial College, London, United Kingdom; and Departments of ‡Pediatrics and §Virology, St Mary's Hospital, Imperial College Healthcare NHS Trust, London, United Kingdom.

Supported by NIHR's Comprehensive Biomedical Research Centre scheme.

J.H. is employed as an NIHR Clinical Lecturer.

Jethro A. Herberg and Kelsey D. J. Jones contributed equally.

Address for correspondence: Jethro A. Herberg, PhD, Department of Paediatrics, Wright-Fleming Institute, Imperial College London, Norfolk Place, London W2 1PG, United Kingdom. E-mail: Jethro.Herberg@imperial.ac.uk.

DOI: 10.1097/INF.0b013e3182040c90

Although most children infected by influenza H1N1/09 had mild disease, a small proportion had severe or fatal influenza.[1] We have compared the presentation, risk factors, and severity of illness of H1N1/09 cases with influenza A cases admitted during the past 5 years at a single center.

METHODS

St. Mary's Hospital in Central London provides general and specialist pediatric services, including infectious disease and intensive care, to central and northwest London. Cases of influenza A infection were identified in the hospital's Diagnostic Virology Laboratory database. The inclusion criteria were all children younger than 18 years with a positive test for influenza A virus at the time of sampling, who were admitted to a ward for any reason. The following 2 cohorts were defined on the basis of presentation date: the seasonal cohort consisted of children admitted with positive influenza A results (by immunofluorescence or cell culture) between January 8, 2004 and January 4, 2009, and the H1N1/09 cohort consisted of children with confirmed H1N1/09 influenza admitted between January 5, 2009 and January 4, 2010, during which time PCR detection of influenza A strains was available.

Medical records from the relevant admission were reviewed, and the following variables were recorded: demographic details (age at admission and gender); underlying medical conditions; clinical features at presentation including shock (defined as ongoing cardiovascular dysfunction despite administration of ≥40 mL/kg of intravenous fluid),[2] neutrophil and lymphocyte counts, and C-reactive protein (CRP) at admission; and bacteriology results. For patients admitted to the pediatric intensive care unit (PICU), severity was assigned using the number of PICU-free days at day 28, an established scoring system for ICU patients that allows comparison of children with different clinical presentations, and gives due weight to children who do not survive or have protracted PICU stays. For those cases not requiring intensive care, the length of stay was recorded. Data from the 2 cohorts were compared using Mann-Whitney *U* and Fisher exact tests as appropriate within SPSS (SPSS Inc, Chicago, IL).

RESULTS

A total of 61 children were admitted with influenza A H1N1/09 infection between May 2009 and April 2010, compared with a mean of 13 children (range, 10–18; total 66 cases) admitted each year with seasonal influenza A during the previous 5 influenza seasons. Of all, 18 H1N1/09 cases and 26 seasonal cases were admitted to the PICU (Table 1).

Demographics. Patients admitted with H1N1/09 were older, with a median age of 55 months (quartiles, 10–108), compared with 18 months (5–39) for those admitted with seasonal influenza ($P = 0.002$). The age discrepancy was most pronounced in the milder cases; the median age of children admitted to the general ward was 74 months (11–149) compared with 10 months (3–33) for H1N1/09 and seasonal cohorts ($P = 0.0001$). In contrast, the median ages of children admitted to PICU were similar, that is, 27 (10–60) and 26 months (18–58) for the H1N1/09 and the seasonal cohorts, respectively. Fifty-six percent of the H1N1/09 cohort were female compared with 44% of the seasonal group ($P = 0.284$).

Risk Factors for Admission. There was no significant difference between seasonal and H1N1/09 cohorts in the proportion of children with underlying medical problems known to predispose to severe infection, irrespective of ward admission (46% of H1N1/09, 53% of seasonal), or PICU admission (28% and

TABLE 1. Comparison of Children in H1N1/09 Cohort and Seasonal Influenza A Cohort Who Required Admission to PICU

	H1N1/09 Cohort	Seasonal Cohort	*P*
No. cases	18	26	—
Median age in months (quartiles)	27 (10–60)	26 (18–58)	NS
Male sex (%)	8 (44)	16 (61)	NS
Risk factors (%)			
None	9 (50)	11 (42)	NS
Neurodevelopmental	8 (44)	9 (35)	NS
Respiratory	3 (17)	4 (15)	NS
Presentation			
Lower respiratory tract infection	11 (61)	12 (46)	NS
Neurologic	4 (22)	15 (58)	0.030
Refractory shock	13 (72)	9 (35)	0.031
Median PICU-free days at day 28 (quartiles)	12.5 (0–21)	24 (20–26)	0.005
Median length of stay on PICU (survivors)	7.5 (3–16)	3 (2–6)	NS
Mortality (%)	6 (33)	2 (8)	0.048

PICU indicates pediatric intensive care unit.

35%, respectively). The most common risk factor in both groups was underlying neurologic or developmental disease, including epilepsy.

Clinical Features. In both groups, the most common presenting symptoms were of respiratory tract infection with other features typical of an influenza-like illness. In the seasonal cohort, 23% of children had a primarily neurologic presentation with seizures or status epilepticus, the most frequent reason for PICU admission, compared with 5% of those in the H1N1/09 cohort ($P = 0.004$). For the H1N1/09 cohort, the most common reason for admission to PICU was shock present in 72% of PICU admissions compared with 35% of seasonal PICU cases ($P = 0.002$).

Lymphocyte and Neutrophil Counts, CRP. There was no significant difference in neutrophil count, lymphocyte count, or CRP at presentation between the 2 cohorts. Lymphopenia was a common finding, with counts below the 10th centile for age in 68% of both cohorts. Median (quartile) neutrophil counts for H1N1/09 and seasonal influenza are as follows: 5.5 (2.4–9.1) and 4.7 (3.1–7.5) $\times$ 10^9 cells/L, respectively; lymphocyte counts, 1.9 (1.1–3.0) and 1.9 (0.9–4.0) $\times$ 10^9 cells/L, respectively; and CRP, 20 (6–53) and 12 (3–28) mg/L, respectively.

Microbiology. The incidence of microbiologically confirmed secondary bacterial infection was low. In each cohort, there were 3 patients with clinically significant bacteria detected at a sterile site (4.9% of H1N1 cases, 4.5% of seasonal cases). These were *Staphylococcus aureus* in blood, *Escherichia coli* in urine, and *Streptococcus pyogenes* in postmortem spleen in the H1N1 group. In the seasonal group, these were *Streptococcus pneumoniae*, *S. pyogenes,* and *Salmonella typhi* in blood cultures. Of these 6 children, 1 H1N1/09 and 2 seasonal influenza patients presented with shock.

Severity. The median length of stay for patients not requiring PICU was the same in both groups (3 days), but patients requiring PICU due to H1N1/09 had a longer length of stay (for survivors), and among all admissions, the number of PICU-free days at day 28 was significantly lower (Table 1). The proportion of deaths in children admitted to the PICU was higher in the H1N1/09 group (Table 1). In the H1N1/09 cohort, all children who died presented with shock. Of these children, 5 had ARDS at the time of death. Table 2 summarizes the clinical findings in the 8 patients who died. The total number of PICU bed-days was 202 and 219 for the H1N1/09 and seasonal cohorts, respectively.

DISCUSSION

Our analysis from a single center demonstrates that H1N1/09 causes a severe illness in a small number of children, which differs from severe seasonal influenza. Among children with severe disease (those admitted to PICU), children with seasonal influenza were more likely to present with neurologic symptoms, whereas children with H1N1/09 often presented with shock. This is not explained by age difference or frequency of underlying medical condition, and appears to represent a novel pathologic feature of the H1N1/09 strain. A high incidence of shock at presentation has been described in other cohorts of H1N1/09 PICU patients,[1,3] but ours is the first study directly comparing H1N1/09 with seasonal influenza characteristics in a single center.

In addition, outcome was worse in the H1N1/09 group with severe disease, with longer admission times and increased mortality. The proportion of deaths at our center (33%) lies between Canadian (7%) and Argentinian (39%) figures for H1N1/09 deaths in PICU.[3,4]

Children with milder illness (admitted to the general pediatric ward) were older in the H1N1/09 group. We suggest that this reflects the higher susceptibility of older children to H1N1/09 than to influenza strains that were circulating in the previous years. This hypothesis is supported by a UK prevalence study which found that the highest rates of seroconversion (up to 40%) in the first wave of H1N1/09 were in older children (aged 5–14 years).[5]

H1N1/09 and seasonal cohorts were similar in other respects, including risk factors for disease, length of stay in non-PICU cases, and the age of those admitted to PICU. Neurodevelopmental delay has previously been cited as a specific risk factor for severe H1N1/09 disease,[6] but we found no significant difference between H1N1/09 and seasonal cohorts among children admitted to PICU. Lymphopenia has been noted in H1N1/09 and previous influenza A outbreaks,[7] and the prevalence of lymphopenia was equal in our H1N1/09 and seasonal cohorts, suggesting a species- rather than strain-dependent effect.

