EFFECT OF A PREPREGNANCY PERTUSSIS BOOSTER DOSE ON MATERNAL ANTIBODY TITERS IN YOUNG INFANTS

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Abstract: To examine the influence of a pertussis booster vaccination on the transfer of maternal antibodies, 24 nonpregnant women received a tetanus, diphtheria, acellular pertussis booster vaccine between 2 consecutive pregnancies. Blood was drawn from mothers and off-spring. Efficient transplacental antibody transfer and significantly higher antibody titers against 3 pertussis antigens were observed in cord blood and in blood of 1-month-old infants born after a maternal booster vaccination compared with results in their siblings born before the booster administration.

Key Words: neonatal pertussis, maternal antibodies, booster vaccination

METHODOLOGY

Design. A prospective multicenter study is currently conducted in Antwerp, Belgium, in accordance with the Helsinki Declaration, International Conference on Harmonisation-Good Clinical Practice, and procedures established by Belgian law. Ethical approval was obtained at the University Hospital of Antwerp. Women participating in a study on maternal antibodies against other vaccine-preventable diseases were recruited.6 Informed consent was obtained from the women and from both parents for the children. Exclusion criteria are low birth weight (<2400 g), prematurity (<36 weeks of gestation), immunologic disorders, and the administration of immunoglobulin.

All women had been vaccinated against pertussis during childhood and received no documented pertussis booster since 14 to 15 months of age. Serum samples were taken from every woman at delivery of her first included newborn (10 mL), from the cord (10 mL), and from the off-spring at 1 month of age (2 mL) (Group A children). Women were offered a tetanus, diphtheria, acellular pertussis (TdP) booster vaccine (Boostrix®, GlaxoSmithKline Biologicals, Rixensart, Belgium) after breast-feeding was ceased, according to the patient information leaflet. In the entire study, 86 women have been vaccinated. One month after vaccination, a blood sample (10 mL) was taken. At the moment of a following delivery, blood is taken from the woman (10 mL), the cord (10 mL), and from the next off-spring (Group B children) at 1 month of age (2 mL).

All serum samples are centrifuged at 2000 rpm within 8 hours after withdrawal, and stored at a temperature between −20°C and −40°C.

Laboratory: An in-house enzyme-linked immunosorbent assay (ELISA) was used to test all samples for anti-PT, anti-FHA and anti-PRN IgG antibodies at GSK Biologicals, Belgium. The limit of detection of the assay was 5 ELU/mL for all 3 antibodies.7

Immunogenicity. A booster response was defined as a postvaccination antibody concentration ≥20 ELU/mL with a prevaccination antibody concentration <5 ELU/mL, a postvaccination rise of at least 4 times the prevaccination antibody concentration in subjects with a prevaccination antibody concentration ≥5 and <20 ELU/mL, or at least twice the prevaccination antibody concentration in subjects with a prevaccination antibody concentration ≥20 ELU/mL.8 The prevaccination value was the maternal sample taken at the first delivery.

Statistics. Antibody geometric mean titers (GMT) with 95% confidence interval were calculated from the log transformed values. A paired student’s t test was used to compare the GMT antibody concentrations of the women pre- and postvaccination and at subsequent delivery, and to compare the siblings with samples of cord blood and blood of 1-month-old infants. A linear regression was used to evaluate potential influence of some variables on the titer of pertussis antibodies in 1-month-old infants: age of the mother, prevaccination pertussis antibody values in women, birth weight, and duration of pregnancy. Time between administration of the booster dose and delivery will be a variable in the end analysis of the study. Analyses were performed with SPSS version 16.0 for Windows.

RESULTS

Maternal Results. Of the 86 participating women in the ongoing study, 24 women were included in this interim analysis. The mean interval between the vaccination with Tdap and the next delivery was 12.7 (range, 8–18.4) months. Three women had a negative

pre- and postbooster. The aim of the study is to evaluate whether a prepregnancy booster helps to increase antibody titers to pertussis in neonates.
pregnancy test at the time of vaccination but turned out to be pregnant 1 month after vaccination, despite contraceptive advice. The mean age of the women at first delivery was 29.2 years (23.2–38.7), and 31.5 years (25.8–40.7) at subsequent delivery. Mean duration of both pregnancies was comparable (group A, 39.5 with group B, 38.9 weeks), as well as mean birth weight of the siblings (3483–3491 g); 16 of 24 children in group A and 17 of 25 in group B (1 twin) were vaginally born. There were no gender ratio differences.

Before the booster dose, 29% of women had detectable anti-PT antibodies, 87.5% anti-FHA and 75% anti-PRN antibodies (Table 1). Of 24 women, 20 women (83%; confidence interval, 63%–95%) responded to the Tdap booster, with rises in anti-PT antibodies according to the definition of vaccine response, 100% responded to FHA antigen, and 100% to PRN antigen (Table 1). Three women had positive anti-PT antibody levels 1 month after vaccination, although not high enough to comply with the protocol-defined definition of vaccine response. One woman did not turn seropositive for anti-PT antibodies, but responded well to FHA and PRN antigens. GMT increased significantly between prevaccination status and postvaccination status and between prevaccination status and the moment of the next delivery (\(P < 0.0001\)) (Table 1). All antibodies declined between the vaccination and the next delivery, but only for anti-PT antibodies, this led to a decrease in percentage of women with detectable levels. Infant Results. The cord/maternal GMT ratio is a measure for the efficiency of the placental transport of IgG. There was an active preferential transport to the newborn of all 3 antibodies: ratio 1.7/1 for anti-PT in both groups, 1.6/1 for anti-FHA in both groups, and 1.4/1 (group A) and 1.75/1 (group B) for anti-PRN.

Antibody titters for all 3 antigens differed significantly between both cords (Table 1). Not all cords were seropositive for anti-PT in group A, and all but 1 cord was seropositive for anti-PT in group B. Also for antibody titters at 1 month of age, GMT differed significantly between both groups of infants, although titers declined already strikingly in comparison with cord values. At 1 month of age, 35% of group A children had detectable anti-PT antibody levels, compared with 81% of group B children (\(P = 0.005\)). For anti-FHA, 71% was seropositive in group A and 100% in group B, anti-PRN antibodies were present in 57% in group A and 100% in group B (\(P < 0.0001\)) (Table 1).

No influence was found by age of the mother, parity, birth weight of the child, or breast-feeding.

**DISCUSSION**

High immunologic response to acellular pertussis (aP) booster vaccination is found in adult women, as well as efficient transplacental transfer and significantly higher titers in 1-month-old infants, born after a maternal booster vaccination compared with siblings born before the maternal booster. Other possible confounding factors such as breast-feeding, birth weight, parity, and age of the mother have no influence on these preliminary results.

Because no correlate of protection is known for pertussis, it is uncertain whether this increase in antibodies can be considered clinically protective. High antibody concentrations are likely to protect better than low values. Moreover, GMT in infants at 12 months of age, after priming with 3 doses of hexavalent vaccine, are comparable with GMT in children at 1 month of age after a prepregnancy booster. This suggests that GMT in group B would be comparable with clinical protection.

Maternal antibodies against pertussis do not last long; a rapid decay is found mostly within 2 months of age. Particularly, antibodies to PT wane rapidly in the present study. It will be difficult to know how often the vaccine would need to be readministered during the time of childbearing age to confer protection to the infants. The results of the entire study, with time since vaccination as a variable, will offer more insight in the decay rate of the antibodies in the mothers after booster vaccination. Ideally, maternal antibodies should offer protection until the primary vaccination schedule starts to confer protection. But prolonging the protection of newborns even with a few weeks could reduce susceptibility to pertussis.

Other strategies are currently being investigated to reduce the pertussis burden in neonates. Cocoon strategies are promoted in several industrialized countries, as is also adolescent/young adult booster vaccination. The latter will not only boost the person’s individual immunity, but also avoid susceptibility and further spreading to others, whereas for women’s future children, it could offer early protection in life. The present results could support the argument for adolescent or adult booster vaccination, especially in the female population, as a prepregnancy booster. An

| TABLE 1. Geometric Mean Titers (GMT) in Women and Children at Different Time Points for 3 Pertussis Antibodies (anti-PT, anti-FHA, anti-PRN) and P Values Indicating the Differences in GMT Between the Different Time Points (Paired Student t Test) |
|-------------------------------------------------|-------------------------------------------------|-------------------------------------------------|
| GMT women                                        | anti-PT (95% CI) (Npos/N) (%)†                   | anti-FHA (95% CI) (Npos/N) (%)†                  | anti-PRN (95% CI) (Npos/N) (%)†                  |
| Prevaccination                                   | 3.6 (2.3–5.8) (7/24) (29%)                       | 13.9 (8.3–23.5) (21/24) (87.5%)                 | 14.4 (8.8–23.7) (18/24) (79%)                  |
| Postvaccination month 1                          | 53.7 (22.2–89.5) (23/24) (96%)                  | 913.5 (650–1283.8) (24/24) (100%)               | 586.9 (389–885.5) (24/24) (100%)               |
| At next delivery                                 | 12.1 (7.3–19.9) (18/22) (81%)                   | 151.2 (99–199.4) (22/22) (100%)                 | 146.4 (94.6–271.8) (22/22) (100%)              |
| P value pair 1‡                                   | <0.0001                                         | <0.0001                                         | <0.0001                                         |
| P value pair 2‡                                   | <0.0001                                         | <0.0001                                         | <0.0001                                         |
| P value pair 3‡                                   | <0.0001                                         | <0.0001                                         | <0.0001                                         |
| GMT children                                     |                                                |                                                |                                                 |
| Cord group A‡                                     | 6.1 (3.5–10.6) (12/22) (54%)                    | 22.2 (12.9–38) (19/22) (86%)                    | 20.3 (11.8–35) (19/22) (86%)                    |
| Cord group B‡                                     | 19.0 (11.7–30.7) (21/22) (95%)                  | 247.0 (161–379) (22/22) (100%)                  | 278.0 (154–502) (22/22) (100%)                  |
| P value both groups of cord samples               | <0.0001                                         | <0.0001                                         | <0.0001                                         |
| Infant group A, month 1‡                          | 3.1 (1.6–6.0) (5/14) (35%)                      | 10.6 (5.2–22) (10/14) (71%)                    | 9.8 (5.2–18) (8/14) (57%)                       |
| Infant group B, month 1‡                          | 10.3 (6.3–16.8) (18/22) (81%)                   | 152.1 (104–220) (22/22) (100%)                  | 167.4 (102–274) (22/22) (100%)                  |
| P value both groups of children                   | <0.0001                                         | <0.0001                                         | <0.0001                                         |

*Npos = number of positive samples/N = total number of available samples and % positive samples.

*P value pair 1‡ = prevaccination and postvaccination month 1; P value pair 2‡ = postvaccination month 1 and at next delivery; P value pair 3‡ = prevaccination and at next delivery.

Group A: first born children, before the maternal booster dose; Group B: second cohort of children, born after the maternal booster dose.

PT indicates pertussis toxin, FHA, filamentous hemagglutinin; PRN, pertactin; CI, confidence interval.
aP birth dose is another strategy to close the susceptibility gap, and has been shown to induce both humoral and cellular immune responses in neonates.11 Caution should be exercised on vaccine interference using this scenario. However, maternal vaccination protects the infant in the early weeks after birth, whereas neonatal vaccination still needs some weeks to build up immunity.

The possible interference of high titers of maternal antibodies on the immunogenicity of the infant pertussis immunization program is of concern. However, literature reports suggest that humoral and cellular immune responses to aP vaccines are not influenced by the presence of maternal antibodies.12 Additional blood samples during the first year of life are taken in the entire study population, but results are not yet available. In the frame of this study, it is important to evaluate the immune response to aP vaccines in infants, because the intervention triggers maternal antibodies through vaccination of the mothers.

**REFERENCES**


**CLOSTRIUM DIFFICILE INFECTION AMONG CHILDREN WITH CANCER**

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**Abstract:** We used data from the Kids’ Inpatient Database to examine *Clostridium difficile* infection (CDI) among children with cancer. The CDI rate was 15 times greater among children with cancer compared with those without cancer. Children with cancer accounted for 21% of all pediatric CDI cases. Increased adherence to infection control recommendations is needed to address CDI in children with cancer.

**Key Words:** *Clostridium difficile*, children, cancer, epidemiology

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The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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**N**ew trends in the epidemiology of *Clostridium difficile* have emerged in recent years. There have been increases in both hospital- and community-acquired *C. difficile* infections (CDI).1,2 CDI traditionally has been found in persons with recent antimicrobial use and in elderly hospitalized patients. However, children, a population once considered to be at low risk for CDI, are increasingly at risk, and incidence of CDI among hospitalized children has recently increased.2 Children who develop CDI, particularly those who are immunosuppressed, can experience severe complications and high mortality rates.3 Children with cancer may have an increased risk of developing CDI, in part because of immunosuppression and increased exposure to healthcare settings. Nonetheless, large epidemiologic studies examining CDI in children with cancer are lacking. We examined national estimates of CDI among children with cancer by using a large hospital discharge database.

