HUMAN METAPNEUMOVIRUS-ASSOCIATED LOWER RESPIRATORY TRACT INFECTIONS IN KOREAN INFANTS AND YOUNG CHILDREN

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Abstract: To define the role of human metapneumovirus (hMPV) in previously healthy Korean children, a retrospective study was done on 166 children with lower respiratory tract infections and on their stored nasal aspirates. The *hMPV* gene was detected by reverse transcriptase-polymerase chain reaction. Twenty-six of 166 individuals tested positive for hMPV. The clinical diagnoses of hMPV infection were pneumonia in 15 children and bronchiolitis in 11 children.

Key Words: human metapneumovirus, lower respiratory tract infection, pneumonia, bronchiolitis

Accepted for publication May 20, 2005.

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Supported by grant 04-2003-029-0 from the Seoul National University Hospital research fund.

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DOI: 10.1097/01.inf.0000190042.65120.23

Human metapneumovirus (hMPV) has been identified as an important respiratory pathogen in many parts of the world. Recent reports from Netherlands, Canada, Australia, the United Kingdom, Finland, France and the United States have chronicled patients exhibiting acute respiratory tract infections associated with hMPV, indicating that hMPV infections can occur over a wide age range and worldwide.[1–7]

To determine the role of hMPV in lower respiratory tract infections occurring in immunocompetent Korean children, we conducted reverse transcription-polymerase chain reaction (RT-PCR) tests on respiratory specimens collected during a 32-month period and stored at the Seoul National University Children's Hospital. The hMPV results were analyzed and compared with the results of previously performed tests for other respiratory viruses.

MATERIALS AND METHODS

Patients and Specimens. Nasal aspirates were obtained by mucus traps and catheters from hospitalized children with lower respiratory tract infection (LRTI) at the Seoul National University Children's Hospital. This institute is a tertiary referral hospital with >300 pediatric beds and is located in the center of Seoul. The obtained specimens were refrigerated immediately after collection without stabilizing media and processed in the laboratory within 72 hours. The specimens were divided into 3 test samples each. One sample was used for antigen detection by indirect immunofluorescent staining for respiratory syncytial virus (RSV), adenovirus, influenza virus A and B and parainfluenza viruses 1, 2 and 3. The second was used to isolate respiratory viruses. The remaining portion was kept frozen at −70°C for later use.[8,9]

Among the stored respiratory specimens, we selected those obtained from children younger than 5 years of age who were hospitalized from August 1997 to March 2000 (32-month period covering 3 winters) and then excluded those from children with underlying disease. Finally 166 specimens from previously healthy children were included.

RT-PCR Assay for hMPV and Phylogenetic Analysis. The 166 nasal aspirates were assessed for the presence of hMPV with a RT-PCR assay, using primers in the *L* gene that are able to detect both genotypes of the virus.[1] The primer sequences were as follows: 5′-CATGCCCACTATAAAAGGTCAG-3′(sense);5′-TTTCAAGAA-AGACTGGG-3′ (antisense).

The primer sequence and amplification conditions were provided by Albert D. M. E. Osterhause, and a virus stain that was used as a positive control was provided by Dr. Guy Boivin (a Centre de Recherche en Infectiologie Investigator, Canada).

The viral RNA in the nasal aspirates was extracted with a commercially available kit (QIAamp Viral RNA Mini Spin Kit; QIAGEN, Hilden, Germany). This prepared viral RNA was then used as a template for complementary DNA synthesis. Complementary DNA was generated in a 20-μL reaction volume containing Hexanucleotide Mix (Roche Diagnostics, Mannheim, Germany) and Expand Reverse Transcriptase (Roche Diagnostics). The *hMPV* gene was investigated via PCR using an RT product, AccuPower-PCR PreMix (Bioneer Corp., Daejeon, Korea) and the primers. Optimized conditions were as follows: 2-μl primers in a total reaction volume of 50 μl, with thermal cycling of 5 minutes at 94°C; 40 cycles of PCR for 30, 30 and 45 seconds at 94°C, 52°C and 72°C, respectively; and 10 minutes at 72°C. The *hMPV* gene was confirmed via amplicon sequencing. The sensitivity of the hMPV assay was tested in our laboratory with the use of a clinical isolate that belonged to group 2 provided by Dr. Guy Boivin, and the assay was found to be sensitive to a quantity of 10^{-2} dose infective to 50% of tissues/mL of virus.