TABLE 2. Summary of Clinical Findings in Children Who Died With Seasonal or H1N1/09 Influenza

Influenza, Age, Sex	Comorbidity	Presentation	Microbiology	LOS Days	Progress
Seasonal, 15 mo, male	Prematurity, short gut syndrome, neurodevelopmental delay, home oxygen	Respiratory distress	No positive bacteriology	71	Poor response to escalating respiratory support
Seasonal, 4 mo, male	Ter Harr syndrome, neurodevelopmental delay, home oxygen	Respiratory distress, apneas	No positive bacteriology	12	Poor response to escalating respiratory support
H1N1/09, 7.5 yr, female	Cerebral palsy, epilepsy, hydrocephalus	Pneumonia	No positive bacteriology	16	Worsening ARDS, rising CRP, shock
H1N1/09, 10 mo, male	Prematurity, chronic lung disease, home oxygen	Pneumonia, increased oxygen requirement	No positive microbiology	10	Refractory shock and pulmonary hypertension
H1N1/09, 7 yr, female	None	Abdominal pain, fever	Group A strep detected at postmortem	1	Refractory shock
H1N1/09, 5 yr, female	None	Seizures, coryza, diarrhea	No positive bacteriology	9	Pulmonary hemorrhage, shock, ARDS
H1N1/09, 5.5 yr, male	Cerebral palsy, epilepsy, recurrent chest infections	Respiratory failure	No positive bacteriology	20	ARDS, shock, poor response to escalating respiratory support
H1N1/09, 1.6 yr, male	Lissencephaly	Respiratory failure	No positive bacteriology	37	Prolonged ventilation, with shock

CRP indicates C-reactive protein; LOS, length of stay.

Evidence from past pandemics highlights the importance of secondary bacterial infection in severe disease, particularly with Gram-positive organisms.[8] We found low but similar rates of confirmed infection in both seasonal and H1N1/09 groups. We recognized that diagnosing invasive bacterial disease or pneumonia on the basis of positive blood culture has low sensitivity. However, clinical features indicative of bacterial disease (including elevated CRP or focal consolidation on chest radiography) have not been validated in patients with H1N1/09 disease.

The geographic variation in the reported incidence and severity of H1N1/09 influenza complicates the comparison of severity data between studies. A strength of our study is that it compares cohorts cared for at the same center. Limitations include the expected difference in sensitivity of the diagnostic methods used in each cohort (immunofluorescence and culture in previous years, compared with PCR during the H1N1/09 pandemic). Our analysis considers children admitted to the hospital. Influenza was also detected in children with mild illness who were seen in the emergency department but did not require admission—47 and 7 children during the pandemic H1N1/09 and seasonal periods, respectively. The higher number of H1N1/09 cases in this group reflects both expected increased assay sensitivity and increased testing during the first wave of the pandemic. However, increased diagnostic capture over a range of severities suggests that PCR-based diagnostics are not disproportionately sensitive in diagnosing the most severe cases. The finding of increased disease severity in children admitted to PICU with H1N1/09 does not, therefore, reflect an ascertainment bias in this group. In summary, our data demonstrate that H1N1/09 causes a devastating illness in a small number of children; compared with previous seasonal influenza, children with H1N1/09 requiring intensive care were more likely to present with shock, and more likely to die.

ACKNOWLEDGMENTS

The authors are grateful to Simon Nadel for critical review of the manuscript.

REFERENCES

1. Lister P, Reynolds F, Parslow R, et al. Swine-origin influenza virus H1N1, seasonal influenza virus, and critical illness in children. *Lancet*. 2009;374: 605–607.
2. Goldstein B, Giroir B, Randolph A. International pediatric sepsis consensus conference: definitions for sepsis and organ dysfunction in pediatrics. *Pediatr Crit Care Med*. 2005;6:2–8.
3. Farias JA, Fernández A, Monteverde E, et al. Critically ill infants and children with influenza A (H1N1) in pediatric intensive care units in Argentina. *Intensive Care Med*. 2010;36:1015–1022.
4. Jouvet P, Hutchison J, Pinto R, et al. Critical illness in children with influenza A/pH1N1 2009 infection in Canada. *Pediatr Crit Care Med*. 2010;11:603–609.
5. Miller E, Hoschler K, Hardelid P, et al. Incidence of 2009 pandemic influenza A H1N1 infection in England: a cross-sectional serological study. *Lancet*. 2010;375:1100–1108.
6. Centers for Disease Control and Prevention. Surveillance for pediatric deaths associated with 2009 pandemic influenza A (H1N1) virus infection—United States, April-August 2009. *Morb Mortal Wkly Rep.* 2009;58:941–947.
7. Cunha BA, Pherez FM, Schoch P. Diagnostic importance of relative lymphopenia as a marker of swine influenza (H1N1) in adults. *Clin Infect Dis*. 2009;49:1454–1456.
8. Brundage J, Shanks GD. Deaths from bacterial pneumonia during 1918–19 influenza pandemic. *Emerg Infect Dis*. 2008;14:1193–1199.

THE SPECTRUM OF PARVOVIRUS B19 INFECTION IN A PEDIATRIC HEMATO-ONCOLOGIC WARD

Herwig Lackner, MD, Petra Sovinz, MD,* Martin Benesch, MD,* Stephan W. Aberle, MD,† Wolfgang Schwinger, MD,* Sandrin Schmidt, MD,* Volker Strenger, MD,* Sonja Pliemitscher, MD,* and Christian Urban, MD**

Abstract: Of 1059 children, 35 children with various hemato-oncologic diseases were diagnosed with parvovirus B19 infection. The clinical spectrum included 11 immunocompromised patients presenting with prolonged pancytopenia, 7 patients with delayed hematologic recovery after stem cell transplantation, 5 patients with parvovirus B19 as possible cause of severe aplastic anemia or myelodysplastic syndrome, and 12 children with hemolytic anemia and transient aplastic crisis.

Key Words: parvovirus, immunosuppression, transplantation, children

Accepted for publication October 11, 2010.

From the *Division of Pediatric Hematology and Oncology, Medical University of Graz, Graz, Austria; and †Department of Virology, Medical University of Vienna, Vienna, Austria.

Address for correspondence: Herwig Lackner, MD, Division of Pediatric Hematology and Oncology, Medical University of Graz, Graz 8036, Austria. E-mail: herwig.lackner@medunigraz.at.

Supplemental digital content is available for this article. Direct URL citations appear in the printed text and are provided in the HTML and PDF versions of this article on the journal's Web site (www.pidj.com).

DOI: 10.1097/INF.0b013e3182014958

In immunocompromised hosts, the lack of efficient immune response may obscure the clinical manifestations of parvovirus B19 (PVB19) illness. The infection can cause persistent bone marrow suppression resulting in severe cytopenias that can be misinterpreted as relapse of the underlying malignancy or drug toxicity.[1] Because these patients might fail to develop an antibody response, the diagnosis of PVB19 infection is consequently on the basis of the direct demonstration of the virus genome by polymerase chain reaction (PCR).[2,3] The aim of the present retrospective study was to describe the wide spectrum of clinical PVB19 manifestations observed during the past 2 decades on pediatric hemato-oncologic ward and to intensify the degree of suspicion for this complication in physicians treating children with hemato-oncologic diseases.

MATERIALS AND METHODS

Study Subjects. Between January 1990 and December 2009, 1059 consecutive children with various hematologic (n = 66) and oncologic (n = 993) diseases were admitted at the Division of Pediatric Hematology and Oncology of the Medical University of Graz, Austria. All patients with malignancies were treated according to the current multicenter treatment protocols used in Germany and Austria. Supportive care included insertion of a central venous line, infection prophylaxis with trimethoprim/sulfamethoxazole, oral amphotericin B, and broad-spectrum antibiotics, if necessary.[4] Patients with malignant diseases were screened for PVB19 by PCR in blood or tissue samples weekly during intensive chemotherapy and additionally, when there was clinical suspicion of infection (eg, unexplained fever or pancytopenia). In patients with nonmalignant hematologic disorders, the diagnosis of PVB19 infection was made by detection of PVB19-specific antibodies. Treatment of PVB19 infection in immunosuppressed patients included intravenous immunoglobulins (IVIG), immunocompetent patients with aplastic crisis were treated with erythrocyte transfusions.[1]

During the period of observation, PVB19 infection was diagnosed in 35 patients who are the subjects of the present study.

A written informed consent was obtained from all patients or their legal guardians. Their medical records were carefully reviewed, and they were classified into 4 groups. Group I included 11 immunocompromised patients developing PVB19 infection during treatment of various malignancies. Group II included 7 patients with PVB19 infection complicating the clinical course after stem cell transplantation (SCT). Group III included 5 patients with PVB19 as possible causative agent of severe aplastic anemia (SAA) or myelodysplastic syndrome (MDS). Group IV included 12 patients with hereditary hemolytic anemia and transient aplastic crisis.