**MATERIALS AND METHODS**

We used data from the Kids’ Inpatient Database (KID), which is part of the Healthcare Cost and Utilization Project. KID is a random sample of pediatric discharges available every 3 years from all acute care hospitals participating in Healthcare Cost and Utilization Project. Each year of KID data contains more than 2 million pediatric inpatient records from more than 2500 hospitals. We combined data on hospital discharges from years 2000, 2003, and 2006. CDI discharges were identified using an International Classification of Diseases, Ninth revision (ICD-9) diagnosis code of 008.45 (previously validated to correlate with laboratory-defined cases*). Cancer patients were identified by using clinical classification software diagnosis codes in the range of 11 to 19 and one month induces antibody responses by two months of age. Pediatr Infect Dis J. 2010;29:209–215.


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tissue infections), and variables describing the hospital stay. We also conducted a descriptive analysis of pediatric cancer patients with CDI. We also used multivariate, logistic regression to determine which factors were independently associated with CDI among pediatric cancer patients. All findings from the logistic regression models are presented as adjusted odds ratios with 95% confidence intervals.

RESULTS
In 2000, 2003, and 2006, we identified 4051 cases of CDI among 297,461 hospitalized children with cancer. The rate of CDI in 2006 was 15 times higher among children with cancer compared with those without cancer (17.7 vs. 1.1 cases per 1000 discharges). CDI discharges of hospitalized children with cancer increased 45%, from 10.2 cases per 1000 discharges in 2000 to 17.7 cases per 1000 discharges in 2006. A similar proportionate increase in CDI was observed among hospitalized children without cancer, from 0.6 cases per 1000 discharges in 2000 to 1.1 cases per 1000 discharges in 2006. Children with cancer accounted for 1% of all hospital discharges of children; however, they accounted for 21% of all pediatric hospitalizations with a CDI diagnosis.

Table 1 displays adjusted odds ratios of cases of CDI among all hospitalized children. CDI was more strongly associated with a cancer diagnosis than other diagnoses for which children commonly receive antimicrobials, such as pneumonia and soft tissue infection. CDI was independently associated with hospitalization in the year 2006 relative to 2000. CDI was associated with a hospital stay of 6 or more days relative to a stay of 1 day or less. In addition, CDI was associated with blood transfusion.

DISCUSSION
Our study highlights the increasing burden and risk for CDI among children with cancer. Our study supports similar findings of increases of observed CDI over time. A study among all children among children with cancer. Our study supports similar findings of more days relative to a stay of 1 day or less.


<table>
<thead>
<tr>
<th>Total Characteristic</th>
<th>CDI OR* n = 20,484</th>
</tr>
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<tbody>
<tr>
<td>Age group (yr)</td>
<td></td>
</tr>
<tr>
<td>&lt;1</td>
<td>0.45 (0.40–0.51)</td>
</tr>
<tr>
<td>1–4</td>
<td>2.04 (1.88–2.20)</td>
</tr>
<tr>
<td>5–9</td>
<td>1.36 (1.25–1.49)</td>
</tr>
<tr>
<td>10–14</td>
<td>1.11 (1.02–1.22)</td>
</tr>
<tr>
<td>15–18</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Cancer diagnosis</td>
<td></td>
</tr>
<tr>
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<td>3.78 (3.35–4.27)</td>
</tr>
<tr>
<td>No</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Sex</td>
<td></td>
</tr>
<tr>
<td>M</td>
<td>1.07 (1.03–1.12)</td>
</tr>
<tr>
<td>F</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>Year of admission</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>1.0 (reference)</td>
</tr>
<tr>
<td>2003</td>
<td>1.10 (0.98–1.23)</td>
</tr>
<tr>
<td>2006</td>
<td>1.52 (1.36–1.69)</td>
</tr>
<tr>
<td>Race/ethnicity</td>
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<tr>
<td>Non-Hispanic white</td>
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<td>Non-Hispanic black</td>
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<td>Asian or Pacific Islander</td>
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<tr>
<td>Emergency department</td>
<td>1.87 (1.73–2.02)</td>
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<tr>
<td>Transfer from another hospital or healthcare facility</td>
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<td>0–1 d</td>
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<tr>
<td>2 d</td>
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<td>3–5 d</td>
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<tr>
<td>6 or more days</td>
<td>14.88 (13.40–16.52)</td>
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<td>Blood transfusion received</td>
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<tr>
<td>Yes</td>
<td>1.86 (1.68–2.07)</td>
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<tr>
<td>Skin and subcutaneous tissue infections</td>
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</tr>
<tr>
<td>Yes</td>
<td>0.87 (0.76–0.99)</td>
</tr>
<tr>
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<td>1.0 (reference)</td>
</tr>
<tr>
<td>Pneumonia (excluding HIV- and tuberculosis-related)</td>
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<tr>
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<td>1.10 (1.02–1.20)</td>
</tr>
<tr>
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*Adjusted odds ratio.
†95% Confidence interval.

CONSISTENT with our finding that increased length of hospital stay is associated with CDI.

CONSISTENT with the increase in intensity and duration of CDI risk, we found that CDI in children with cancer was associated with being 1 to 4 years of age. Leukemia is the most common cancer in young children. Consequently, children in this age group are more likely to be admitted to hospitals for cancer treatment and receive more therapies that may put them at higher risk for CDI.
Another factor that may be associated with immunosuppression is our finding of an association between the receipt of a blood transfusion and CDI. Aside from an intact, lower intestine bacterial flora, a brisk, humoral immune response to *C. difficile* is another important host defense against CDI. Although one cannot exclude the possibility that it is simply a marker of more severe disease, blood transfusion itself is associated with immunosuppression and could possibly increase CDI risk through this mechanism. Efforts are needed to address the high and increasing rate of CDI among children with cancer. Increased surveillance of infections and clinical outcomes among children with cancer is needed to better understand the epidemiologic features of CDI in this high-risk population. A prospective study of the factors associated with CDI among children with cancer is also needed. Although CDI has been considered an important disease in older adult populations, the increase in overall rates among pediatric age groups suggests that efforts to promote better adherence to existing infection control recommendations should include pediatric healthcare settings. Our data suggest that there should be a particular priority in settings where children with cancer receive treatment. Although it may be difficult to implement in some settings, antimicrobial stewardship with the promotion of narrower spectrum agents holds promise as an intervention to reduce CDI. It will be important for future research to examine the role of prophylactic antimicrobial use in the development of CDI, to ensure that the risk-benefit remains favorable. Moreover, because many pediatric cancer patients receive treatment under study protocols, it will be important for investigators and multi-institutional study coordinators to be aware of local and regional CDI protocols, it will be important for future research to examine the role of prophylactic antimicrobial use in the development of CDI, to ensure that the risk-benefit remains favorable. Moreover, because many pediatric cancer patients receive treatment under study protocols, it will be important for investigators and multi-institutional study coordinators to be aware of local and regional CDI rates and the risk posed by cancer therapy received as part of such protocols.

A limitation to our findings is the inability to conduct a patient-level analysis of information in KID data because of the lack of a uniform patient identifier necessary to determine whether individual patients had been admitted multiple times. Also, the KID database does not collect information on the source of infections (nosocomial vs. community acquired). In addition, KID data represents discharges, not admissions. Therefore, we were unable to assess whether children were admitted with CDI or had acquired it during hospitalization. We did not include information on antimicrobial use; KID does not include data on oral antimicrobial administration. The CDI rates we report represent CDI discharge diagnoses based on administrative data and therefore, in clinical or laboratory-identified cases among adult inpatients and, where there are discrepancies, there is a tendency for discharge diagnoses to underestimate rather than overestimate laboratory-defined cases.

**CONCLUSIONS**

Our study highlights the increasing burden and risk for CDI among children with cancer. Increased surveillance and improved adherence to infection control guidelines are needed to better understand and prevent CDI in children with cancer.

**REFERENCES**


**FREQUENCY OF HUMAN RHINOVIRUS SPECIES IN OUTPATIENT CHILDREN WITH ACUTE RESPIRATORY INFECTIONS AT PRIMARY CARE LEVEL IN BRAZIL**

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**Abstract:** This study assessed the occurrence of human rhinovirus (HRV) species in outpatient children attending day-care in Sao Paulo, Brazil. HRV reverse transcriptase polymerase chain reaction and amplicon sequencing were done in 120 samples collected in 2008. HRV was detected in 27.5% of samples. HRV C was detected in 60.7% of wheezers, a frequency not different from that observed in nonwheezers (69.6%).

**Key Words:** rhinovirus, wheezing, sequencing, species

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**Studies based on polymerase chain reaction (PCR) for the detection of respiratory viruses have established that human rhinoviruses (HRV) of species A, B, and C are the most frequent agents of acute respiratory infections (ARI) in all age groups. Moreover, these agents are also associated with up to 70% of virus-related wheezing exacerbations. The most recently identified HRV species C has been detected in association with bronchiolitis, wheezing, and asthma exacerbations requiring hospitalization. However, most studies focusing on HRV-C have been...**
hospital-based, or restricted to inpatients, a selection that may have narrowed the perception of the clinical spectrum that may be caused by HRV-C. We have assessed the frequencies of HRV species in children seen as outpatients at a primary care facility in São Paulo, southeast Brazil.

**PATIENTS AND METHODS**

This study was done with stored nasal aspirates collected from March to December 2008 as part of a previous prospective study of ARI in children of less than 12 years of age (median, 4 years) without any underlying disease (eg, asthma), in the city of São Paulo. Children were seen as outpatients at a primary care facility by a pediatrician who, upon a clinical diagnosis of ARI, filled out a form with clinical and epidemiologic information, and obtained an informed consent from parents or guardians. The follow-up of all patients was accompanied by the physician in subsequent office visits, or by telephone, with data entered on the form, until the resolution of respiratory episode. The median time from the onset of symptoms to the collection of nasal washings was 3 days (1–12 days).

Viral RNA was extracted using QIAamp Viral RNA extraction Kit (Qiagen, Hilden, Germany), according to manufacturer’s instructions. Amplifications of HRV 5’ untranslated region and VP4/VP2 gene were performed by the reverse transcriptase PCR assay as described previously, with minor modifications. Positive (HRV type 39 stock) and negative controls were included in all tested sample batches. The assay was standardized to detect HRV RNA equivalent to $10^{8.25}$ TCID₅₀ of HRV 39.

DNA sequencing was done with BigDye Terminator Cycle Sequencing Ready Reaction Kit, according to the manufacturer’s instructions (Applied Biosystems, Foster City, CA), using the same primers as in the PCR. Phylogenetic relationships were assessed by maximum likelihood by the PhyML software. Trees were replicated 100 times to provide bootstrap support for clades. The analyses were performed using the software Topali v2.5, comparing with HRV sequences available in GenBank (National Center for Biotechnology Information).

Positive samples for different HRV species were distributed in 2 categories, according to the presence of wheezing. χ² (statistical package for social sciences [SPSS] v11.5, SPSS Inc., Chicago, IL) and Fisher exact test (GraphPad InStat v3.06 software) were applied when appropriate. Data on sample positivity for respiratory syncytial virus (RSV) and adenovirus (AdV) by immunofluorescence (SimulFluor Respiratory Screen Kit, Chemicon, US) and for human bocavirus (HBoV) by PCR were reviewed to assess the frequency of coinfections.

**RESULTS**

Samples from 120 patients (1 sample per patient) were analyzed. Although most patients had symptoms of upper ARI, fever was present in 50.8% (61/120) and wheezing in 47.5% (57/120) of them. Rhinovirus RNA was detected in 46.7% of the patients (56/120), and the median age of HRV-positive patients was 3 years (ranging from 1 month to 10 years). Of the HRV positive samples, wheezing was present in 51.8% (29/56), dyspnea in 41.1% (21/56), fever in 41.1%, and all 3 symptoms simultaneously in 33.9% (19/56). Coinfection with other respiratory viruses was found in 14.3% (8/56) of the samples: 7.3% (4/56) with RSV, 5.3% (3/56) with HBoV, and 1.8% (1/56) with AdV.

Partial genome sequencing revealed that of the 56 HRVs detected, 12 (21.4%) were HRV A, 6 (10.7%) were HRV B, and 33 (58.9%) were of HRV C. Therefore, HRV C was detected in 27.5% (33/120) of the study patients.

Five HRVs could not be identified by the partial genome sequencing performed (1 from a wheezing child and 4 from nonwheezing children). Of the 28 wheezing children from whom HRV was sequenced, 60.7% (17/28) were HRV C, 28.5% (8/28) with HRV A, and 10.7% (3/28) with HRV B. In the wheezers group, 13.8% of patients (4/29) were coinfected with RSV and 6.9% (2/29) with HBoV. Of the 23 HRVs obtained from nonwheezing patients, 69.5% (16/23) were HRV C, 17.4% (4/23) were HRV A, and 13% (3/23) were HRV B. Coinfections with HBoV and AdV were detected with the same frequency, 3.7% (1/27), in the nonwheezing group. Differences in frequencies of

![FIGURE 1. Number of samples positive for each HRV species by age group. Light grey bars represent HRV A; dark grey bars, HRV B; black bars, HRV C. Dotted line, tendency line for HRVA; dashed line, tendency line for HRV B; solid line, tendency line for HRV C.](image-url)

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the 3 HRV species between patients with and without wheezing were not significant. When patients were stratified into 8 age groups, HRV C was the predominant species in all age groups between 6 months and 5 years of age (Fig. 1). Association between HRV species frequency and age was performed in both, wheezer and nonwheezer groups. There was no significant association of wheezing with any of the 3 HRV species in any of the age groups.