To characterize genetic relatedness among the isolates, the strains were compared by the neighbor-joining distance method, with the software Vector NTI suite 7.0 (InforMax Inc., Bethesda, MD), and the tree was constructed using PhyloDraw software ver 0.8 (Graphics Application Laboratory, Pusan, Korea), based on the amplicon sequences.

Review of Medical Records. Demographic and clinical data of all patients were collected with positive RT-PCR for hMPV by a retrospective review of medical records, including age, sex, date of admission, clinical symptoms and signs and laboratory and radiologic findings. LRTIs were categorized as "pneumonia," "bronchiolitis" and "croup" as described elsewhere.[10]

RESULTS

Detection of hMPV in Children With LRTIs. We identified 58 respiratory viruses in the 166 nasal aspirate specimens tested and detected the presence of hMPV in 26 (15.7%) specimens. Other respiratory viruses identified included 15 RSVs, 10 parainfluenza viruses, 4 adenoviruses and 3 influenza viruses. Five (19.2%) of the 26 hMPV-positive specimens had previously yielded other viruses, including 2 RSVs, 2 adenoviruses and 1 parainfluenza virus type 3. hMPV was detected in samples taken in all 3 respiratory seasons. However, hMPV was detected principally in the spring and fall, during which parainfluenza virus and RSV epidemics overlapped (Fig. 1).

Phylogenetic Analysis of hMPV Strains. In a phylogenetic analysis based on the RT-PCR product sequences, the 26 hMPV strains were clustered into 2 *L* lineages, as previously reported[1]; 12 strains belonged to group 1 (which includes the prototype strain from the Netherlands; GenBank accession no. af371337), and 14 strains belonged to group 2. Two distinct hMPV genotypes were cocirculating during the study period. At the nucleotide level, these 2 groups were determined to be 91–96% similar, as compared with 96–100% similarity within group 1 and 95–100% similarity within group 2.

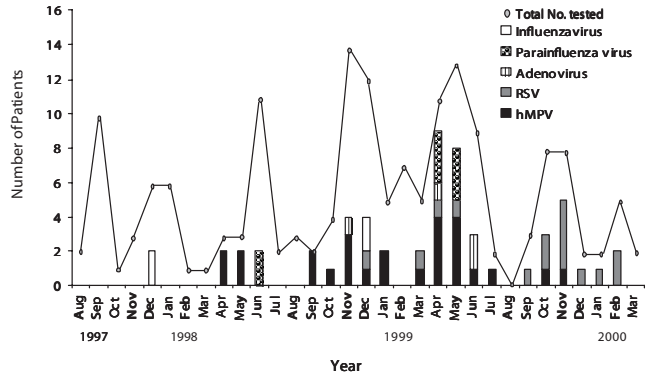

FIGURE 1. Monthly distribution of patients with lower respiratory tract infections and respiratory viruses, identified from patients at the Seoul National University Children's Hospital, August 1997–March 2000. The vertical axis indicates the number of cases.

Demographic and Clinical Findings. Of 26 children with hMPV infection, 17 were boys and 9 were girls (male-female ratio, 1.9:1). The mean patient age was 19.2 months (median age, 15 months; range, 1–49 months). Twelve (46.2%) children were younger than 12 months of age, and 15 (57.7%) children were younger than 24 months of age. The mean duration from symptom onset to hospitalization was 4.2 ± 3.1 days (mean ± SD; median, 3 days; range, 0–14 days). The mean duration of hospitalization in the study patients was 5.8 ± 3.9 days (median, 5 days; range, 1–15 days).

The most frequently reported symptoms and signs included cough (100% of the patients), fever (88.5%), rales (88.5%), tachypnea (80.8%), rhinorrhea (76.9%) and wheezing (42.3%). In addition, diarrhea and/or vomiting occurred in 14 children (53.8%), and viral exanthema occurred in 2 children (7.7%).

We analyzed the clinical symptoms with respective to age (younger than 24 months old versus older than 24 months old) but could not find any differences in clinical symptoms between 2 groups except duration of fever. The duration of fever was longer in older children than in younger children (6.5 ± 4.6 days versus 3.5 ± 2.6 days; $P = 0.046$). Three young children did not have fever although all of older children had fever. Patients also tended to exhibit gastrointestinal symptoms, including vomiting and/or diarrhea (53.8%) and also evidenced mild elevations in alanine aminotransferase/aspartate aminotransferase values (6 of 22, 27.3%, within 2-fold increase above normal value). Five patients required oxygen inhalation, but only 1 patient management in the intensive care unit without ventilator support. Chest roentgenograms revealed abnormalities in all but 2 cases, and the most common finding, in this study, was bilateral peribronchial infiltration. All of the 5 children who were infected with copathogens did not experience more severe disease.