Testing for PVB19. For the TaqMan-based PVB19 real-time PCR, 5 μL of DNA extracted with QIAamp Viral RNA Mini Kit (Qiagen, Hilden, Germany) were added to the amplification mixture, using TaqMan universal PCR master mix (PE Applied Biosystems), yielding a total volume of 25 μL. The cycling conditions were 3 minutes at 50°C, 10 minutes at 95°C followed by 45 cycles of 95°C for 15 seconds, 55°C, and 72°C each for 30 seconds. The PVB19 primers and probe are located in the VP2 gene and described by Knöll et al.[5] For the detection of PVB19 IgG and IgM antibodies, the commercial ELISA recomWell PVB19 (Mikrogen, Neuried, Germany) was used.

RESULTS

PVB19 Infection in Immunocompromised Patients (Group I). PVB19 infection was diagnosed in 11 patients (6 females, 5 males) undergoing therapy for various malignancies (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A656), which illustrates the patient characteristics and clinical course of these patients. Oncologic diagnoses included acute lymphoblastic leukemia (ALL) (n = 6), acute myelogenous leukemia (AML) (n = 3), non-Hodgkin lymphoma (n = 1), and Ewing sarcoma (n = 1). The clinical manifestations of PVB19 infection were prolonged myelodepression in 7 of 11 patients, pure red cell aplasia (PRCA) in 2 of 11 patients, and hemophagocytic lymphohistiocytosis (HLH) in 1 patient. A maculopapular rash was seen in 2 patients. One patient showed positive PCR for PVB19 without any clinical symptoms. In 10 of 11 patients, PVB19 DNA was detected in blood and/or bone marrow, in the remaining 1 patient (R.T.) presenting with myelodepression and rash, positive PCR was found by biopsy of a skin lesion. All patients were treated with IVIG, median duration of symptoms was 28 days. In 7 of 11 patients, chemotherapy had to be discontinued for a median duration of 12 days. Ten patients survived, 1 patient (P.B.) developed HLH during maintenance therapy for AML that was possibly triggered by multiple viruses including PVB19, adenovirus, and Epstein-Barr virus. Despite multimodal therapy according to the HLH treatment protocol combined with IVIG, the clinical situation deteriorated, and the patient died 131 days after first manifestation of HLH.

PVB19 Infection in Patients After SCT (Group II). In 7 patients (5 males, 2 females), PVB19 was complicating the clinical course after allogeneic SCT (Table, Supplemental Digital Content 2, http://links.lww.com/INF/A657), which illustrates the patient characteristics, transplant data, and clinical course of these patients. The underlying malignancies were ALL (n = 4), AML (n = 2), and Hodgkin disease (n = 1). Grafts were obtained from matched sibling donors (n = 3), haploidentical parents (n = 3), and matched unrelated donors (n = 1). Conditioning regimens included fludarabine and melphalan in 4 patients; busulfan, cyclophosphamide, and melphalan in 1 patient; and total body irradiation and Vepesid in 2 patients. PVB19 was detected in peripheral blood (n = 5), bone marrow (n = 5), mucosa of the gut (n = 3), and liver (n = 1). Median day of first detection was day 60 after SCT (range: 0–226 days). In all patients, at least 1 additional virus was identified during regular screening for virus infections. In 6 of 7 patients, SCT was complicated by acute and/or chronic graft versus host disease with subsequent need of intensified immunosuppression. All patients achieved engraftment, and median engraftment time of neutrophils was day +12. However, 6 patients subsequently developed poor graft function, and the median complete hematologic recovery (defined as normalization of blood cell counts) was day +127 (range: 54–482), and the median immunologic recovery (defined as normalization of immunoglobulins and B-T-cell counts) was day 597 (range: 114–1038). The remaining seventh patient (patient PV) developed myocarditis with concomitant positivity of PVB19 in peripheral blood ($>10 \times 10^{10}$ copies/mL) on day +613 after haploidentical SCT for relapsed Hodgkin disease when she was still immunocompromised.

PVB19 Infection in Patients With Aplastic Anemia or MDS (Group III). In 5 patients (3 males and 2 females; median age, 12 years), PVB19 was detected serologically and by PCR at diagnosis of SAA (n = 3) or hypoplastic MDS (n = 2). The 3 patients with SAA achieved remission after uncomplicated allogeneic SCT. The 2 patients with hypoplastic MDS had both negative cytogenetics for MDS and were treated initially with IVIG without success. Subsequently, they underwent allogeneic SCT (peripheral SCT and bone marrow transplantation, respectively) with a post-transplantation course complicated by poor graft function and delayed hematologic engraftment. Both patients are now in complete hematologic remission with persisting positivity of PVB19 PCR in peripheral blood and bone marrow.

PVB19 Infection in Patients With Hereditary Hemolytic Anemia (Group IV). Twelve patients (7 females, 5 males, median age 17 years) with spherocytosis (n = 11) or sickle cell anemia (n = 1), respectively, developed transient aplastic crisis during PVB19 infection. Diagnosis of PVB19 infection was based on positivity of PVB19 IgM and IgG in serum, and the median hemoglobin values were 5.3 mg/dL (range: 3.5–8.1). All patients were treated with erythrocyte transfusions and subsequently with substitution of folic acid, and all patients recovered within 2 weeks. In 2 patients, aplastic crisis was the first clinical manifestation of the underlying hereditary hemolytic anemia, thus posing some diagnostic challenge.

DISCUSSION

Since its discovery in 1974, PVB19 has been associated with a wide spectrum of clinical manifestations, the outcome of which depends on the immune response against the virus.[1] Immunocompromised patients were shown to be at increased risk of PVB19 infection.[6,7] These patients can acquire PVB19 infection either as nosocomial infection or from endogenous reactivation of latent virus. In addition, transfusions of blood products are also reported as possible routes of infection.[7] Because of impaired immune response, the diagnosis may be difficult in immunocompromised patients when only antibody levels are measured, and determination of PVB19 DNA by PCR appears to be the most appropriate diagnostic protocol for the correct diagnosis of PVB19 infection.[2,3,7] In these patients, PVB19 infection usually presents with nonspecific symptoms including fever and prolonged pancytopenia, and therefore is easily overlooked.[2,6,8] There are several reports describing PVB19 infection in children with different malignant diseases including solid tumors[3,7,8] or leukemias treated with chemotherapy.[9–14] The children presented with nonspecific clinical symptoms such as fever, pallor, fatigue, and pancytopenia mimicking drug-related side effects and/or leukemic relapse. Lindblom et al recently reported on 18 of 117 children with ALL who became PVB19 DNA-positive and cytopenic, leading to significant prolonged interruptions of chemotherapy compared with PVB19

DNA-negative patients (59 vs. 30 days).[13] In our series of 11 immunocompromised children receiving chemotherapy, 7 patients developed pancytopenia and 2 developed PRCA. Treatment with IVIG was initiated immediately resulting in resolution of symptoms after a median of 28 days and a relatively short period of treatment interruption (median, 12 days).

Little is known about PVB19 infection complicating SCT in children. Broliden estimates the overall prevalence of PVB19 infection in children with solid-organ and bone marrow transplantation to be about 1% to 2%.[14] Data on adult transplant recipients show that PVB19 infections may manifest as refractory anemia and/or pancytopenia during the posttransplantation period.[15,16] Our series of 7 children with PVB19 infections complicating the clinical course after SCT is the largest series in the pediatric setting published so far. Our data clearly show that successful SCT in patients with PVB19 infection is possible. In all patients, the posttransplantation course was complicated by graft versus host disease with subsequent need of prolonged immunosuppression, delayed hematologic engraftment, and multiple virus infections in association with PVB19 infection. Therefore, PVB19 alone cannot be considered as the only causative agent responsible for the clinical course.

There are only few reports in the literature that have implicated PVB19 as the possible cause of SAA in immunocompetent children.[17,18] Osaki et al reported the first case of a previously healthy boy who developed SAA following PVB19 infection, and who was successfully treated with immunosuppression.[17] Qian et al described 6 additional children with SAA associated with PVB19 infection.[18] In our series, 3 children with SAA and PVB19 infection underwent successful allogeneic SCT. An interesting observation is the association of PVB19 with MDS that was seen in 2 of our patients. Data in literature describing children with PVB19 infection mimicking MDS are extremely rare.[19–21] Less than 5 children with PVB19 infection and MDS have been reported. Our 2 patients developed typical morphologic appearance of MDS in association with chronic PVB19 infection, however, with negative cytogenetics. After nonresponse to multiple IVIG and prolonged cytopenia, both children underwent allogeneic SCT that was complicated by delayed hematologic engraftment and persisting positivity of PVB19 DNA.