DISCUSSION

In this outpatient study, HRV C was the most frequently detected HRV species in association with ARI in children, including the very young. All ARI episodes in this study had a favorable outcome, none of the patients required hospitalization and no difference was detected between wheezers and nonwheezers regarding the frequencies of HRV species. These results are in contrast with those from previously published studies, in which HRV C infections were frequent in hospitalized children with respiratory diseases and were associated with asthma, recurrent wheezing, and bronchiolitis. However, most studies incriminating HRV C as a frequent cause of severe lower respiratory tract ARI and asthma have been hospital-based, in which very young children may have been overrepresented, thereby introducing a possible selection bias. In this regard, Calvo et al (2010) described that HRV C infections were not associated with more severe disease than HRV A infections in hospitalized patients. Assessment of the full range of clinical manifestations of HRV species in children must include not only patients at different levels of health care, but also in the households. Therefore, HRV species determination in samples obtained in community-based studies should be done to address this issue.

REFERENCES


HIGH HUMAN IMMUNODEFICIENCY VIRUS-FREE SURVIVAL OF INFANTS BORN TO HUMAN IMMUNODEFICIENCY VIRUS-POSITIVE MOTHERS IN AN INTEGRATED PROGRAM TO DECREASE CHILD MORTALITY IN RURAL RWANDA

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Abstract: We retrospectively examined infant mortality and human immunodeficiency virus (HIV)-free survival among 211 infants who received a comprehensive package of health services, including breast milk substitution and clean water access, to prevent maternal-to-child transmission of HIV and improve child survival. The cumulative 12-month infant survival probability was 0.97 (95% confidence interval: 0.94–0.99). The cumulative 12- to 18-month HIV-free survival probability was 0.95 (confidence interval: 0.91–0.97).

Key Words: HIV-free survival, prevention of maternal to child transmission, child survival, rural, infant mortality

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In 2000, the United Nations included, among the Millennium Development Goals (MDGs), the aim of reducing <5 years of age mortality by two-thirds between 1990 and 2015. As of 2006, most countries in sub-Saharan Africa had not made progress toward this goal. Human immunodeficiency virus (HIV) burden may be a key impediment to achieve significant reductions in child mortality. In 2005, Partners In Health and Rwanda’s Ministry of Health implemented a child survival program in 6 rural clinics. The aim of the program was to improve overall survival of children <5 years of age through strengthened hospital, health center, and community-based services, and to improve HIV-free survival among children born to HIV-infected women. Because the risk of HIV transmission through breast milk might offset the benefits of breast-feeding among infants born to HIV-infected women, the option to replace feeding HIV-exposed infants was a key program component. We report mortality, HIV-free survival, and HIV transmission among infants in this program whose mothers chose to replace feeding.

METHODS

Study Setting and Population. We conducted this evaluation in eastern Rwanda, where the infant mortality rate is estimated to be 84 deaths/1000 live births. A total of 34% of Rwandans living in rural areas derive water from sources that are not reliably safe (open public wells, rivers, streams, ponds/lakes). For analysis, we

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included the children born to HIV-infected women who enrolled in the program at, or before, delivery between August 1, 2005 and February 28, 2007 and chose to replacement feed. We excluded 2 second-born and 1 randomly-selected twin infants from analysis. Prevention of Maternal-to-child Transmission Services and Care. Key program components are shown in Figure, Supplemental Digital Content 1, http://links.lww.com/INF/A719. Mother–infant pairs received the complete child survival package including free replacement feeding with infant formula from birth through 8 months, and a locally produced porridge of vitamin-fortified sorghum, soy, maize flours, and sugar, from 6 months until 18 months. To facilitate clean water preparation and exclusive replacement feeding, women received a stove, a thermos, kerosene, feeding bottles, and a pot and jug for boiling and storing water. Clinicians and community health workers conducted ongoing assessments of diarrheal disease, nutritional status, and replacement feeding adherence. Women visited clinics biweekly to receive supplies. During clinic visits, nurses regularly trained mothers on clean water and formula preparation, hygiene, family planning, recognition of diarrheal disease and dehydration, and other health topics. Infants had regular physical exams and received cotrimoxazole prophylaxis from 6 weeks of life until the first negative HIV test result.

We received antiretroviral medications to prevent vertical HIV transmission according to Rwanda national protocols (Fig., Supplemental Digital Content 1, http://links.lww.com/INF/A719). Community health workers provided home-based care, including daily directly observed therapy, to mothers and infants receiving antiretroviral medications, and other routine visits to address problems and support formula preparation. Social workers visited mother–infant pairs who missed more than one distribution and closely monitored losses-to-follow-up and mortality. Because of the increased risk of HIV transmission from mixed feeding, replacement feeding was suspended in women repeatedly identified as breast-feeding.

HIV Testing. In accordance with national protocols, infants received their first HIV test after 6 weeks of life using a dried blood spot polymerase chain reaction (PCR) test. We retested infants between the ages of 12 and 18 months, using HIV antibody testing. Children were also tested on parental request or when clinically indicated. Because of the occasional nationwide shortages of PCR reagents, the timing of initial HIV testing varied throughout the study period, and some children <1 year of age were initially tested using antibody tests.

Data Collection. We retrospectively reviewed electronic and paper program registries and patient charts for demographic and clinical data and outcomes of infant mortality, loss to follow-up, and HIV transmission.

Outcome Definitions. A woman was defined as lost-to-follow-up if she missed 2 consecutive distributions and could not be traced by a social worker. A child was confirmed to be HIV-infected following any positive PCR test result and HIV-uninfected following a negative PCR or antibody test result after 12 months of age. To ensure that every mother–infant pair was eligible to be followed up for at least 1 year, follow-up for outcomes of mortality and losses-to-follow-up ended on the program completion date or February 28, 2008, whichever came first.

Data Analysis. We calculated the cumulative probability of survival to 12 months using the product-limit estimator. We also calculated the Kaplan–Meier HIV-free survival probabilities for the infancy (0–365 days) and 12- to 18-month follow-up (366–541 days) periods and computed the cumulative HIV-free survival probability through the follow-up period. For the cumulative survival probability analysis, children were administratively censored at 365 days or on the date of their last visit if they exited the program for a reason other than death before 365 days. For the HIV-free survival analysis, we considered the event date to be the earlier of the date of death or first HIV-positive test result. Children who exited the program before 1 year were censored at the beginning of the follow-up period (ie, 366 days). Confidence intervals (CI) for cumulative survival probabilities were calculated using the log cumulative hazard transformation. Because a small number of children exited the program before initial HIV testing, we conducted a sensitivity analysis in which we presumed them to be HIV-infected. This evaluation received institutional review board approval by Partners Human Research Committee, Boston, USA and Rwanda National Ethics Committee.

RESULTS

Mother–infant pairs were followed up for a median of 477 days (interquartile range: 400–517). See Table, Supplemental Digital Content 2, http://links.lww.com/INF/A720, for clinical and demographic characteristics of the cohort.

Mortality and Other Outcomes. Six infants died during the study period (2.8%). HIV-test results were available for 4 of them, and 1 had confirmed HIV infection. The median time from enrollment to death was 174 days (range: 0–362). The 12-month survival probability was 0.97 (95% CI: 0.94–0.99). Seventeen infants (7.1%) exited the program before 1 year (ie, were censored) because they moved out of the catchment area (N = 2); became lost to follow-up (N = 3); left the program early when a new sibling enrolled in the program (N = 4); were suspended for breast-feeding (N = 3); or for repeatedly selling formula or enrolling at multiple sites (N = 3). HIV Transmission. HIV test results were available for 205 of the 211 children (97.2%) in the program during the infancy period and for 179 of the 187 children (95.7%) who reached 1 year and remained at risk for HIV infection. Reasons for missing test results during the infancy period included death (N = 2), transfer out of the area (N = 2), or early exit from the program (N = 2). Five infants were documented as HIV-infected, of which, 3 children were diagnosed at the time of their first HIV test (at 36, 64, and 110 days) and 2 children with initial HIV-negative test results were subsequently confirmed to be HIV-infected.

HIV-free Survival. Table 1 displays the HIV-free survival probabilities for the infancy and follow-up periods. The 12- to 18-month cumulative HIV-free survival probability was 0.95 (95% CI: 0.91–0.99).

<table>
<thead>
<tr>
<th>TABLE 1. HIV-free Survival During the Infancy and Follow-up Periods</th>
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<tr>
<td>Infancy period 12-18 months</td>
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<tr>
<td>Follow-up period</td>
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</table>

<sup>1</sup>Infancy period: 0 to 365 days; follow-up period: 366 to 541 days. One HIV-infected child died during the infancy period; therefore, the total number of events during the infancy period was 9.

<sup>2</sup>Seventeen children exited the program during the infancy period and were censored at the beginning of the follow-up period.
DISCUSSION

Our data show that high child survival and HIV-free survival rates are achievable in rural, resource-poor settings, even among high-risk children such as those exposed to HIV. We believe that the close follow-up of mothers and infants and improved access to basic health services and clean water were essential program components, and were likely responsible for the high HIV-free survival probabilities observed in this cohort. This hypothesis is consistent with previous reports of high HIV-free survival probabilities in cohorts of infants that were closely followed and supported while receiving replacement food or ART during breast-feeding.8,9 We advocate that these elements be included as the cornerstone of any prevent maternal-to-child transmission strategy, whether it includes replacement feeding or ART while breast-feeding.

Few previous reports have described successful program models for HIV-free survival that included replacement feeding, and higher rates of death have been reported among infants who replacement fed compared with those who breast-fed.10 Because nearly all women in this program elected to replacement feed, we did not have a reference group with whom we could compare HIV-free survival. Nonetheless, given the high background infant mortality rate in this setting, this cohort’s high HIV-free survival probabilities are remarkable and among the highest reported till date for a cohort of HIV-exposed infants.

In conclusion, although HIV has threatened progress toward the MDG of reducing <5 years of age mortality, investment in HIV care has offered a launching pad from which to strengthen primary care services through improved health infrastructure, hiring and training of additional staff, and establishment of reliable drug supplies. If the MDG is to be achieved, the scale-up of HIV services must be used as an opportunity not only to develop targeted services for HIV-infected and HIV-exposed children, but also to buttress services for all children <5 years of age. Such efforts should be conducted in tandem with initiatives toward poverty alleviation, food security, and clean water access for all individuals, regardless of HIV status.

ACKNOWLEDGMENTS

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REFERENCES


ROTAVIRUS ENTERIC INFECTION IN CHILDREN OF NORTHWEST IRAN

Ziaedin Ghorashi, MD, Afshin Ghahehgolab Behbahan, MD, and Shahram Abdoli Osouei, MD

Abstract: This is a cross-sectional and analytical-descriptive study of 511 children less than 36 months of age who were admitted to Tabriz Children’s Hospital with acute gastroenteritis during a period of 2 years (from October 2007 to October 2009). Rotavirus was found in stool specimens of 284 (55.6%) of 511 children with diarrhea. Two-thirds of them were admitted during autumn and winter for a mean hospital stay of 3.1 ± 1.8 days.

Key Words: rotavirus, gastroenteritis, diarrhea, children

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Diarrhea is the leading cause of death in children worldwide, as it causes 2.1 million deaths per year, while 85% of these childhood deaths happen in low-income countries.1,2 According to epidemiologic studies, known viral pathogens are responsible for 50% to 80% of cases of infectious diarrhea. Rotavirus is the most common viral cause of severe watery diarrhea in infants and children worldwide.3,4 In 1985, De Zoyas and Feachem published their prominent study about ubiquitous prevalence of rotavirus; they declared that 6% of all episodes of diarrhea and 20% of diarrhea-related mortality among children <5 years of age in both developing and developed countries have been caused by rotavirus. Their finding showed that improvements in public health including hygienic water supply and safe sewage cannot control this disease. According to the most recent announcement of World Health Organization6 (June 2009), rotavirus vaccination has been recommended in all countries, especially in those areas of the world where diarrhea is responsible for 10% of pediatric deaths or more. Because the epidemiology and clinical manifestations of rotavirus gastroenteritis had not been studied in children of Northwest Iran, this study was designed to cover the subjects among children of this area, who were admitted to Tabriz Children’s Hospital.

METHODS AND MATERIALS

This was a hospital-based, cross-sectional, and analytical-descriptive study of 511 children <36 months of age who...
were admitted to the infectious disease ward of Tabriz Children’s Hospital with acute gastroenteritis, during period of 2 years (from October 2007 to October 2009). Tabriz Children’s Hospital is a tertiary referral hospital in Northwest Iran affiliated to Tabriz University of Medical Sciences. Admitted cases of acute diarrhea were selected by convenience sampling method but only those patients were included whose parents signed a written consent form to signify their voluntary participation in this study. However, no emotional or financial burden was imposed on patients for the sake of this study; therefore, the research proposal was approved by the Medical Research Ethics Committee of Tabriz University of Medical Sciences.

The following exclusion criteria were considered before enrollment:

● Being older than 36 months.
● Detection of either bacterial or parasitic pathogens in stool examination by light microscopic study and/or cultures.

Every patient’s stool specimen was transported to the hospital laboratory in a special closed container and initially tested for bacterial or parasitic pathogens and negative samples (lacking bacterial or parasitic pathogens) were checked by enzyme-linked immunosorbent assay ELISA method; for antigen of rotavirus using the ELISA kit: “IDEIA Rotavirus, Dako, United Kingdom.”

All needed clinical, demographic, and epidemiologic data were confidentially collected from patients’ hospital files and recorded in nameless (code-identified) data collection forms. SPSS software (version 15) was used for the statistical analysis of the variables; descriptive parameters are presented as frequencies (percent) and quantitative findings as mean ± SD (and range). Independent Samples T test and K square test were used to compare quantitative and descriptive parameters, respectively. P values of less than 0.05 were considered to be statistically significant.

RESULTS

Between October 2007 and October 2009, 1125 children <36 months of age were admitted to the infectious disease ward of Tabriz Children’s Hospital with acute diarrhea; 511 (45.4%) of them had no parasitic or bacterial pathogen in stool examination and culture. These negative stool specimens then underwent antigenic surveillance by ELISA method for rotavirus antigen that were positive in 284 (55.6%) cases. Acute diarrhea was defined as 3 or more episodes of defecation per day with loose or watery stool for a period of 14 days or less. The patients with rotavirus gastroenteritis had a mean age of 13.4 ± 7.3 (1.0–36) months and a mean weight of 9035 ± 2358 (1050–15,000) g. Table 1 shows the epidemiologic findings including age and sex distribution, season at presentation, area of residence, type of feeding, and sex-matched weight-for-age percentiles. There was a meaningful seasonal pattern of presentation with a predilection for cold months of autumn and winter (P < 0.001) (Table 1).

The mean body temperature of children with rotavirus gastroenteritis was 37.7°C ± 0.9°C (36°C–42°C) at admission and most had a positive history of watery diarrhea (99.3%) since 3.7 ± 1.6 (1–14) days prior to admission with mean bowel movements of 7.3 ± 3.5 (1–20) episodes at first day of admission. They often complained of repeated vomiting with a frequency of 5.6 ± 3.1 (1–20) episodes of the day which had been begun since 2.8 ± 1.5 (1–14) days prior to admission. Their mean serum level of sodium was 139.9 ± 5.4 (129–159) mEq/L. Children were mostly treated with intravenous or combined intravenous and oral rehydration therapies (46.9% and 51%, respectively) and only 2.1% by the oral route alone. We observed no case mortality due to rotavirus gastroenteritis during this study.

DISCUSSION

Rotavirus was responsible for 55.6% of nonbacterial nonparasitic acute diarrheal episodes among children under 36 months of age who were admitted to our hospital; this frequency is quite similar to those of other countries as mentioned in reports from Vietnam (59%), Japan (58%), Finland (54%), Myanmar (53%), China, Malaysia, and Australia (50%).7–9 Smaller proportions of acute diarrheal diseases in children are caused by rotavirus in reports from other countries including Taiwan, Thailand, Indonesia (43%), England (39%), Hong Kong (28%), and Germany (25%).10–12

The mean age of children with rotavirus gastroenteritis in patient population of our study was 13.4 months and 47.5% of them were under 1 year of age. A similar study in Shanghai-China showed that 60% of children with rotavirus gastroenteritis were less than 1 year old.13 According to these findings, children <1 year of age are at higher risk for catching rotavirus diarrheal diseases. The male preponderance in our study (M/F = 1.5/1) resembles that of other studies worldwide.1–3 We observed the highest seasonal distribution of rotavirus gastroenteritis in the cold months of autumn and winter encompassing 64.1% of our studied patients, especially more frequently in autumn (33.5%) and less in summer (13.4%). Similar results have been declared by many researchers, who have determined a seasonal pattern for incidence of rotavirus diarrheal diseases in children worldwide. The peak incidence of rotavirus gastroenteritis was during winter in Australia and Germany, through February in Netherlands, and from October to December in Shanghai-China.7,10–13–15

The most common clinical manifestations of rotavirus gastroenteritis observed in our patient population include watery diarrhea (99.3%) and vomiting (97.9%), causing moderate-to-severe dehydration (92.3%); these are similar to the results of 2 separate studies from Australia and Indonesia.9,14 Hospital-based studies like ours have the limitation that they only cover the admitted patients whose illnesses are more severe than those who

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**TABLE 1. Epidemiologic Findings of 284 Children With Rotavirus Gastroenteritis**

<table>
<thead>
<tr>
<th>Epidemiologic Characteristics</th>
<th>Frequencies</th>
<th>Number (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age distribution (mo)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;12</td>
<td>135</td>
<td>(47.5%)</td>
</tr>
<tr>
<td>12–23</td>
<td>116</td>
<td>(40.8%)</td>
</tr>
<tr>
<td>24–36</td>
<td>33</td>
<td>(11.7%)</td>
</tr>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>171</td>
<td>(60.2%)</td>
</tr>
<tr>
<td>Female</td>
<td>113</td>
<td>(39.8%)</td>
</tr>
<tr>
<td><strong>Season at presentation</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Spring</td>
<td>64</td>
<td>(22.5%)</td>
</tr>
<tr>
<td>Summer</td>
<td>38</td>
<td>(13.4%)</td>
</tr>
<tr>
<td>Autumn</td>
<td>95</td>
<td>(33.5%)</td>
</tr>
<tr>
<td>Winter</td>
<td>87</td>
<td>(30.6%)</td>
</tr>
<tr>
<td><strong>Type of feeding</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Milk*</td>
<td>31</td>
<td>(10.9%)</td>
</tr>
<tr>
<td>Milk* and supplemental foods</td>
<td>223</td>
<td>(78.5%)</td>
</tr>
<tr>
<td>Regular food</td>
<td>30</td>
<td>(10.6%)</td>
</tr>
<tr>
<td><strong>Weight-for-age percentiles (sex-matched)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>&lt;5</td>
<td>68</td>
<td>(24%)</td>
</tr>
<tr>
<td>5–25</td>
<td>76</td>
<td>(28.8%)</td>
</tr>
<tr>
<td>25–50</td>
<td>63</td>
<td>(22.1%)</td>
</tr>
<tr>
<td>50–75</td>
<td>25</td>
<td>(8.8%)</td>
</tr>
<tr>
<td>&gt;75</td>
<td>38</td>
<td>(13.4%)</td>
</tr>
<tr>
<td>&gt;90</td>
<td>14</td>
<td>(4.9%)</td>
</tr>
</tbody>
</table>

*Breastfeeding and/or formula.
have been managed as out-patients or even milder states as subclinical rotavirus infections that may not seek any medical attention; therefore, the relative frequencies mentioned for clinical manifestations in admitted patients may not be applicable for general pediatric population. The mean hospital stay for an episode of rotavirus gastroenteritis in our center was 3.1 ± 1.8 days which was shorter than those of Poland, United States, and Netherlands, but longer than those of Australia.7,12,15

In our area, as in other parts of the world, rotavirus is a common viral pathogen that plays a significant role in the epidemiology and pathogenesis of acute diarrhea specially in children <1 year of age; therefore, routine immunization against rotavirus in early infancy, using a vaccine with acceptable safety and efficacy should be highly cost-effective.

REFERENCES


PREVALENCE OF HEPATITIS C VIRUS ANTIBODY IN NEWBORN INFANTS IN SOUTHERN CALIFORNIA IN 2003

John S. Bradley, MD,* Steve Graham, MPH, † Gaston R. Picchio, PhD,‡ Duc J. Vugia, MD, MPH,§ and Martin Kharrazi, PhD¶

Abstract: The prevalence of hepatitis C virus (HCV) antibody in newborn infants in 3 counties in southern California in 2003 was found to be 2.5 per 1000 live births using dried blood spot testing. With advances in HCV antiviral therapy providing decreasing morbidity from chronic HCV infection, prenatal HCV screening to identify both mothers and at-risk infants should be reconsidered.

Key Words: hepatitis C virus, newborn screening, epidemiology

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Hepatitis C virus (HCV) is one of the most common causes of chronic hepatitis in developed countries and represents the most common indication for liver transplantation in adults in the United States.1,2 In 1990, blood donor screening for HCV was implemented in the United States and dramatically reduced the number of new cases of HCV infection. However, infection continues to occur within certain populations, including those who engage in injection drug use, those with multiple sexual partners, and infants born to HCV-infected pregnant women.3 The sero-prevalence of HCV in the United States was approximately 1.8% in 1988 to 1994 during the Third National Health and Nutrition Examination Survey,4 although within high-risk groups, considerably higher rates of infection existed. Centers for Disease Control and Prevention estimated that 17,000 new infections caused by HCV and 43,000 caused by hepatitis B virus occurred in the US population in 2007, with the total disease burden of persons with chronic infection estimated at 2.7 to 3.9 million for HCV and 800,000 to 1.4 million for hepatitis B virus.5 The rate of HCV infection as assessed by the Third National Health and Nutrition Examination Survey for children 6 to 12 years of age was 0.2%, whereas the rate for those 12 to 19 years of age was approximately 0.4%.6

Although transfusion-transmitted infection has been virtually eliminated as a cause of hepatitis C in children, vertical transmission from HCV-viremic mothers at the time of delivery continues as a source of HCV infection for children born in the United States.6 Data from Europe, Asia, and the United States have prospectively documented vertical transmission rates of hepatitis C in children born to HCV-seropositive mothers, with risk of infection of 2% to 7% in an infant born to an human immunodeficiency virus (HIV)-negative mother. The transmission rate for infants born to mothers coinfected with HIV and HCV is 3-fold higher.7,8 As infants with perinatal acquisition of HCV infection are rarely symptomatic during childhood, the number of cases of HCV from infants and children passively reported to health authorities will underestimate the disease burden.

Routine prenatal screening of all pregnant women for hepatitis C infection has not been deemed to be cost-effective.9 However, local or regional HCV prevalence data should provide a more accurate assessment for state and local health officials to better address cost-effectiveness for HCV treatment in mothers and possible prevention of infection in the newborn infant. We assessed the HCV serostatus of mothers in southern California through their newborn infants by means of a drop of blood routinely collected by heel stick onto filter paper.10
MATERIALS AND METHODS

Human Research Authorization. The study protocol was approved by the State of California Health and Human Services Agency Committee for the Protection of Human Subjects (Project No. 02-10-07). Information on the newborn’s sex; birth weight; parity; mother’s and father’s age, race, and level of education; month of pregnancy when prenatal care began; prenatal care health insurance (private vs. public); and mother’s birth place (the United States vs. Mexico vs. other) was obtained from live-birth records linked to screening-program data and specimens. The Scripps Research Institute institutional review board and the Sharp Healthcare institutional review board approved this project for both obtaining samples of blood from adults with chronic HCV infection to establish assay conditions, as well as for the analysis of neonatal heel stick blood samples for antibody to HCV.

Sample Acquisition. Heel stick blood spot specimens are routinely collected on filter paper from nearly all newborn infants to perform screening tests for genetic disorders through the California Department of Public Health, Genetic Disease Screening Program. Unused samples are stored indefinitely at −20°C. Dried blood spots from 2806 infants born from February 1 through 16, 2003, and received from the regional laboratory serving birth hospitals in San Diego, Orange, and Imperial counties in southern California were selected. A single deidentified blood spot sample was assayed for antibodies to HCV. Specimens that tested positive for HCV were subsequently tested for antibody to HIV.

Sample HCV Antibody Assay. The standard 15 mm diameter filter paper circle containing the newborn blood was cut into 2 equal half-sample portions. A single half-sample was placed in a single well within a 24 well plate (BD Falcon 24 Well Cell Culture Plate, BD Biosciences Discovery Labware, Bedford, MA) with 500 microliters of sample buffer provided by the manufacturer of the HCV enzyme immunoassay (EIA) assay (HCV 3.0 EIA, Ortho Clinical Diagnostics, Raritan, NJ). The plate was incubated at room temperature for 30 minutes with orbital shaking, yielding approximately 300 μL of eluted material from each sample.