In conclusion, during the past 2 decades we observed a wide spectrum of clinical manifestations of PVB19 infections ranging from unclear and prolonged pancytopenia or PRCA in immunocompromised children, to delayed hematologic engraftment in children after SCT, to PVB19 as possible causative agent in children with SAA or MDS, and to aplastic crisis in patients with underlying hemolytic anemia. Especially in immunocompromised patients, the diagnosis of PVB19 infection might be difficult since clinical symptoms are frequently nonspecific and laboratory diagnosis needs detection of PVB19 by PCR. Physicians treating children with hematooncologic diseases should be aware of this complication. Weekly screening of blood for PVB19 by PCR is recommended in immunocompromised children during intensive chemotherapy as well as after SCT to intervene as early as possible by application of IVIG and to avoid unnecessary interruptions of chemotherapy.

REFERENCES

1. Broliden K, Tolfvenstam T, Norbeck O. Clinical aspects of parvovirus B19 infection. *J Intern Med*. 2006;260:285–304.
2. Florea AV, Ionescu DN, Melhelm MF. Parvovirus B19 infection in the immunocompromised host. *Arch Pathol Lab Med*. 2007;131:799–804.
3. El-Sayed Soliman O, Abd El-Aal Hegazi Hasan M, El-Ashri R, et al. Parvovirus B19 infection in pediatric oncology patients. Diagnostic value of clinical and serologic parameters compared with nested PCR. *J Pediatr Hematol Oncol.* 2009;31:173–176.
4. Lackner H, Sovinz P, Benesch M, et al. Management of brain abscesses in children treated for acute lymphoblastic leukemia. *Pediatr Blood Cancer*. 2009;52:408–411.
5. Knöll A, Louwen F, Kochanowski B, et al. Parvovirus B19 infection in pregnancy: quantitative viral DNA analysis using a kinetic fluorescence detection system (TaqMan PCR). *J Med Virol*. 2002;67:259–266.
6. Sung-Hsin K, Liang-In L, Chee-Jen C, et al. Increased risk of parvovirus B19 infection in young adult cancer patients receiving multiple courses of chemotherapy. *J Clin Microbiol.* 2002;40:3909–3912.
7. Flunker G, Peters A, Wierbitzky S, et al. Persistent parvovirus B19 infections in immunocompromised children. *Med Microbiol Immunol*. 1998;186:189–194.
8. Zaki ME, Hassan SA, Seleim T, et al. Parvovirus B19 infection in children with a variety of hematological disorders. *Hematology*. 2006;11:261–266.
9. Smith MA, Sha NR, Lobel JS, et al. Severe anemia caused by human parvovirus in a leukemia patient on maintenance chemotherapy. *Clin Pediatr*. 1998;27:383–386.
10. McNall RY, Head DR, Razzouk BI. Parvovirus B19 infection in a child with acute lymphoblastic leukemia during induction therapy. *J Pediatr Hematol Oncol*. 2001;23:309–311.
11. Heegaard ED, Schmiegelow K. Serologic study on parvovirus B19 infection in childhood acute lymphoblastic leukemia during chemotherapy: clinical and hematologic implications. *J Pediatr Hematol Oncol*. 2002;24:368–373.
12. Fattet S, Cassinotti P, Popovic MB. Persistent human parvovirus B19 infection in children under maintenance chemotherapy for acute lymphocytic leukemia. *J Pediatr Hematol Oncol*. 2004;26:497–503.
13. Lindblom A, Heyman M, Gustafsson I, et al. Parvovirus B19 infection in children with acute lymphoblastic leukemia is associated with cytopenia resulting in prolonged interruptions of chemotherapy. *Clin Infect Dis*. 2008;46:528–563.
14. Broliden K. Parvovirus B19 infection in pediatric solid-organ and bone marrow transplantation. *Pediatr Transplant*. 2001;5:320–330.
15. Eid AJ, Brown RA, Patel R, et al. Parvovirus B19 infection after transplantation: a review of 98 cases. *Clin Infect Dis*. 2006;43:40–48.
16. Plentz A, Hahn J, Holler E, et al. Long-term parvovirus B19 viraemia associated with pure red cell aplasia after allogeneic bone marrow transplantation. *J Clin Virol*. 2004;31:16–19.
17. Osaki M, Matsubara K, Iwasaki T, et al. Severe aplastic anemia associated with human parvovirus B19 infection in a patient without underlying disease. *Ann Hematol*. 1999;78:83–86.
18. Qian XH, Zhang GC, Jiao XY, et al. Aplastic anaemia associated with parvovirus B19 infection. *Arch Dis Child*. 2002;87:436–437.
19. Hasle H, Kerndrup G, Jacobsen BB, et al. Chronic parvovirus infection mimicking myelodysplastic syndrome in a child with subclinical immunodeficiency. *Am J Pediatr Hematol Oncol*. 1994;16:329–333.
20. Yetgin S, Cetin M, Yenicesu I, et al. Acute parvovirus B19 infection mimicking juvenile myelomonocytic leukemia. *Eur J Haematol*. 2000;65:276–278.
21. Yarali N, Duru F, Sipahi T, et al. Parvovirus B19 infection reminiscent of myelodysplastic syndrome in three children with chronic haemolytic anemia. *Pediatr Hematol Oncol*. 2000;17:475–482.

HOSPITALIZATIONS DUE TO RESPIRATORY SYNCYTIAL VIRUS IN CHILDREN WITH CONGENITAL MALFORMATIONS

Philip Zachariah, MB BS, Margaret Ruttenber, MSPH,† and Eric A. F. Simões, MB BS, DCH, MD**

Abstract: Statewide respiratory syncytial virus (RSV) lower respiratory tract infection hospitalization data of Colorado children with congenital malformations was used to estimate the population-based risk and severity of disease of RSV hospitalizations. Spina bifida without anencephaly, cleft palate, lung agenesis or dysgenesis, and biliary atresia were associated with a higher risk of being hospitalized with RSV lower respiratory tract infection and an increased severity of disease when hospitalized.

Key Words: respiratory syncytial virus, congenital malformations, respiratory virus

Accepted for publication October 13, 2010.

From the *Section of Infectious Diseases, Department of Pediatrics, University of Colorado Denver, and The Children's Hospital, Denver, CO; †Colorado Responds to Children with Special Needs, Colorado Department of Public Health and Environment, Denver, CO.

Supported by a research grant from MedImmune.

Research support and honoraria from MedImmune and Abbott Inc (to E.A.F.S.).

Address for correspondence: Eric A. F. Simoes, MB BS, DCH, MD, Section of Pediatric Infectious Diseases, The Children's Hospital, 13123 East 16th Ave, Box B055, Aurora, CO 80045. E-mail: eric.simoes@ucdenver.edu.

DOI: 10.1097/INF.0b013e318201813b

Respiratory syncytial virus (RSV) is a cause of significant morbidity[1] in infants and young children. Prematurity,[2] chronic lung disease (CLD),[3] congenital heart disease (CHD),[4] immunodeficiency,[5] neuromuscular impairment,[6] and Down syndrome[7] have been associated with an increased risk of being hospitalized with RSV lower respiratory tract infection (LRTI). Children with major congenital malformations are known to have significant comorbidities and frequent hospitalizations. This study examines the population-based risk of being hospitalized with RSV LRTI and severity of disease when hospitalized in children with representative congenital malformations.

METHODS

Data Sources. A population-based retrospective cohort study design was used with data obtained from the Colorado Responds to Children with Special Needs (CRCSN) Program of the Colorado Department of Public Health and Environment (CDPHE), the Colorado Health and Hospital Association (CHA), and the Annual Colorado state census.

The CHA provided RSV hospitalization data for children and adolescents younger than 21 years of age in Colorado for the years 1995–2006. This database, used by us previously,[8,9] records hospital discharge data from 76 of 88 hospitals in Colorado. Of the 12 hospitals excluded from the CHA database, only 3 hospitals admit children <5 years of age, 2 in rural areas, and the other usually transports children to a larger facility. Records identifying a Colorado zip code and RSV-associated International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) codes 079.6 (RSV), 466.11 (acute bronchiolitis due to RSV) and 480.1 (RSV pneumonia) in the first 7 of 15 potential diagnostic fields, were used to indicate a RSV LRTI hospitalization.[8,9] Congenital malformations, risk factors for RSV LRTI (CHD, CLD, pulmonary hypertension, prematurity, neuromuscular disease, immunodeficiency), and severity (need for mechanical ventilation) of an RSV LRTI episode were identified by specific ICD-9 codes.

Colorado Responds to Children with Special Needs (CRCSN), Colorado's birth defects monitoring and surveillance program, maintains a database of young children with birth defects, developmental disabilities and risks for developmental delay provided data on number of children diagnosed with representative congenital malformations born between 1997 and 2004. This database has a 98% coverage rate (available at: http://www.cdphe.state.co.us./cohid/crcsndata.html). Birth data for the same years for children without malformation was obtained from the Colorado annual state census. The Colorado Multiple Institutional Review Board approved this study.

Calculation of Population-based Rate Ratios–Birth Cohort Study. Hospitalizations in the malformation cohort were stratified based on the presence or absence of known clinical risk factors for RSV (CHD, CLD, prematurity, pulmonary hypertension, neuromuscular disease, and immunodeficiency) recorded in any of the 15 diagnostic fields. Only hospitalizations without additional risk factors were used in the analysis.

The state-wide average rate for RSV LRTI hospitalization per child year for each malformation was estimated by combining birth data from CRCSN during the years 1997 to 2004 with a specific congenital malformation (denominators), with the number of RSV hospitalizations (without risk factors for RSV) in the first 2 years of life, for the corresponding birth cohort from the CHA database (numerators). The 2-year time period was chosen since the risk of severe RSV is highest in children younger than 2 years of age, and to limit chances of migration out of the state. Infant and child deaths in the first 2 years of life were accounted for while calculating the child-years denominator. For the cohort without malformations, birth data for children during the same time period from the Colorado state census was combined with RSV hospitalization data for the first 2 years of life.

Comparison of Severity and Course of Hospitalization: Population-based Retrospective Cohort. All nonduplicate hospitalization episodes for children from 1995 to 2006 from the CHA database were used to compare the severity of disease in the 2 cohorts. The 3M All Patient Refined Diagnosis-Related Groups (APR DRG) severity coding is an extension of the basic DRG with 4 severity-of-illness levels within each DRG assigned according to a clinical logic that simultaneously evaluates the interactions of multiple comorbidities, age, procedures, and principal diagnosis. An APR DRG severity of >2 (more than moderate severity) was used as an indicator of severe disease.

Statistical Analysis. All analyses were performed using SAS Version 8.2, (SAS Institute Inc., Cary, NC). Confidence intervals at the 95% level were calculated for rate ratios. The χ^2 test and Fisher exact test were used to compare outcomes of interest between categorical data. Differences in duration of hospitalization for RSV LRTI among children were analyzed using analysis of variance with a Bonferroni correction. A $P \leq 0.05$ was considered significant.

RESULTS

Hospitalizations With RSV LRTI in Colorado. There were 516,831 children born in Colorado during the years 1997–2004. In this birth cohort, there were 12,522 total hospitalizations with RSV LRTI within the first 2 years of life (12.12 hospitalizations per 1000 child years). During the same time period, there were 3417 live births born with representative major congenital malformations (0.7%) selected for the study. In this subgroup, there were 77 hospitalizations for RSV LRTI in the first 2 years of life, with 65 of these having no risk factors for RSV LRTI in any of the 15 discharge fields.

Population-based Risk Estimates for Hospitalization With RSV LRTI. When compared with the cohort without malformations, children with spina bifida without anencephaly (RR: 2.73, 95% CI: 1.99–3.74), agenesis, hypoplasia, or dysplasia of the lung (RR: 1.42, 95% CI: 1.02–1.98), cleft palate alone (RR: 1.42, 95% CI: 1.02–1.98), and biliary atresia (RR: 3.4, 95% CI: 2.2–5.4) had statistically higher risks of being hospitalized with RSV LRTI in the first 2 years of life (Table 1). Microcephaly, anomalies of the diaphragm, and choanal atresia were not associated with an elevated risk, whereas hypertrophic pyloric stenosis and cleft lip were associated with a lower risk of being hospitalized with RSV LRTI.

Severity of Hospitalizations With RSV LRTI. In comparing severity of hospitalizations with RSV LRTI between 2 cohorts, a majority of the congenital malformations studied were associated with at least 1 indicator of increased severity of disease (increased median duration of stay, severity of >2/4 on the APR-DRG score, or an increased requirement for mechanical ventilation). Congenital hy-

TABLE 1. RSV LRTI Hospitalizations in Each Birth Cohort With Coexisting Congenital Malformations in the First 2 Years of Life and the Rate Ratio When Compared to the Non-CM Cohort—With and Without Documented Risk Factors for RSV; and Severity of Disease in Hospitalized Children

Diagnosis	Population-based Estimates						RSV Hospitalization Severity		
	ICD-9 Code	Birth Cohort	RSV (Total)	RSV (No Risk Factors)	RR	95% CI	Median Duration of Stay	Severity >2/4 on APR-DRG Score	Mechanical Ventilation
Central nervous system anomalies									
Microcephaly	742.1	278	4	4	0.60	0.37–1.00	5	7/15**	1/15
Spina bifida without anencephaly	741.0 or 741.9 without 740.0, 740.1, or 740.2	142	12	11	2.73	1.99–3.74	3	6/13**	3/13**
Congenital hydrocephalus without spina bifida	742.3, without 741.0	376	10	7	0.83	0.57–1.21	5**	7/15**	1/15
Genitourinary anomalies									
Renal agenesis and dysgenesis	753.0	196	3	3	0.54	0.31–0.97	4	2/8	0/8
Respiratory anomalies									
Choanal atresia	748.0	83	3	2	1.15	0.57–2.32	3**	2/5*	2/5**
Anomalies of the diaphragm	756.6	188	4	3	1.15	0.70–1.90	2	5/7**	1/7
Agenesis, hypoplasia, dysplasia of the lung	748.5	253	9	6	1.42	1.02–1.98	4**	14/15**	3/15**
Orofacial anomalies									
Cleft lip with or without cleft palate	749.1, 749.2	529	7	5	0.49	0.33–0.71	5**	3/9*	2/9**
Cleft palate	749.0	426	16	16	1.79	1.40–2.30	6**	28/43**	13/43**
Gastrointestinal anomalies									
Congenital hypertrophic pyloric stenosis	750.5	880	3	3	0.14	0.1–0.2	5	0/6	1/6
Biliary atresia	751.61	66	6	5	3.4	2.2–5.4	3	4/6**	0/6

ICD-9 indicates International Classification of Diseases, Ninth Revision; CM, clinical modification; RSV, respiratory syncytial virus; RR, risk ratio; CI, confidence interval; APR-DRG, All Patient Refined Diagnosis-Related Groups; LRTI, lower respiratory tract infection.
*$P < 0.05$; **$P < 0.001$.

drocephalus without spina bifida, choanal atresia, agenesis, hypoplasia, dysgenesis of the lung, and cleft lip/palate were associated with a longer median duration of stay and a higher rate of mechanical ventilation during the duration of hospital stay ($P < 0.05$). All the congenital malformations other than renal agenesis/dysgenesis and hypertrophic pyloric stenosis were associated with increased severity using the APR-DRG score ($P < 0.05$).

DISCUSSION

Our study shows that children with certain congenital malformations have an elevated risk of being hospitalized with RSV LRTI in the first 2 years of life and also an increased severity of disease when hospitalized even in the absence of other known risk factors for RSV LRTI. Though cardiac malformations are an established risk factor for RSV LRTI, an elevated risk for RSV LRTI hospitalization in infants and children with other congenital malformations has not been reported previously, and, this is the first population-based estimate of risk in this group.

There are potential mechanisms that could contribute to the risk of RSV LRTI in these children. Though hospitalizations with coexisting risk factors for RSV LRTI including congenital heart disease were excluded from the analysis, children with certain malformations (congenital diaphragmatic hernia and pulmonary malformations) could have altered pulmonary circulation dynamics and anatomy, which while not qualifying them for a diagnosis of chronic lung disease or pulmonary hypertension could still make them more susceptible to severe disease.

Though we tried to limit the role of nosocomial infections by using only admissions where RSV was included in the first 7 of 15 diagnostic codes, children with congenital malformations could be exposed to risk of infection through frequent emergency room visits, and specialized medical day care settings. Another predisposing factor could be failure to thrive, which commonly occurs in this group as a result of impaired swallowing (cleft palate), frequent hospitalizations, and surgery (spina bifida, cleft palate, biliary atresia). Craniofacial anomalies including cleft palate can be associated with impaired T-cell immunity as part of DiGeorge syndrome. Malformations such as cleft lip and pyloric stenosis are corrected surgically within the first year of life and might not have effects on upper or lower respiratory tract physiology.

However, since the time of surgery was not available from the database, the 2-year follow-up period could be an overestimation, which could possibly explain the "lower" risk seen in the study. Another explanation could be that corrective surgery could take into account correction of gastroesophageal reflux, a potential risk factor for RSV LRTI.[10]

This report has limitations. Children with congenital malformation may have a lower threshold for hospital admission, because of their co morbidities. However, in this study, they also demonstrated more objective evidence of severe disease (need for mechanical ventilation), and the APR-DRG severity codes, which are adjusted for underlying comorbidities. The CRCSN could not provide information on coexisting malformations so the same child-years denominator was used to calculate rate ratios for the subgroups (with and without RSV LRTI risk factors). However, the larger denominator would only underestimate the rate ratio in this cohort. In addition to the constraints of analyzing a large data base, the use of self-reported zip codes is a caveat. Boundaries change and residents being unaware of the change may inadvertently report an old zip. This study is also limited by the use of

ICD-9-CM codes and not laboratory testing for identifying the outcome of interest.