Optimal assay conditions for dried samples of blood on filter paper as assessed by HCV EIA analysis were evaluated from samples of EDTA-whole blood obtained from known adult HCV-positive patients (n = 10). The mean optical density (OD492) of the HCV-negative control was 0.048. A blood spot sample at a 1/5 dilution of the filter paper eluate was 0.302; the OD492 of a 1/20 dilution was 0.048. At the 1/5 and 1/20 dilutions, 100% and 50% of the HCV positive samples, respectively, provided a positive HCV EIA result when analyzed according to the manufacturer’s recommendations (HCV 3.0 EIA, Ortho Clinical Diagnostics), using a customized assay cutoff. A 1/5 dilution of the eluted neonatal sample material was subsequently used to assay for HCV antibody.

To assess the titer of anti-HCV antibody present in the newborns, serial 2-fold dilutions were performed with filter paper eluate samples, with an assay for HCV antibody performed at each dilution to identify the highest dilution of eluate at which antibody was still detectable. Titters of 1/40 or greater were defined as true positives.

Sample HIV Antibody Assay. The second half of specimens that tested positive for HCV were subsequently tested for antibody to HIV using the FDA approved bioMerieux Vironostika HIV-1 Microelisa System. The assay is an enzyme-linked immunosorbent assay for the qualitative determinate of antibody to HIV-1 in human serum, plasma, and dried blood spots.

Risk Factor Analysis. To assess potential risk factors for HCV infection, the demographic traits of the de-identified HCV-positive neonates were statistically compared with HCV-negative neonates, using Fisher exact test.

RESULTS

We tested 2806 samples and in the preliminary analysis, identified 10 HCV antibody-reactive samples. On retesting these samples in the EIA assay from the same eluted material, all 10 were confirmed as positive. In the confirmatory experiment to assess the titer of antibody present, sufficient eluent remained for subsequent testing from only 3 samples. For 7 newborns with insufficient sample to test from the initial half-circle, the remaining half-circle of the dried-blood filter paper disc was eluted. The 3 samples from the initial elution process remained positive, but out of the 7 newly eluted samples, only 5 (71%) tested positive. Of the 8 repeatedly positive samples, 7 had a titer ≥1/40, whereas the eighth sample was only positive at the lowest dilution tested, ie, 1/5. The 7 high-titer samples, defined as true positives, yielded an HCV seroprevalence of 7 of 2806 (0.25%) in women delivering live-born infants in southern California.

The 7 positive samples for HCV antibody were then assayed for HIV antibody and were all documented to be negative.

Selected characteristics of the 7 positive samples were compared with those of the control population of samples collected during the same interval from the same hospitals. Of the 2806 coded samples assayed for HCV antibody, epidemiologic data were available from the birth record and analyses conducted on 2575 (92%) infants, including all those whose samples tested positive for HCV antibody. In our test population, 51% of mothers were Hispanic, 32% were white, and 17% were “Other.” Although the number of HCV positive samples was small, statistically significant associations were found between lower levels of education in both mother (71% vs. 28% with less than a high school education, P = 0.02) and father (57% vs. 24% with less than a high school education, P = 0.05) for HCV-positive samples compared with HCV-negative. Of the 7 HCV-positive infants, 6 were females (86% of HCV-positive samples were female vs. 49% in HCV-negative samples, P = 0.06). No statistically significant associations were found when comparing HCV-positive samples with negative samples for birth weight, gestational age, parity, mother’s and father’s age, race, month of pregnancy when prenatal care began, private versus public prenatatal care health insurance, and mother’s birth place (the United States vs. Mexico vs. other).

DISCUSSION

HCV infections in infants and children represent a serious public health concern, yet virtually all infections remain unidentified during childhood, with long-term morbidity and healthcare costs becoming apparent only later in life.1,11 Of approximately 4000 women who delivered infants at the Sharp Mary Birch Hospital for Women in San Diego during January to June 2001, only 6 had been diagnosed with hepatitis C infection at some time prior to or during the pregnancy, and carried a discharge diagnosis of hepatitis C infection following delivery of their infants (data not shown). We documented a rate of maternal infection of at least 7/2806 (0.24%), almost twice that found by discharge diagnosis. Although our definition of HCV-positivity was based on a high neonatal titer of HCV antibody, a more accurate assessment of HCV infection using recombinant immunoblot techniques or by using HCV rtPCR was not possible in our study given the limited volume of sample eluent to test, and a priority to test for HIV infection.

Both HIV and HCV are known to coinfect certain high-risk populations, and have been well-documented to occur in pregnant women.7,8,12 We were surprised that none of the HCV-positive neonatal samples were also positive for antibody to HIV, as common risk factors exist for both infections. The epidemiology of acquisition of HCV infection in women prior to pregnancy in southern California may be different than for other populations in...
the United States in which a greater association with HIV exists.\(^7\) In our demographics analysis, we also found that both the mother and the father of HCV-positive infants reported a level of education below than that found from parents of HCV-negative infants. The reasons for this association are not clear, and it was not possible to collect additional epidemiologic data that might have provided insight into this risk factor.

Although some authorities have indicated that routine screening of all pregnant women is not cost-effective and therefore is not advocated.\(^9\) Centers for Disease Control and Prevention and American College of Obstetrics and Gynecology have both recommended that pregnant women with HCV risk factors, including injection drug use or receipt of blood transfusion before July 1992, be targeted for screening and counseling.\(^13\) Unfortunately, selective HCV screening of high-risk pregnant women may not identify all pregnant women with HCV infection as documented in Scotland where selective antenatal screening failed to identify 72\% of HCV infections.\(^14\) Routine screening of pregnant women for HCV at the prenatal visit may provide a potential means to prevent transmission for that pregnancy and identify women who may choose to undergo treatment for HCV infection at the end of the pregnancy. Infants who become infected can be identified and can benefit from newer antiviral therapy regimens that are more effective and better tolerated than regimens currently used for children.\(^15,16\)

The data from this study can be used to help assess the overall cost-effectiveness of screening and early treatment for hepatitis C for both mothers and their offspring. Given a rate of infection of at least 0.24\% in our pregnant population and a perinatal vertical transmission rate of approximately 5\%, the prevalence of actual HCV infection in newborn infants in southern California can be estimated to be approximately 0.0125\% from this 2003 birth cohort. Of the approximately 540,000 live births in California in 2003, 70 infants will develop hepatitis C virus infection. Of infants who develop perinatal infection, about one-fourth will experience a spontaneous clearing of infection, but 50 infants may develop chronic HCV infection annually, representing a significant illness burden to the California health care system for liver failure and hepatocellular carcinoma later in life.\(^15,17\) The ability to detect asymptomatic HCV infection in pregnant women and to treat both mothers (postpartum) and their infants to prevent chronic liver disease are compelling reasons to reassess the benefits of routine prenatal screening for HCV.

ACKNOWLEDGMENTS

The authors thank Donald E. Mosier, MD, PhD (The Scripps Research Institute) for collaboration on the study of vertical transmission of HCV infection; John G. McHutchison, MD (The Scripps Clinic) for collaboration on development of the filter paper assay from adult HCV patients; Linda M. Bradley, PhD (The Sidney Kimmel Cancer Center) and Evan S. Bradley for performing the HCV EIA antibody assays, and the State of California Department of Public Health, Retrovirus Diagnostic Section, Viral and Rickettsial Diseases Laboratory for performing testing for HIV on HCV-positive samples.

REFERENCES


ETIOLOGY OF MENINGITIS AMONG PATIENTS ADMITTED TO A TERTIARY REFERRAL HOSPITAL IN BOTSWANA

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Abstract: This retrospective review evaluated records of cerebrospinal fluid samples between 2000 and 2008 at Princess Marina Hospital in Gaborone, Botswana. Of the 7501 cerebrospinal fluid samples reviewed, Streptococcus pneumoniae (n = 125) and Haemophilus influenzae (n = 60) were the most common bacteria cultured. There were also 1018 cryptococcal and 44 tuberculous meningitis cases. Antimicrobial susceptibilities are described. Public health interventions could decrease the burden of meningitis in Botswana.

Key Words: meningitis, tuberculosis, Africa, vaccine, Pneumococcus, Botswana

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Meningitis is a major cause of mortality and morbidity in developing countries, with mortality rates as high as 30% to 50%. Descriptive meningitis data have been published in other countries in Sub-Saharan Africa but are incomplete in Botswana. We retrospectively reviewed the laboratory records of cerebrospinal fluid (CSF) samples that were collected at the nation's largest public hospital in Gaborone, Botswana, to help guide empirical therapy decisions, to inform decisions about antibiotic availability in specific regions, and to advocate for the introduction of appropriate vaccines.

MATERIALS AND METHODS

Setting. Laboratory data from CSF samples collected between January 1, 2000 and May 31, 2008 at the Princess Marina Hospital in Gaborone were retrospectively reviewed.

Laboratory Methods. After collection, CSF samples were processed at the Botswana National Health Laboratory. They were plated onto sheep blood agar, chocolate agar, and Sabouraud dextrose agar and incubated at 35°C in 5% CO2 (Sabouraud and chocolate agar) or onto sheep blood agar, chocolate agar, and Sabouraud dextrose agar incubated at 35°C in 5% CO2 (Sabouraud and chocolate agar) or ambient air (Sabouraud). Plates were checked daily for 72 hours. Any suspicious for a contaminant was excluded if the sample had a positive culture with organisms that may be contaminants. Except for Cryptococcus, a positive culture with organisms other than Staphylococcus, Streptococcus, and non-lactose fermenting was culture-positive. The CSF leukocyte count was performed using an improved Neubauer counting chamber.

If requested, samples sent to the National Tuberculosis Reference Laboratory (NTRL) were acid-fast stained and examined by fluorescent microscopy. They were also cultured for up to 12 weeks on Lowenstein-Jensen media and susceptibilities to isoniazid, rifampin, ethambutol, streptomycin, and para nitrobenzoic acid were determined.

Case Identification. Laboratory record books and electronic data bases from the Botswana National Health Laboratory were reviewed. Any sample received from a patient who had a second sample submitted within 3 weeks of the first sample was counted as a single positive case. Any positive CSF culture that was suspicious for a contaminant was excluded if the sample had <10 leukocytes/mL. Cryptococcal meningitis was defined by a positive cryptococcal culture. India ink testing was performed on all samples and results were reviewed. Additionally, laboratory record books from the National Tuberculosis Reference Laboratory were reviewed to capture all samples that were processed for workup of tuberculous meningitis (TBM).

RESULTS

Study Profile. There were 7501 CSF samples received during the study period. Overall numbers and age ranges of each organism are listed in Table 1. Due to absent record books at National Health Laboratory and NTRL, 17% and 25% of the months were not available, respectively, for analysis.

Bacterial Causes. Although Streptococcus pneumoniae was the most common bacterial organism overall, Haemophilus influenzae was the most frequent organism in the ≤12-year-olds and ≤5-year-olds, and made up 50% and 55% of the cases, respectively. H. influenzae was seen most commonly between May and September (P < 0.01), whereas S. pneumoniae was seen most commonly between May and October (P < 0.001). Susceptibility of S. pneumoniae samples was 64% to penicillin G (n = 110 samples), 88% to cefotaxime (n = 8), 95% to chloramphenicol (n = 120), 97% to ampicillin (n = 76), and 99% to vancomycin (n = 86). Susceptibility of H. influenzae samples was 38% to amoxicillin/clavulanate (n = 26), 74% to chloramphenicol (n = 42), and 94% to cefotaxime (n = 18). Between the early and latter halves of the study, there was a significant decrease in H. influenzae and S. pneumoniae susceptibility to chloramphenicol (P < 0.05).

Tuberculous Meningitis. Of the 7501 study samples, 876 (12%) were sent to the NTRL for workup of tuberculosis. Of the 44 cases of TBM identified, 14 samples were positive for acid-fast bacilli and 34 were culture-positive. The susceptibilities to streptomycin, isoniazid, rifampin, ethambutol, and para nitrobenzoic acid were 81%, 96%, 96%, 100%, and 100%, respectively.

**TABLE 1.** Organisms Isolated From Cerebrospinal Fluid Samples From Princess Marina Hospital in Gaborone, Botswana

<table>
<thead>
<tr>
<th>Organism</th>
<th>All Ages</th>
<th>Patients &lt;2-mo-old</th>
<th>Patients 2–9-mo-old</th>
<th>Patients 2–9-mo-old</th>
<th>Patients ≥18-yr-old</th>
<th>Cases With Patient of Unknown Age</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cryptococcus spp.</td>
<td>1018</td>
<td>1</td>
<td>3</td>
<td>20</td>
<td>834</td>
<td>160</td>
</tr>
<tr>
<td>Streptococcus pneumoniae</td>
<td>125</td>
<td>2</td>
<td>22</td>
<td>11</td>
<td>79</td>
<td>11</td>
</tr>
<tr>
<td>Haemophilus influenzae</td>
<td>60</td>
<td>3</td>
<td>45</td>
<td>5</td>
<td>5</td>
<td>2</td>
</tr>
<tr>
<td>Mycobacterium tuberculosis*</td>
<td>44</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>37</td>
<td>7</td>
</tr>
<tr>
<td>Salmonella spp</td>
<td>10</td>
<td>0</td>
<td>5</td>
<td>0</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>7</td>
<td>1</td>
<td>0</td>
<td>0</td>
<td>5</td>
<td>1</td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>7</td>
<td>1</td>
<td>1</td>
<td>0</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>Staphylococcus aureus</td>
<td>6</td>
<td>0</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Group B Streptococcus</td>
<td>3</td>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Other</td>
<td>11</td>
<td>1</td>
<td>3</td>
<td>1</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Total positive cultures per age group</td>
<td>1291</td>
<td>11</td>
<td>81</td>
<td>39</td>
<td>975</td>
<td>185</td>
</tr>
<tr>
<td>Total negative cultures per age group</td>
<td>6210</td>
<td>98</td>
<td>701</td>
<td>244</td>
<td>3167</td>
<td>2000</td>
</tr>
</tbody>
</table>

*For M. tuberculosis, culture positive and/or acid-fast bacilli positive cases are included in this number.