In conclusion, congenital malformations contribute to the risk and severity of RSV LRTI. Larger studies should be done to more closely analyze these subgroups.

ACKNOWLEDGMENT

The authors acknowledge the technical assistance of Dexiang Gao PhD in statistical methods.

REFERENCES

1. Shay DK, Holman RC, Newman RD, et al. Bronchiolitis-associated hospitalizations among US children, 1980–1996. *JAMA*. 1999;282:1440–1446.
2. Cunningham CK, McMillan JA, Gross SJ. Rehospitalization for respiratory illness in infants of less than 32 weeks' gestation. *Pediatrics*. 1991;88:527–532.
3. Groothuis JR, Gutierrez KM, Lauer BA. Respiratory syncytial virus infection in children with bronchopulmonary dysplasia. *Pediatrics*. 1988;82:199–203.
4. MacDonald NE, Hall CB, Suffin SC, et al. Respiratory syncytial viral infection in infants with congenital heart disease. *N Engl J Med*. 1982;307:397–400.
5. Hall CB, Powell KR, MacDonald NE, et al. Respiratory syncytial viral infection in children with compromised immune function. *N Engl J Med*. 1986;315:77–81.
6. Wilkesmann A, Ammann RA, Schildgen O, et al. DSM RSV Ped Study Group. Hospitalized children with respiratory syncytial virus infection and neuromuscular impairment face an increased risk of a complicated course. *Pediatr Infect Dis J*. 2007;26:485–491.
7. Bloemers BL, van Furth AM, Weijerman ME, et al. Down syndrome: a novel risk factor for respiratory syncytial virus bronchiolitis–a prospective birth-cohort study. *Pediatrics*. 2007;120:e1076–e1081.
8. Choudhuri JA, Ogden LG, Ruttenber AJ, et al. Effect of altitude on hospitalizations for respiratory syncytial virus infection. *Pediatrics*. 2006;117:349–56.
9. Zachariah P, Shah S, Gao D, et al. Predictors of the duration of the respiratory syncytial virus season. *Pediatr Infect Dis J*. 2009;28:772–776.
10. Calabrese F, Rizzo S, Giacometti C, et al. High viral frequency in children with gastroesophageal reflux-related chronic respiratory disorders. *Pediatr Pulmonol*. 2008;43:690–696.

COMPLETE ATRIOVENTRICULAR BLOCK AS A COMPLICATION OF VARICELLA IN CHILDREN

Tomohiro Katsuta, MD, Hitoshi Kato, MD, PhD,† Yukihiro Kaneko, MD, PhD,‡ Masahide Kaneko, MD,† Yasushi Misaki, MD,† Mitsutoshi Kimura, MD,‡ Kensuke Shoji, MD,* Hiro Nakao, MD,§ and Akihiko Saitoh, MD, PhD**

Abstract: Although varicella is a benign and self-limited disease in children, serious complications can occur. We herein report a case of a 15-month-old boy who required a permanent pacemaker because of complete atrioventricular block as a complication of varicella. Universal vaccination is warranted to prevent such a potentially fatal complication in Japan where varicella is still endemic.

Key Words: varicella, atrioventricular block, vaccination

Accepted for publication October 11, 2010.

From the Divisions of *Infectious Diseases and †Cardiology, Department of Medical Specialties, National Center for Child Health and Development, Tokyo, Japan; ‡Division of Cardiovascular Surgery, Department of Surgical Specialties, National Center for Child Health and Development, Tokyo, Japan; and §Division of General Pediatrics, Department of Interdisciplinary Medicine, National Center for Child Health and Development, Tokyo, Japan.

Supported by the Grant of National Center for Child Health and Development (21A-2) (to A.S.).

Address for correspondence: Akihiko Saitoh, MD, PhD, FAAP, 2-10-1 Okura, Setagaya-ku, Tokyo 157-0074 Japan. E-mail: saitoh-aki@ncchd.go.jp.

DOI: 10.1097/INF.0b013e3182011286

Although most children with varicella recover from the disease uneventfully, serious complications can sometimes occur. Myocarditis is one of the most critical complications of varicella, and it is associated with common viral infections in children, including coxsackie virus, Epstein-Barr virus, respiratory syncytial virus, mumps virus, etc.[1] In the present study, we report the case of a 15-month-old boy who developed complete atrioventricular block (CAVB) without myocarditis and required a permanent pacemaker as a complication of varicella.

CASE REPORT

A previously healthy 15-month-old boy developed fever up to 38.5°C and several vesicular lesions on his abdomen 1 day before admission. Significant bradycardia (heart rate, 50 beats/min) was noted on physical examination at his primary care doctor's office, and he was immediately transferred to our pediatric intensive care unit for further evaluation and treatment. He had no significant medical or family history related to heart diseases. His recent visit at our hospital was 1 month before admission when he was diagnosed with a febrile seizure and required hospitalization for 2 days, with a normal heart rate and blood pressure during the entire hospitalization. His immunizations were up to date according to the Japanese National Immunization Program (NIP), but he had not received the varicella vaccine because it had not been included in the NIP. He had no history of varicella. Of note, his mother had developed vesicular lesions at her right Th2 dermatome and was diagnosed of having herpes zoster 2 weeks before the onset of his disease, and he had experienced close contact with her through breast-feeding.

On admission, his vital signs were as follows: heart rate, 45/min; respiratory rate, 26/min; body temperature, 36.5°C; and blood pressure, 96/42 mm Hg. His general appearance was good without acute distress. Physical examination was significant for several vesicular lesions over his abdomen. No heart murmur was audible, and S_1 and S_2 were normal. Laboratory findings were within normal ranges; the creatine phosphokinase level was 40 IU/L (normal range, 57–197 IU/L) and the troponin-T level was <0.1 μg/mL (normal range, <0.1 μg/mL). A chest radiograph showed a normal heart silhouette without cardiomegaly. An electrocardiogram was significant for third-degree heart block. An echocardiogram demonstrated normal cardiac contraction (ejection fraction, 62%; normal range, 60%–70%) without pericardial effusion.

The diagnosis of varicella was confirmed by characteristics of the skin lesions and a history of close contact with his mother having herpes zoster. It was also confirmed by the real-time polymerase chain reaction, demonstrating positivity for varicella zoster virus (VZV) DNA in serum. Intravenous acyclovir was started (30 mg/kg/d, every 8 hours), and a temporal transvenous pacing system was placed when the diagnosis of CAVB was made. The vesicular lesions with different stages progressed in his trunk within a few days. On day 10 of hospitalization, he started to have his own heart beat, and subsequently the pacing system was removed. Intravenous acyclovir was continued for 10 days. However, he started to have CAVB after the removal of the pacing system, and eventually a permanent pacemaker was replaced on day 12. The patient was subsequently discharged on day 28.

DISCUSSION

CAVB is a very rare medical condition in children, but it is a fatal condition that is frequently associated with sudden death or

TABLE 1. A Summary of Treatment and Clinical Outcomes of Atrioventricular Block Complicated With Varicella Infection

Authors	Age (yr)	Sex	LVEF (%)	CK on Admission (IU/L)	CK-MB Fraction (%)	Cardiomegaly on Chest X-Ray	ECG	Myocarditis	Treatment (Duration)	Requirement of Permanent Pacemaker
Rich and McErlean[5]	6	F	N/A	1726	14	−	CAVB	+	Acyclovir 1500 mg/m^2/d (unknown)	No
Ettedgui et al[6]	1	M	35	N/A	N/A	+	CAVB	+	Acyclovir 15 mg/kg/d q8 h (7 d)	Yes
Nurnberg et al[7]*	9	M	N/A	21	N/A	−	SAVB	−	—	No
Current report	1	M	62	40	10	−	CAVB	−	Acyclovir 30 mg/kg/d q8 h (10 d)	Yes

*Article in German.

LVEF indicates left ventricular ejection fraction; CK, creatine phosphokinase; CK-MB, creatine phosphokinase-myocardial band; ECG, electrocardiogram; CAVB, complete atrioventricular block; SAVB, second degree atrioventricular block; N/A, not available.

near death, and therefore requires urgent evaluation and management. It is usually acquired congenitally or postsurgically, and rarely recognized as a complication of myocarditis associated with various infections.[1,2]

Information regarding the etiologies of myocarditis in children is lacking. One study tried to evaluate the etiologies of myocarditis in 40 children with CAVB[3]; however, the causes were confirmed in only 12 cases (30%); coxsackie B virus (n = 4), respiratory syncytial virus (n = 2), *Mycoplasma pneumoniae* (n = 2), and VZV (n = 1). Among all cases, 11 patients (28%) required a permanent pacemaker and 2 patients (5%) died. Persistent CAVB >1 week was associated with the need for a permanent pacemaker. Furthermore, there are limited data describing myocarditis complicated with varicella. Waagner and Murphy reviewed 18 children with myocarditis complicated with varicella.[4] Although no CAVB was reported, 7 (39%) patients died, 1 (6%) patient experienced persistent cardiac failure, and 2 (11%) patients required a permanent pacemaker, thus demonstrating the poor prognosis of this disease. CAVB associated with acute myocarditis as a complication of varicella has been reported in the previous literature[5–7] (Table 1); however, to our knowledge, there have been no reports in the English literature regarding CAVB without myocarditis complicated with either varicella or other viral infections.

In the current case, the cardiac muscle enzyme levels remained within the normal range, demonstrating no signs of myocarditis. The most probable pathologic mechanism leading to heart block is inflammatory cell infiltration by varicella in the atrioventricular node and/or bundle of His because VZV is known to be a neurotropic virus; however, it is unclear whether direct infection of the virus into the node and/or bundle of His or a viral-induced immunologic process is responsible for the inflammation.

Acyclovir is the treatment of choice for children with complicated varicella; however, the use of acyclovir has a limited window of opportunity to affect the clinical outcome of VZV infection. Successful acyclovir therapy for myocarditis and CAVB associated with varicella has been reported[6]; however, there is still no definitive evidence that acyclovir treatment improves the disease course of varicella-associated CAVB.

Given that treatment options are limited and the efficacy is unknown for the serious disease complications, prevention is the key to avoid the complications of varicella. The varicella vaccine was developed in Japan in 1985[8] and its efficacy has been proven in many countries.[9] Unfortunately, the overall vaccine coverage rate for varicella is only 32% in Japan, because the varicella vaccine is not in the NIP and is classified as a voluntary vaccine, for which caregivers are required to pay out-of-pocket up to US $70 for each injection. In addition, outbreaks of varicella in tertiary hospitals in Japan have been a significant issue, requiring several interventions, including antiviral and immunoglobulin therapy for immunocompromised hosts, vaccinations of the susceptible exposed patients, ward closure, and a significant loss of hospital income.[10] The addition of the varicella vaccine in the NIP is the only way to prevent such a serious complication of varicella in Japan, where varicella is still endemic.

ACKNOWLEDGMENT

Authors acknowledge Dr. Emily Knaup in the Division of Intensive Care at the National Center for Child Health and Development for translating a German article to English and Dr. Tatsuo Kato at the National Center for Child Health and Development for helpful discussion.

REFERENCES

1. Maury P, Chilon T, Dumonteil N, et al. Complete atrioventricular block persisting after regression of infectious myocarditis. *J Electrocardiol*. 2008;41:665–667.
2. Wang JN, Tsai YC, Lee WL, et al. Complete atrioventricular block following myocarditis in children. *Pediatr Cardiol*. 2002;23:518–521.
3. Batra AS, Epstein D, Silka MJ. The clinical course of acquired complete heart block in children with acute myocarditis. *Pediatr Cardiol*. 2003;24:495–497.
4. Waagner DC, Murphy TV. Varicella myocarditis. *Pediatr Infect Dis J*. 1990;9:360–363.
5. Rich R, McErlean M. Complete heart block in a child with varicella. *Am J Emerg Med*. 1993;11:602–605.
6. Ettedgui JA, Ladusans E, Bamford M. Complete heart block as a complication of varicella. *Int J Cardiol*. 1987;14:362–365.
7. Nurnberg JH, Senft G, Sperling P, et al. A delayed life-threatening complication after uneventful varicella infection: transient complete heart block [in German]. *Klin Padiatr*. 2002;214:113–116.
8. Sadzot-Delvaux C, Rentier B, Wutzler P, et al. Varicella vaccination in Japan, South Korea, and Europe. *J Infect Dis*. 2008;197(suppl 2):S185–S190.
9. Marin M, Meissner HC, Seward JF. Varicella prevention in the United States: a review of successes and challenges. *Pediatrics*. 2008;122:e744–e751.
10. Katsuta T, Sugawara Y, Kato T, et al. Bed control simulation based on patient's immune status and past medical history—preparing for varicella outbreak in a pediatric tertiary care hospital in Japan. In: The 8th East Asian Conference on Infection Control and Prevention; November 12–13, 2009; Tokyo, Japan.

ACHALASIA AND *MYCOBACTERIUM GOODII* PULMONARY INFECTION

Daniel Martínez-González, MD, José Franco, PhD,**
*David Navarro-Ortega, PhD,†‡ Carmen Muñoz, PhD,**
Roberto Martí-Obiol, PhD,§ and Rafael Borrás-Salvador, PhD†‡

Abstract: Achalasia is a common adult disorder that rarely manifests in children and infrequently can be associated with pulmonary nontuberculous mycobacteria infections. We describe here the first case of *Mycobacterium goodii* pulmonary infection associated with achalasia in a pediatric patient. Heller myotomy with Dor fundoplication and 12 months of treatment with ciprofloxacin and doxycycline resulted in complete clinical and radiologic improvement.

Key Words: *Mycobacterium goodii*, nontuberculous mycobacteria, achalasia, pulmonary infection

Accepted for publication October 18, 2010.

From the *Servicio de Neumología, Hospital Clínico Universitario, Valencia, Spain; †Servicio de Microbiología, Hospital Clínico Universitario, Valencia, Spain; ‡Sección Departamental de Microbiología, Facultad de Medicina y Odontología, Universidad de Valencia, Valencia, Spain; and §Servicio de Cirugía, Hospital Clínico Universitario, Valencia, Spain.

The authors and their relatives have no conflicts of interest and have received no compensation in any form for the elaboration of this manuscript at any time. There was no external sponsor.

This work was performed at Hospital Clínico Universitario de Valencia, Spain.

Address for correspondence: Daniel Martínez-González, MD, Servicio de Neumología, Hospital Clínico Universitario, Avenida Blasco Ibáñez, 17, PC 46010 Valencia, Spain. E-mail: danimargo@gmail.com.

DOI: 10.1097/INF.0b013e3182024c1c

Esophageal and other swallowing disorders can be associated with pulmonary infections due to nontuberculous mycobacteria (NTM),[1] frequently masquerading as aspiration pneumonia.[2] NTM pulmonary infection in achalasia is almost always associated with rapidly growing mycobacteria (RGM), particularly *Mycobacterium fortuitum-chelonei.* Other rapid growers such as *Mycobacterium smegmatis*[3] and recently *Mycobacterium abscessus*[4] have also been reported in patients with achalasia.

M. goodii is a nonpigmented or late-pigmented RGM, formerly named *M. smegmatis* group 2. This organism is closely related to *M. smegmatis* sensu stricto (formerly *M. smegmatis* group 1) and *Mycobacterium wolinsky* (formerly *M. smegmatis* group 3) which together form a phenetic group named *M. smegmatis* group. Respiratory disease caused by *M. goodii* was reported in 6 of 28 patients (21%) from the initial microbiologic report by Brown et al,[5] of them, 1 patient was a 53-year-old man with achalasia and pulmonary infiltrates. So far, no other report of *M. goodii* associated with achalasia has been published.

We present here another case of a 15-year-old girl, which is to our knowledge, the first case reported of the association between *M. goodii* pulmonary disease and achalasia in a pediatric patient.

CASE REPORT

A 15-year-old white girl presented to the hospital with fever and productive cough for 1 week before admission. She had a history of marked delay in speech and had been treated by a speech therapist. Four years earlier, she was treated for presumed rickettsial infection because of fever of unknown origin and positive serologies for *Rickettsia conorii*. Since then, she had suffered from intermittent fever up to 39.5°C, mainly at night, and had a history of chronic dry cough, which worsened at night, that was attributed to bronchospasm; it led to multiple visits to the emergency room. Allergy tests were negative and pulmonary function tests were normal.

On examination at the time of current admission she appeared ill with normal skin color and turgor. The patient had a fever of 37.5°C and oxygen saturation of 95% while breathing room air. Laboratory investigations revealed only leukocytosis (19.8×10^9 with neutrophils at 79%) and increased C-reactive protein (178.6 mg/L). Cultures from blood and urine were negative. A chest radiograph demonstrated bilateral pulmonary consolidation areas with mediastinal enlargement. The computed tomography scan revealed a ground glass opacity pattern associated with parenchymal consolidation areas with air bronchograms mainly in right upper lobe and left lower lobe (Fig. 1). Severe esophageal dilation with "bird's beak" distal esophagus and bilateral mediastinal lymphadenopathy were seen. These findings suggested achalasia and aspiration pneumonia. Endoscopic examination confirmed the diagnosis of achalasia and esophageal candidiasis was also documented.