†Other total: Staphylococcus epidermidis (3), other Streptococcus spp. (3), Acinetobacter (1), Citrobacter (1), Neisseria meningitidis (1), nonlactose fermenter (1), Pseudomonas aeruginosa (1). In certain settings, these organisms may be contaminants. Except for Cryptococcus, a positive culture with ≥8 leukocytes/mm³ in the CSF of any of the listed cultures was defined as a contaminant and excluded.
Cryptococcal Meningitis. There were 1155 cases of cryptococcal meningitis during the study period. India ink results were recorded on 7499 of the 7501 samples. India ink testing had a sensitivity of 91% (95% confidence interval: 89, 93) and a negative predictive value of 98.6% (95% confidence interval: 98.3, 98.9). A higher proportion of Cryptococcus cases were reported between the May and October months than in the months between November and April (P<0.05).

**DISCUSSION**

These results have provided new data describing the burden of meningitis seen in Botswana. A retrospective review in nearby Pretoria, South Africa, showed similar findings to our data, with 57% of cases being caused by H. influenzae and 33% caused by S. pneumoniae. The Integrated Management of Childhood Illness (IMCI) standard recommendation for empiric meningitis treatment in the absence of known resistance is chloramphenicol plus either ampicillin or benzylpenicillin. When considering the H. influenzae and S. pneumoniae pediatric (≤12 years) cases in this study, these regimens had antimicrobial susceptibilities of 87% (66/76) and 79% (64/81); a third generation cephalosporin (eg, cefotaxime) would have provided 100% (17/17) coverage. The Botswana-specific IMCI committee is now recommending cefotaxime as empiric meningitis coverage for pediatric patients more than 2 months of age.

With increasingly drug-resistant S. pneumoniae and H. influenzae, the implementation of effective vaccines assumes even greater priority. The vaccine program of Botswana is very effective, with coverage rates as high as 96% for the third DTP vaccine. This study should prove useful to national decision makers as they prioritize vaccinations in Botswana—neither the H. influenzae type b (Hib) nor pneumococcal vaccine are included in current national guidelines. After the introduction of a nonavalent pneumococcal conjugate vaccine in South Africa, first invasive pneumococcal disease events in children were reduced by 65% to 83%. A S. pneumoniae nasopharyngeal carriage study in Botswana found that 4 of the invasive serotypes found in the population are present in the nonavalent pneumococcal vaccine. Commencing pneumococcal vaccination in children would also likely lead to decreased invasive pneumococcal disease in the adult population. Similarly, the introduction of Hib vaccines in South Africa precipitated a 65% decrease in the absolute number of invasive Hib cases seen in children. Given our proximity to South Africa, one could estimate that Hib is likely to be responsible for 97% of H. influenzae meningitis cases.

Other regional studies have described higher rates of TBM than this study’s finding that 3.1% of all causes of meningitis were TBM. This was surprising given the high rates of immune suppression and tuberculosis in Botswana. Our results could be due to patient (or caregiver) refusal of lumbar punctures, the cumbersome physician ordering process that currently exists, or the substandard handling of specimens that might limit the yield of cultures. With increasing rates of resistance to first-line antibiotic therapy in Southern Africa, clinicians should prioritize sending CSF samples for TB culture and susceptibility studies.

Our study had a number of limitations that likely limited the magnitude of meningitis cases. First, we were missing 21% of the study period’s laboratory books. Laboratory personnel have now been trained to use Microsoft Excel for electronic record keeping. Second, the number of CSF samples may have been affected by patients declining lumbar puncture due to the misconception that the procedure is what causes the patient’s death. Additionally, many clinical meningitis cases may have been pretreated before hospital CSF analysis, thereby limiting culture yield. S. pneumoniae and H. influenzae were the leading causes of bacterial meningitis. Public health measures could combat these vaccine-preventable diseases to limit sequelae and mortalities. Early diagnosis, improved lay public knowledge, enhanced antibiotic availability, and adherence to Botswana-specific IMCI empiric antibiotic choices will also improve the outcome for those who do become infected. The variance in specific patterns of meningitic organisms across Africa highlights the need for continued local surveillance to determine appropriate healthcare interventions.

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**REFERENCES**


**2009 INFLUENZA A H1N1 INFECTIONS**

**DELAYS IN STARTING TREATMENT WITH OSELTAMIVIR WERE ASSOCIATED WITH A MORE SEVERE DISEASE**

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**Abstract:** Respiratory failure has been the main severe complication described in pediatric patients with influenza A H1N1 2009 (pandemic H1N1) infection. We describe the pandemic H1N1 2009 disease in children who required hospital admission and the patients’ data associated with pediatric intensive care unit admission. Respiratory failure was the main complication. Extrapulmonary manifestations were also observed. Of the
As of June 2009, the infection due to influenza A H1N1 (pandemic H1N1) 2009 was declared a global pandemic by the World Health Organization (WHO). Data on the pandemic H1N1 2009 infection in pediatric patients was scarce at the outset of the pandemic wave. In the first published reports, respiratory failure was the main severe complication described in pediatric patients. Neurologic, cardiologic, and gastrointestinal manifestations, as well as mortality, were also observed.1–4 We present our experience with pandemic H1N1 2009 infection within a large cohort of children admitted to our institution, describing patients’ data associated with pneumococcal coinfection and with PICU admission.

PATIENTS AND METHODS

Our institution (Hospital Sant Joan de Déu) is a 345-bed tertiary pediatric hospital located in Barcelona, Spain. In our region, the incidence of pandemic H1N1 2009 infection among children aged 5 to 14 years old reached its peak between weeks 43 and 46 (1200 infections per 100,000 inhabitants).5 Epidemiologic and clinical data, and information on therapies of admitted patients younger than 18 years with pandemic H1N1 2009 infection were prospectively collected from the 30th to the 50th week of 2009. Patients with respiratory symptoms who required admission and those younger than 36 months admitted with fever without a source were tested for pandemic H1N1 2009. Antiviral treatment with oseltamivir was started without awaiting confirmation in admitted patients who fulfilled influenza-like illness diagnostic criteria of the WHO. This study was approved by the institutional ethics committee.

Detection of Pandemic H1N1 2009 Virus. The pandemic H1N1 2009 infection was confirmed with real-time reverse-transcriptase polymerase-chain-reaction (RT-PCR) (Roche Diagnostics’ Influenza A/H1N1 Detection Set kit, which has a detection limit of <100 copies per PCR reaction).

Definitions. The WHO case definition of influenza-like illness was as a person with sudden onset of fever of more than 38°C and cough or sore throat in the absence of other diagnoses.6 Definitions for community-acquired pneumonia were based on draft guidelines for industry from the US Food and Drug Administration.7 Invasive bacterial disease was defined as isolation by culture of bacterial pathogens in any sterile fluid and/or DNA detection of Streptococcus pneumoniae by real-time PCR.

Statistical Analysis. Descriptive statistics of noncontinuous variables were reported in terms of absolute frequencies and/or rates, and data comparisons were performed using Pearson χ2 test or Fisher exact test when the expected count in any category was <5. Continuous non-normal distributed variables were described in terms of median value with interquartile range (IQR: 25th percentile–75th percentile) and compared using Mann-Whitney U test. A multivariable logistic regression model using the “Enter” method evaluated the association of PICU admission and variables with a univariate P < 0.2. The Enter method builds the equation by entering all of the variables at once.

RESULTS

There were 127 cases admitted (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A714). None had received pandemic H1N1 2009 vaccination. Of 127 patients, 31 were treated with antibiotics before admission.

Clinical Data. The influenza-like illness (ILI) diagnostic criteria of the WHO were fulfilled in 107 of 127 patients. The others had respiratory distress without fever (n = 13), fever without a source (n = 4), atypical febrile seizures without respiratory symptoms (n = 2), and gastrointestinal symptoms without respiratory symptoms (n = 1).

Pneumonia With/Without Pneumococcal Coinfection. Of 127 patients, 78 fulfilled the diagnostic criteria of community-acquired pneumonia, but antibiotics were started or were continued in 95 of the 127 patients at the time of admission. Thirteen of 127 patients had demonstrated pneumococcal coinfection. Comparing pneumococcal-coinfected patients and noncoinfected patients, there were no differences in age (coinfected patients: 50 months [IQR: 18–152]; noninfected patients: 49 months [IQR: 14–103], P = 0.1), but coinfected patients had longer time from onset of fever to admission (96 hours [IQR: 52–132] vs. 32 hours [IQR: 12–120], P = 0.01), higher rate of band neutrophils (0.3 [IQR: 0.08–0.38] vs. 0 [IQR: 0–0.03], P < 0.01), higher levels of C-reactive protein (CRP) (265 mg/L [IQR: 130–290] vs. 14 [IQR: <5–51], P < 0.01), higher temperature (39.9°C [IQR: 39.5–40] vs. 39 [38.5–39.5], P < 0.01) and longer admission stay (5 days [IQR: 3–7] vs. 4 [IQR: 2–6], P < 0.02).

Extrapulmonary Manifestations and Severe Cases. Two previously-healthy infants and a school-age girl with dermatomyositis had acute encephalopathy. Brain magnetic resonance image was normal in all and they had normal sterile cerebrospinal fluid except for one. This was a 3-month-old patient in whom RT-PCR for pandemic H1N1 2009 was positive in cerebrospinal fluid.6 Oseltamivir was started at onset of neurologic symptoms in the 2 infants, after 1 to 5 days of ILI. All completely recovered from the acute episode in 24 to 72 hours but the patient diagnosed at 3 months of age was receiving antiepileptic drugs because of persistence of an altered EEG at 6-month follow-up.

Two previously healthy children had myopericarditis. They were a 4-year-old girl who died in the first hours after admission, and a 14-month-old boy who recovered completely in 4 days. Oseltamivir had been started at the time of admission of both of them, after 5 days of ILI. Three previously healthy school-age children had arrhythmias (2 had frequent ventricularextrasystoles and 1 had paroxysmal supraventricular tachycardia) without electrocardiographic findings of myocarditis or increased levels of troponins. Arrhythmias resolved in 3 days, coinciding with the improvement of respiratory symptoms and fever. One of them developed the arrhythmia after 4 days of fever without respiratory symptoms, and the RT-PCR results were received when he was improving. In the other 2 patients, oseltamivir was started at the time of admission, after 3 to 5 days of ILI. No patients had further episodes at 6 months of follow-up.
A 6-year-old boy receiving antiepileptic treatment (valproate, levetiracetam, and carbamazepine) developed transient hepatic and renal failure, requiring extrarenal support for 3 weeks. The hepatic failure resolved in 5 days after discontinuation of antiepileptic drugs. Oseltamivir was started at the time of admission after 3 days of ILI.

Of 127 patients, 24 were admitted to the PICU. Median PICU stay was 4 days (IQR: 2–7). The main reason for PICU admission was acute respiratory failure (n = 18). Fifteen of 24 patients had previously known diseases (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A714). Noninvasive bi-level positive airway pressure support was effective in 14 of 18 patients with acute respiratory failure. It was used for a median time of 3 days (IQR: 2–4). Nine of 24 needed conventional mechanical ventilation for a median of 4 days (IQR: 2–5): 4 due to respiratory failure, 3 due to consciousness alteration, and 2 due to hemodynamic instability. Bronchoalveolar lavage bacterial culture was performed in those who required conventional mechanical ventilation, all with negative results. No patient required extracorporeal cardiopulmonary support. Table 1 shows detailed differences between patients admitted to PICU and patients nonadmitted to PICU.

Of 127 patients, 4 died. Three with a previously known severe neurologic condition died after withdrawal from life-support, and the fourth died of fulminant myocarditis.

**Antiviral Treatment.** Oseltamivir was started within the first 24 hours of admission in 110 of 127. The duration of antiviral treatment was 5 days except for 7 immunocompromised patients in whom the treatment was prolonged. Oseltamivir was started within the first 24 hours in 110 of 127 (IQR: 3–6). Ten of 24 patients with acute respiratory failure had previously known diseases (n = 18). Fifteen of 24 patients had previously known diseases (Table, Supplemental Digital Content 1, http://links.lww.com/INF/A714). Noninvasive bi-level positive airway pressure support was effective in 14 of 18 patients with acute respiratory failure. It was used for a median time of 3 days (IQR: 2–4). Nine of 24 needed conventional mechanical ventilation for a median of 4 days (IQR: 2–5): 4 due to respiratory failure, 3 due to consciousness alteration, and 2 due to hemodynamic instability. Bronchoalveolar lavage bacterial culture was performed in those who required conventional mechanical ventilation, all with negative results. No patient required extracorporeal cardiopulmonary support. Table 1 shows detailed differences between patients admitted to PICU and patients nonadmitted to PICU.

DISCUSSION

Epidemiologic data in international reports showed that children and young adults were the main age group target for the pandemic H1N1 2009 requiring hospitalization.6 In our series, the school-age children were the most important age-group, as in other series.1,2 Even though young children, especially infants, were those with a higher hospitalization rate,9 the total number of pandemic H1N1 2009 infections was greater among school-age children in our country.

Pulmonary and neurologic chronic diseases were the high-risk medical conditions reported for pediatric patients.3,4 We found similar results.

Not all the patients fulfilled the WHO initial ILI diagnostic criteria. This fact has to be taken into account to control the transmission in admitted patients with respiratory symptoms during the pandemic H1N1 2009 high-prevalence seasons. Extrapulmonary manifestations were observed, and some of them were severe in previously healthy children. In contrast to previously published pediatric series on seasonal and pandemic influenza3,4 cardiac manifestations were notable in our study. Encephalopathy, dehydration, and exacerbation of underlying chronic disease were the other observed complications, as reported by others.1–4,10 Thus, it may still be necessary to test the pandemic H1N1 2009 during high-prevalence seasons in children with unexplained neurologic and cardiac symptoms.

Many patients required PICU admission. As described in the literature,1–4 the most frequent complication was respiratory failure. Noninvasive ventilation support with a bilevel positive airway pressure device was effective for most of the severe respiratory cases.

A large percentage of patients were received antibiotics before admission to the hospital. This might explain the low number of children with proven bacterial coinfection. In our series, the rate of band neutrophils and CRP values could have been used to detect patients with pneumococcal coinfection. In contrast to the results of others,10 in our series coinfection was not a necessary condition for developing a more severe disease in those who required PICU admission.

As most of the patients received antiviral treatment, evolution without it cannot be inferred. However, longer disease evolution before initiation of oseltamivir was found in patients who required admission to PICU. As we did not find any major adverse effects, it is likely that oseltamivir was safe for our patients.
REFERENCES


CASE PRESENTATIONS

Three patients admitted to the National University Hospital with clinically suspected oseltamivir resistance had a series of sequentially collected nasopharyngeal swabs tested for H275Y drug resistance. Clinical suspicion of oseltamivir drug resistance arose when there was a lack of improvement in the patient’s clinical condition, despite treatment with oseltamivir, as perceived by the treating physicians.

Patient J.D.C. was an 8-year-old Chinese boy with a history of chronic renal disease who was on immunosuppressive drugs including prednisolone (20 mg alternate days), mycophenolate mofetil (500 mg twice daily), and cyclosporine (30 mg twice daily) at the time of presentation. J.D.C. presented to the outpatient clinic with 2-day history of a cough and sore throat with no fever or signs involving his lower respiratory tract on August 3, 2009. He was given oseltamivir (60 mg twice daily) on that day and continued on the drug until August 19, 2009. During this period no adjustments were made to his immunosuppressive medications and he had 5 serial respiratory samples taken for drug resistance (H275Y) testing while he was on oseltamivir. His infection eventually resolved and was limited to a mild upper respiratory tract infection. As his fever settled after oseltamivir was started, no screening for bacterial coinfection was thought necessary as his condition had improved.

Patient C.Y.Z. was a 4-year-old Chinese boy with common variable immunodeficiency syndrome. He was initially admitted for 5 days (January 8–12, 2010) for presumed community-acquired pneumonia for which he received ceftriaxone followed by amoxicillin-clavulanate. An influenza reverse transcription polymerase chain reaction (RT-PCR) test done during this admission (January 8, 2010) was negative for all viral subtypes. Blood cultures were also negative. He was readmitted on January 15, 2010 as he had a new onset of fever up to 40°C. On admission, his total white cell count was 11.2 × 10^9/L, with absolute lymphopenia (0.47 × 10^9/L). His repeat chest radiograph showed further progression with additional right lung infiltrates and left lower lobe consolidation. During this time, Pseudomonas aeruginosa and Streptococcus pneumoniae (serotype 23 F) were cultured from sputum (January 15, 2010). The child had not had any previous pneumococcal vaccination. Intravenous cefazidime and amikacin were given from January 15 to 22, 2010. He started on a standard 5-day course of oseltamivir (30 mg twice daily, January 16–20, 2010; 10 doses) for influenza detected on nasal swab PCR and eventually had 6 serial samples taken for drug resistance (H275Y) testing. During this time, he was transferred to the pediatric intensive care unit for bilevel positive airway pressure (BIPAP) and his anti-

MIXTURES OF OSELTAMIVIR-SENSITIVE AND -RESISTANT PANDEMIC INFLUENZA A/H1N1/2009 VIRUSES IN IMMUNOCOMPROMISED HOSPITALIZED CHILDREN

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Abstract: We report on 3 immunocompromised children infected with pandemic influenza A/H1N1/2009 in whom mixtures of oseltamivir-susceptible and oseltamivir-resistant viral populations developed, despite them receiving relatively short-term courses of oseltamivir. In addition, it was found that bacterial coinfections were common, indicating that empiric antibiotics should be considered in such patients when infected with influenza virus.

Key Words: influenza, oseltamivir, drug resistance, H275Y, children, mixture

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Supported by a National University Health System (NUHS) Inter-Depart-
Patient A.M.A. was a 2½-year-old boy with a previous liver transplant for biliary cirrhosis at the age of 13 months, for which he received prednisolone 0.5 mg and tacrolimus 0.5 mg daily. He was admitted on June 3, 2010 for the management of his chronic lung disease and complications of portal vein thrombosis. He developed a respiratory infection with *P. aeruginosa* and was treated with intravenous ceftazidime and amikacin. He responded well to 2 weeks of antibiotics but later developed a new onset of fever of 40°C on July 12, 2010 with diarrhea and was found to have *Clostridium difficile* infection. His fever continued with a worsening of his respiratory function and was transferred to pediatric intensive care unit on July 14, 2010 for noninvasive ventilation (BIPAP). A nasopharyngeal swab was positive for H1N1/2009 infection on July 17, 2010 and he was given a 5-day course of oseltamivir (30 mg twice daily). His chest radiograph showed consolidation in the right perihilar and left retrocardiac regions at this point. Repeat respiratory cultures showed that *P. aeruginosa* was still present and ceftazidime was continued for 7 days. His respiratory function deteriorated and he was intubated and mechanically ventilated on July 25, 2010, eventually requiring high frequency ventilatory oscillation on July 26, 2010. A repeat nasal swab was sent on July 24, 2010 because of clinical suspicion of an H275Y oseltamivir-resistant strain and this returned positive. He was treated with intravenous zanamivir 70 mg 12 hourly (dose corrected for creatinine clearance of 33 mL/min/1.73 m²) on July 26, 2010. His fever settled on July 28, 2010 and he was continued on ventilatory support. Additional nasal swabs were taken on July 30, 2010 and August 1, 2010 to check his influenza H1N1/2009 clearance.

**MATERIALS AND METHODS**

For oseltamivir resistance testing at the Molecular Diagnostics Centre, primers and probes targeting a 756 bp region containing the potential H275Y mutation site on the NA (neuraminidase) gene of the H1N1/2009 genome were designed (based on an NA reference sequence from GenBank: A/Washington/28/2009, GQ499337). The viral RNA was extracted, using the EZ1 virus mini kit v2.0 or the QIAasympDNA Virus/ Bacteria mini kit (Qiagen, Valencia, CA), according to the manufacturer’s instructions. RT-PCR amplification was performed using the OneStep RT-PCR Kit (Qiagen) on a GeneAmp 9700 PCR system (Applied Biosystems Inc, Foster City, CA). The amplified PCR products were separated and visualized on an ethidium bromide-stained 1% agarose gel. The appropriate positive bands were further gel-purified using the QIAquick PCR Purification Kit (Qiagen) and sequenced to detect the presence (or absence) of H275Y, using the BigDye Terminator v3.1 Cycle Sequencing Kit (ABI) on an ABI Prism 3130 Genetic Analyzer. Sequence alignment and inspection were performed using the proprietary software.

Mixtures of wild-type and mutant viral populations were identified by the presence of both *CA* (coding for His, H) and *TC* (coding for Tyr, Y) nucleotides within the sample at codon 275 (ie, H275Y).

For independent confirmatory testing at the National Public Health Laboratory (NPHL), Singapore, pyrosequencing of NA-H275Y was performed according to US CDC protocol published by the World Health Organization on PyroMark Q96 (Qiagen GmbH, Hilden, Germany). The percentage of viral population containing H275Y was determined using the single nucleotide polymorphism module as implemented in the accompanying PyroMark ID software.

**RESULTS**

Each of the patient’s influenza H1N1/2009 test results are summarized in Table 1.

Patient J.D.C.’s 5 serial samples showed an evolution towards the mutant H275Y oseltamivir-resistant phenotype: August 3, 2009 (wild-type), August 7, 2009 (mixed), August 11, 2009 (mixed), August 13, 2009 (mixed), and August 14, 2009 (resistant H275Y mutant). These results were independently confirmed by NPHL testing with their pyrosequencing method. His sixth and last sample was taken on August 17, 2009, which was negative for influenza.

Patient C.Y.Z.’s 6 serial samples demonstrated a detectable fluctuation between oseltamivir-sensitive and -resistant viral populations: January 15, 2010 (wild-type), January 21, 2010 (mutant), January 23, 2010 (predominantly mutant, with emergence of wild-type; reported as a mixture by NPHL), January 26, 2010 (predominantly wild-type, remnants of mutant; reported as a mixture by NPHL), January 29, 2010 (not amplifiable at National University Hospital, reported as low titer wild-type by NPHL), and February 1, 2010 (wild-type).

Patient A.M.A.’s influenza H1N1/2009 initially wild-type viral population (July 17, 2010) developed an oseltamivir-resistant population while receiving oseltamivir, which remained predominant and detectable until July 26, 2010. Subsequently, even longer

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample Date</th>
<th>NUH Results</th>
<th>NPHL Results (% of Mutant)</th>
</tr>
</thead>
<tbody>
<tr>
<td>J.D.C.</td>
<td>August 3, 2009</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>August 7, 2009</td>
<td>Mixture</td>
<td>Mixture (21.2%)</td>
</tr>
<tr>
<td></td>
<td>August 11, 2009</td>
<td>Mixture</td>
<td>Mixture (67.8%)</td>
</tr>
<tr>
<td></td>
<td>August 13, 2009</td>
<td>Mixture</td>
<td>Mixture (68.9%)</td>
</tr>
<tr>
<td></td>
<td>August 14, 2009</td>
<td>Mutant</td>
<td>Mutant</td>
</tr>
<tr>
<td></td>
<td>January 15, 2010</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>January 21, 2010</td>
<td>Mutant</td>
<td>Mutant</td>
</tr>
<tr>
<td></td>
<td>January 23, 2010</td>
<td>Mutant</td>
<td>Mutant (86.2%)</td>
</tr>
<tr>
<td></td>
<td>January 26, 2010</td>
<td>Wild type</td>
<td>Wild type (24.9%)</td>
</tr>
<tr>
<td></td>
<td>January 29, 2010</td>
<td>Not amplified</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>February 1, 2010</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>July 17, 2010</td>
<td>Wild type</td>
<td>Wild type</td>
</tr>
<tr>
<td></td>
<td>July 26, 2010</td>
<td>Mutant</td>
<td>Mutant</td>
</tr>
<tr>
<td></td>
<td>July 30, 2010</td>
<td>Mixture</td>
<td>Mixture (70.8%)</td>
</tr>
<tr>
<td>A.M.A.</td>
<td>August 1, 2010</td>
<td>Not amplified</td>
<td>Mixture (13.7%)</td>
</tr>
</tbody>
</table>

NUH indicates National University Hospital; NPHL, National Public Health Laboratory.
after the end of the 5-day oseltamivir course, wild-type and mutant viruses coexisted as a mixture (July 30, 2010), after which the virus became nondetectable (August 1, 2010).

DISCUSSION

As reported previously, these pediatric cases have demonstrated that oseltamivir resistance can arise within the normal, recommended 5-day course of treatment, as well as when receiving longer treatment regimens. However, while all these previous cases describe an evolution of the virus population from a susceptible to a resistant phenotype, one of our patients (C.Y.Z.) showed a reversible change from susceptible-to-resistant-to-susceptible viral populations, despite receiving only the conventional 5-day course of oseltamivir treatment. Intriguingly, his H275Y mutant viral population remained detectable for up to 4 days after the end of oseltamivir therapy (on January 26, 2010). The eventual replacement of this mutant population with wild-type virus (on February 1, 2010) in the absence of oseltamivir, rather than complete viral clearance, may be a result of his underlying immunodeficiency, which, together with the influenza infection, probably contributed to an exacerbated secondary bacterial infection with P. aeruginosa and S. pneumoniae.