The patient could not tolerate bronchoscopy but 2 separate sputum samples were submitted to the microbiology laboratory for bacteriologic study. Acid-fast bacilli were seen in one of them and an RGM was recovered in both the samples from cultures in both Mycobacterial Growth Indicator Tubes (BD BACTEC MGIT 9600) and Lowenstein-Jensen medium (Biomedics, Tres Cantos, Madrid, Spain), which was identified as *M. goodii* by phenetic methods[5] and a reverse hybridization assay (GenoType *Mycobacterium* CM assay; Hain Lifescience GmbH, Nehren, Germany). Antimicrobial susceptibility testing was performed by a microdilution method as previously reported[6] and the results were interpreted

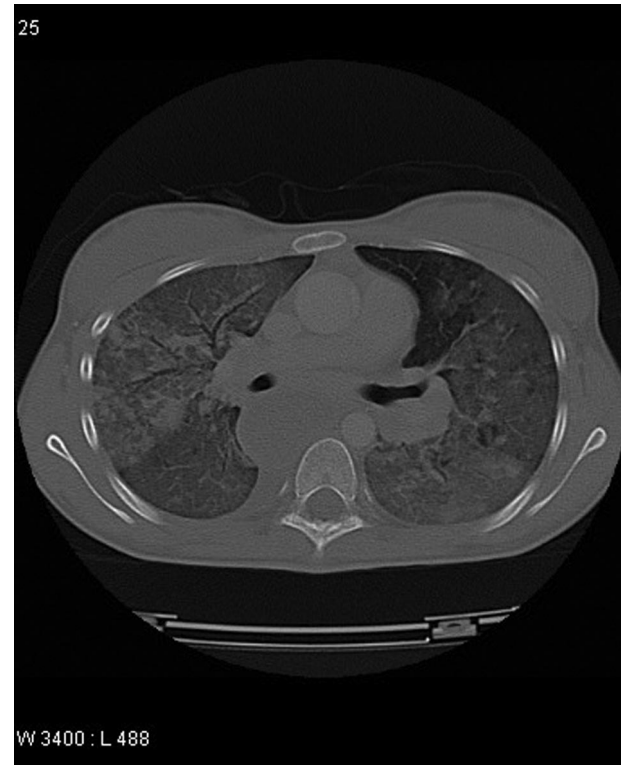

FIGURE 1. Computed tomography scan revealing ground glass opacity pattern associated with parenchymal consolidation areas with air bronchograms mainly in the right upper lobe and the left lower lobe.

following Clinical and Laboratory Standards Institute criteria for RGM.[7] The isolate was susceptible to amikacin (1 mg/L), ciprofloxacin (0.25 mg/L), doxycycline (1 mg/L), ethambutol (1 mg/L), linezolid (2 mg/L), minocycline (1 mg/L), moxifloxacin (0.125 mg/L), tigecycline (0.25 mg/L), and tobramycin (1 mg/L), and resistant to clarithromycin (32 mg/L).

The patient underwent a laparoscopic Heller myotomy with Dor fundoplication for esophageal achalasia. Ciprofloxacin 500 mg twice and doxycycline 100 mg daily were initiated and continued for 12 months. At follow-up examinations, an increase in body weight up to 13 kg and cessation of fever and night-time cough were observed. Mycobacterial cultures were negative after 4 months of treatment and the chest radiograph revealed complete resolution of infiltrates a year after treatment.

DISCUSSION

M. goodii pulmonary disease associated with esophageal achalasia is a rare condition and has not been previously reported in a pediatric patient. *M. goodii* is an RGM that has been involved in both human and animal infections.[8] Although the environment is the most probable reservoir, *M. goodii* has never been isolated from environmental sources. Nosocomial outbreaks caused by *M. goodii* have been reported and demonstrated by clonal analysis. Infections due to NTM are related to local or systemic immunity alterations. Clinical disease caused by *M. goodii* mainly involves postsurgical and post-traumatic infections. A few cases of respiratory disease due to *M. goodii* have been published, mostly associated with underlying exogenous lipoid pneumonia.[8] To date, the association between achalasia and *M. goodii* pulmonary infection has been reported only once, in an adult man.[5]

Achalasia is a common adult disorder that rarely manifests in children. It is a primary esophageal motility disorder characterized by the absence of esophageal peristalsis and lower esophageal sphincter relaxation due to damage to the myenteric plexus. The consequent stasis of food and regurgitation can lead to chronic aspiration, aspiration pneumonia, and occasionally to NTM infection mainly due to RGM. Stasis of food in the esophagus and recurrent aspiration seem to play a primary role in the NTM infection in patients with esophageal dysmotility.

NTM pulmonary infection involving lipoid pneumonia or achalasia is rare and clinical symptoms are nonspecific and reflect the type and extent of disease, the underlying condition, and comorbidities. Chronic cough is common, whereas fever and sweats occur less frequently; hemoptysis and systemic symptoms suggest advanced disease. Patients with achalasia seem to have a radiographic appearance which differs from that of other patients with pulmonary NTM infection. As in the case presented here, patchy bilateral infiltrates that resemble aspiration pneumonia is the most common pattern referred in the literature.[9]

According to the American Thoracic Society, clinical and microbiologic criteria are required to diagnose NTM lung disease.[10] In our case, the diagnosis was made on the basis of isolation of *M. goodii* from 2 separate sputum samples along with respiratory symptoms, and a radiologic-pattern characteristic of achalasia related NTM pulmonary infections. The isolate was identified as an RGM closely related to both *M. smegmatis* sensu stricto and *M. goodii* on the basis of its culture features, the lack of 3-day arylsufatatase activity, and susceptibility to tobramycin. The 2 species were differentiated by a reverse hybridization assay; a molecular tool used by other authors to identify this organism at the species level. Antimicrobial susceptibility testing showed a susceptibility pattern similar to that described for *M. smegmatis* group, with tobramycin minimum inhibitory concentration (MIC) of 1 mg /L. Although intermediate susceptibility of M. *goodii* to tobramycin (MIC of 2–8 mg/L) was described in previous reports for taxonomic purposes,[5,8] later observations have reported MIC values between 0.5 and 1 mg/L.

The length of therapy for RGM pulmonary disease should be at least 12 months.[8] Our patient was treated with doxycycline and ciprofloxacin for 12 months, combined with surgical procedures for achalasia, which resulted in clinical and radiologic improvement.

ACKNOWLEDGMENTS

The authors thank Professor Zaza Cohen from the Pulmonary Disease and Critical Care Medicine Division, Medicine Department of the University of Medicine and Dentistry Hospital of New Jersey for his assistance in the preparation of this manuscript.

REFERENCES

1. Hadjiliadis D, Adlakha A, Prakash UB. Rapidly growing mycobacterial lung infection in association with esophageal disorders. *Mayo Clin Proc*. 1999;74:45–51.
2. Church AC, Watkins S. Non-tuberculous mycobacteria masquerading as aspiration pneumonia in patients with gastrointestinal problems. *Respir Med*. 2006;100:1663–1665.
3. Newton JA Jr, Weiss PJ. Aspiration pneumonia caused by *Mycobacterium smegmatis*. *Mayo Clin Proc*. 1993;68:1025–1026.
4. Cramer JP, Sudeck H, Burchard GD. Pulmonary infection with rapidly growing mycobacteria in a singer with achalasia: a case report. *J Infect*. 2007;54:e219–e221.
5. Brown BA, Springer B, Steingrube VA, et al. *Mycobacterium wolinsky* sp. nov. and *Mycobacterium goodii* sp. nov., two rapidly growing species related to *Mycobacterium smegmatis* and associated with human wound infections: a cooperative study from the International Working Group on Mycobacterial Taxonomy. *Int J Syst Bacteriol*. 1999;49:1493–1511.
6. Guna R, Muñoz C, Domínguez V, et al. In vitro activity of linezolid, clarithromycin and moxifloxacin against clinical isolates of *Mycobacterium kansasii*. *J Antimicrob Chemother*. 2005;55:950–953.
7. Woods GL, Brown-Elliot BA, Desmond EP, et al. *Susceptibility Testing of Mycobacteria, Nocardia and Other Actinomycetes.* Approved Standard M24-A. Wayne, PA: National Committee for Clinical Laboratory Standards; 2003.
8. Brown-Elliott BA, Wallace RJ Jr. Clinical and taxonomic status of pathogenic nonpigmented or late-pigmenting rapidly growing mycobacteria. *Clin Microbiol Rev*. 2002;15:716–746.
9. Miller WT Jr. Spectrum of pulmonary nontuberculous mycobacterial infection. *Radiology*. 1994;191:343–350.
10. Griffith DE, Aksamit T, Brown-Elliott BA, et al. An official ATS/IDSA statement: diagnosis, treatment, and prevention of nontuberculous mycobacterial diseases. *Am J Respir Crit Care Med*. 2007;175:367–416.