Patient A.M.A. experienced similar trends in the changes in the virus population, but went on to be treated with intravenous zanamivir to clear the virus. This was the only patient who we treated with intravenous zanamivir. Despite this or perhaps because of it, he did not have reversion to wild type or dominance of the mutant form at the end of therapy.

All 3 children were immunocompromised and it is possible that the second and third cases acquired their infections in hospital. These children are the most at risk for severe influenza requiring assisted ventilation which C.Y.Z. and A.M.A. did. Bacterial coinfections, as seen in patients C.Y.Z. and A.M.A., almost certainly contributed to this ventilation demand. Continued replication and shedding of influenza virus in such immunocompromised patients may lead to ongoing damage to the respiratory tract, especially the clinal ladder with a subsequent risk of secondary bacterial infections.

During the earlier stages of the pandemic, alternative drugs used to treat oseltamivir-resistant influenza viral infections included a new neuraminidase inhibitor, peramivir, under emergency use authorization, though this authorization has now been withdrawn and the drug is undergoing formal licensing trials. Intra venous zanamivir therapy has been reported in children previously with some degree of success. Importantly, the timely detection of such mutant, drug-resistant viral populations requires rapid, reliable, accurate diagnostic assays for the results to be immediately, clinically applicable.

ACKNOWLEDGMENTS

The authors thank Ms Shiau Phuah Pheng, Ms Meng Li Teo, and Ms Shu Meng at the NPHE for their assistance with this study.

REFERENCES


ISOLATED NASAL SEPTUM NECROSIS CAUSED BY ASPERGILLUS FLAVUS IN AN IMMUNOCOMPROMISED CHILD

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Abstract: Nontraumatic isolated nasal septal aspergillosis in the absence of sinusitis is a rare but serious infection in immunocompromised patients. Adequate management requires early diagnosis, prompt empiric antifungal therapy, and surgical debridement to prevent progression of life-threatening complications. With the increasing population of immunocompromised children, it is essential for timely management that clinicians have a high index of suspicion for this unusual presentation of aspergillosis.

Key Words: nasal septum, immunocompromised, Aspergillus, children

Acknowledgments

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DOI: 10.1097/INF.0b013e31820b7c3a

Fungal rhino-sino-cerebral infection is a well-described complication of myelosuppressive therapy in patients with malignancy or recipients of stem cell or bone marrow transplant. Reported cases with localized fungal infection involving only the nasal septum are rare and usually described as a complication of recent trauma, dental extraction, or surgical intervention in immunocompromised patients. Nasal septal infection can also result from progression of the fungal infection from the adjacent sinuses. Extensive search of the English language literature involving pediatric patients has identified only 1 case of a 15-year-old boy, an autologous bone marrow transplant recipient for acute myelogenous leukemia, who developed nasal septal necrosis caused by Aspergillus flavus without any antecedent trauma or surgery. We report the second pediatric case of nasal septum necrosis due to...
Aspergillus infection in a child with acute lymphoblastic leukemia with no history of trauma or surgical procedures.

**PATIENT**

A 5-year-old boy was diagnosed with pre-B-cell acute lymphoblastic leukemia in November 2009. On day 11 of remission induction chemotherapy, he was hospitalized for fever and neutropenia without any focal signs or symptoms of infection. Because of his ill appearance, empiric broad-spectrum antimicrobial therapy was initiated with vancomycin and cefepime. After 12 hours, the blood cultures collected from the central venous catheter at the time of presentation showed growth of Gram-positive cocci and Gram-negative bacilli, which were identified as viridans groups *Streptococcus* and *Escherichia coli*, respectively. On day 4 of hospitalization and after fever resolution for 2 days, he was discharged to complete a 14-day course of cefepime. One day after discharge, he was rehospitalized for recurrent fever and left nasal and oral pain. The physical examination was normal. Empiric therapy with vancomycin, meropenem, and micafungin was initiated. Radiographs of the sinuses and panoramic radiograph of dentition were normal. Blood cultures remained negative. Fever resolved within 24 hours of hospitalization, and the left nasal and oral pain was managed with oral morphine and gabapentin. On day 5 of hospitalization, he was discharged with the plan to continue cefepime and micafungin for 7 additional days.

Three days after completion of the antimicrobial therapy, he developed fever, nasal obstruction, and swelling at the left anterior portion of the nasal septum with no history of trauma. He denied headache or nasal drainage. Physical examination revealed a spherical greenish mass, 1 cm in diameter, which was attached to the anterior inferior part of the left nasal septum. The inferior turbinate was mildly enlarged. He had no facial swelling, erythema, or warmth on palpation. Computed tomography scan without contrast of the nose and sinuses showed mucosal thickening with a focal area of soft-tissue prominence in the anterior aspect of the left nasal cavity near the left naris and falk demineralization at the anterior aspect of the nasal septum without evidence of sinusitis (Figure A, Supplemental Digital Content 1, http://links.lww.com/INF/A730). CT scan with contrast of sinuses: normal. Nasal abscesses, septal abscesses, soft-tissue edema of the nasal septum, focal demineralization at the anterior aspect of the left nasal septum. The inferior turbinate was mildly enlarged.

Three days after completion of the antimicrobial therapy, he developed fever, nasal obstruction, and swelling at the left anterior portion of the nasal septum with no history of trauma. He denied headache or nasal drainage. Physical examination revealed a spherical greenish mass, 1 cm in diameter, which was attached to the anterior inferior part of the left nasal septum. The inferior turbinate was mildly enlarged. He had no facial swelling, erythema, or warmth on palpation. Computed tomography scan without contrast of the nose and sinuses showed mucosal thickening with a focal area of soft-tissue prominence in the anterior aspect of the left nasal cavity near the left naris and focal demineralization at the anterior aspect of the nasal septum without evidence of sinusitis (Figure A, Supplemental Digital Content 1, http://links.lww.com/INF/A730). Computed tomography scan of the chest and abdomen showed no evidence of fungal disease. He had been neutropenic for 4 weeks with an absolute neutrophil count ≤100 cells/mm³; however, on the day of evaluation, his absolute neutrophil count began to recover to 800 cells/mm³. He was hospitalized, and empiric therapy with vancomycin, cefepime, and micafungin was initiated. The patient underwent fiberoptic endoscopic evaluation and surgical debridement of the necrotic tissue on the left anterior nasal septum. Pathologic examination and fungal staining of the debris revealed necrotic mucosa with invasive branching septate hyphae (Figure A, Supplemental Digital Content 1, http://links.lww.com/INF/A730). Fungal culture of tissue showed growth of *Aspergillus flavus* (Figure C, Supplemental Digital Content 1, http://links.lww.com/INF/A732).

Blood cultures collected before the initiation of antimicrobial agents did not show growth. *Aspergillus* antigen detection in the serum was negative. On the fifth day of hospitalization, he was discharged after fever resolution for 3 consecutive days. He completed 16 weeks of therapy initially with oral voriconazole and intravenous micafungin followed by oral voriconazole monotherapy, 100 mg twice daily. The patient did not receive granulocyte colony-stimulating factors or granulocyte transfusions during the course of therapy. Chemotherapy was resumed after a 2-week delay without any worsening of the infection. There was complete resolution of the nasal lesion 12 weeks after completion of antifungal therapy.

### TABLE 1. Clinical Course of Patients With Isolated Nasal Septum Aspergillosis

<table>
<thead>
<tr>
<th>Age, Gender</th>
<th>Underlying Disease</th>
<th>Clinical Presentation</th>
<th>Pathology and Microbiologic Tests</th>
<th>Treatment and Duration</th>
<th>Outcome</th>
<th>Recovery at 1-yr follow-up</th>
<th>Recovery at 6-wk follow-up</th>
<th>Recovery at 18-mo follow-up</th>
<th>Recovery at 54-mo follow-up</th>
<th>Recovery at 12-wk follow-up visit after completion of therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td>15 yr, male</td>
<td>ALL</td>
<td>Fever, facial swelling, and tenderness</td>
<td>Nasal abscesses, septal abscesses, soft-tissue edema of nasal septum, focal demineralization at anterior aspect of nasal septum</td>
<td>Surgical drainage of nasal septal abscess, intravenous antifungal therapy (not specified) for 6 wk</td>
<td>Recovery at 54-mo follow-up</td>
<td>Recovery at 12-wk follow-up visit after completion of therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>17 yr, male</td>
<td>ALL</td>
<td>Nasal trauma, erythema, edema, and tenderness</td>
<td>Nasal abscesses and focal demineralization at anterior aspect of nasal septum</td>
<td>Surgical drainage of nasal septal abscess, voriconazole (duration not reported)</td>
<td>Recovery at 18-mo follow-up</td>
<td>Recovery at 12-wk follow-up visit after completion of therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>64 yr, male</td>
<td>ALL</td>
<td>Nasal abscesses, septal abscesses, soft-tissue edema of nasal septum, focal demineralization at anterior aspect of nasal septum</td>
<td>Nasal abscesses, septal abscesses, soft-tissue edema of nasal septum</td>
<td>Surgical drainage of nasal septal abscess, voriconazole (duration not reported)</td>
<td>Recovery at 18-mo follow-up</td>
<td>Recovery at 12-wk follow-up visit after completion of therapy</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 yr, male, present</td>
<td>ALL</td>
<td>Fever, nasal mass, obstruction, and tenderness</td>
<td>Nasal abscesses, septal abscesses, soft-tissue edema of nasal septum</td>
<td>Surgical drainage of nasal septal abscess, intravenous antifungal therapy (not specified) for 6 wk</td>
<td>Recovery at 18-mo follow-up</td>
<td>Recovery at 12-wk follow-up visit after completion of therapy</td>
<td></td>
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</tr>
</tbody>
</table>

AML indicates acute myeloblastic leukemia; BMT, bone marrow transplant; CT, computed tomography; G-CSF, granulocyte-colony stimulating factor; ALL, acute lymphoblastic leukemia.
DISCUSSION

This case report illustrates that isolated nasal septal aspergillosis in immunocompromised patients can present without any history of nasal trauma or progression of infection from adjacent paranasal sinuses. Nasal septal fungal abscesses complicating nasal trauma, surgical procedures, sinuses, and dental infections have been previously reported. To our knowledge, there has been only one report of isolated nasal septal aspergillosis in an immunocompromised child without any antecedent traumatic event. The case was that of a 15-year-old boy with necrosis of the nasal septum because of Aspergillus infection after bone marrow transplantation for acute myelogenous leukemia. The patient had no evidence of sinus involvement and there was no history of trauma. He responded to therapy with amphotericin B and granulocyte colony-stimulating factor. This presentation of aspergillosis is likewise rare in adults. Walker et al reported a 64-year-old man with Crohn disease and pulmonary fibrosis treated with imipenem-sulfamethoxazole, who developed nasal septal abscess due to Aspergillus flavus without sinusitis or history of traumatic event. A summary of the adult and pediatric cases of isolated nasal septum aspergillosis that has been identified by literature review is presented in Table 1. Predisposing risk factors for isolated nasal septum aspergillosis include prolonged neutropenia, severe immunosuppression, trauma or surgical procedures, and broad-spectrum antibiotics therapy.

Patients with nasal septal fungal infection present clinically with nasal obstruction and pain, and possibly with rhinorhhea, fever, and bleeding. Direct clinical inspection reveals swelling with some necrotic tissue at the site of infection as was observed in our patient. Early diagnosis and treatment are essential to prevent the associated complications that range from cosmetic disfigurement such as saddle deformity of nasal bridge to life-threatening invasive fungal infections such as brain abscess, fungal sinusitis, and cavernous sinus thrombosis. Comprehensive direct inspection of the nasal cavity is important. Focal signs of abscess or mass can usually be appreciated. Definitive diagnosis of nasal septal aspergillosis often requires histopathologic examination and fungal staining with culture of the debrided nasal tissue or mass that may show invasive septate hyphae.

The past decade has witnessed significant advances in the diagnosis of invasive aspergillosis using noninvasive assays that detect a polysaccharide cell wall component, galactomannan, in the serum. However, the existing knowledge about the diagnostic role of galactomannan assay has been derived from studies evaluating patients with invasive pulmonary or disseminated aspergillosis. Little is known about the role of galactomannan detection assay because of micafungin that our patient was receiving is a plausible explanation.

When nasal septal fungal infection is suspected, treatment that includes surgical debridement and antifungal agents must be promptly initiated because of the potentially life-threatening consequences of delayed therapy. Voriconazole is the primary drug for the treatment of invasive aspergillosis. The optimal duration of therapy is unknown; extrapolating from the Infectious Diseases Society of America treatment guidelines for pulmonary aspergillosis infection, a duration of at least 6 to 12 weeks of therapy is reasonable. Therapy can be extended beyond this period depending on the clinical response and immune status of the patient. Our patient received almost 16 weeks of therapy to avoid recurrence of infection while receiving intense chemotherapy.

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