The clinical diagnoses of the hMPV-infected children were pneumonia (15 of 26; 57.7%) and bronchiolitis (11 of 26; 42.3%).

DISCUSSION

This study adds to the evidence suggesting that young children are most susceptible to hMPV infection and that this virus can induce pneumonia and bronchiolitis. This study also reveals the high rate of hMPV infection occurring among children with LRTIs and demonstrates the cocirculation of 2 distinct hMPV genotypes.

Some investigators[10,11] reported that hMPV is much more frequently associated with bronchiolitis than pneumonia. In this study, hMPV was more frequently associated with pneumonia.

We detected hMPV in 16% of our specimens. This percentage is higher than in the results of most of the other studies, although a few studies have reported even higher rates.[10,12]

In this study, hMPV was detected with the highest frequently, followed by RSV, the parainfluenza virus, adenovirus and the influenza virus, in decreasing order of frequency. In previous studies from Korea, in which the presence of hMPV was not assessed, RSV was detected more frequently than any other respiratory viruses, in samples collected during the late fall and winter. However, comparisons of the frequency with which different viruses occur should be interpreted with caution, especially when different assays are used. Nucleic acid amplification is, in general, more sensitive with regard to respiratory viruses than are antigen detections and cultures.[13–15] In this study, the assay used for the detection of hMPV was a nucleic acid amplification assay (RT-PCR), whereas other viruses were detected by antigen detection, via indirect immunofluorescent staining and culture. Therefore the possibility remains that the determined order of frequency would have been different if the same method had been used for the detection of all individual viruses.

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MASTITIS IN CHILDREN FROM BIRTH TO 17 YEARS

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Abstract: Twenty-two cases of mastitis were evaluated between 1995 and 2003. Nine of the children were younger than 2 months of age, and 12 were older than 8 years of age. Girls accounted for 82% of the cases. Seven of the infections were true abscesses. Pathogens included *Staphylococcus aureus* in 5, Gram-negative bacilli in 3, group A *Streptococcus* in 1 and enterococcus in 1. These data suggest that mastitis in children occurs in 2 distinct age groups, neonates and pubescent/postpubescent; however, the clinical disease is similar in both populations.

Accepted for publication May 20, 2005.

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DOI: 10.1097/01.inf.0000190031.59905.9f

Mastitis is uncommon in children. In the past 30 years, there have been at least 4 reviews and 5 case reports in the pediatric medical literature.[1–9] All of the articles describe neonatal disease exclusively. The disease occurs in full term infants and most often in the first 3 weeks of life. Girls account for two-thirds of the cases. In general, systemic signs are mild. Concurrent bacteremia or meningitis is extremely uncommon. *S. aureus* causes >75% of the cases. Gram-negative bacilli, such as *Escherichia coli*, account for most of the other cases. The infection responds well to antibiotics administered for 7–10 days and surgical drainage in cases of abscess. The present report was designed to review mastitis in children younger than 18 years of age in a large children's hospital during a 9-year period. A total of 22 cases are described; 13 of the cases were older than 2 months.

METHODS

The charts were selected for review if they had the International Classification of Diseases, 9th revision, code for mastitis/breast abscess, 611.0, or newborn breast abscess, 771.5. Cases were restricted to nonpregnant, nonpostpartum children younger than 18 years of age. Only cases with definite red, warm, indurated and tender areas in a breast were selected for review.

RESULTS

Population. The review of the medical records from January 1, 1995, through December 31, 2003, identified 22 cases of mastitis/breast abscess. The children ranged in age from 3 days to 17 years with a median age of 10 years. The age distribution was bimodal with 9 children younger than 2 months of age and 12 children between 8 and 17 years of age. One child was 6 months of age. There were 18 girls (82%) and 4 boys (18%). The racial/ethnic composition was 15 white, 4 Hispanic and 3 black.

Presentation. All of the children presented with a red, warm, indurated, tender area in the left (14) or right (8) breast. The illness had been present 1–7 days (median, 2 days) before to coming to the hospital. Five children had been treated with antibiotics. Six children were febrile. The white blood cell count was obtained in 13 cases and was elevated in 6.

Diagnosis and Treatment. Fifteen children had mastitis without abscess and 7 children had mastitis with abscess. The 14 children who were admitted to the hospital included all of the infants and all the children with abscesses. Blood cultures done in all hospitalized children were negative. Cerebrospinal fluid was collected from all infants, and all were normal. Eight children were managed as outpatients. All but one of the children admitted to the hospital received parenteral antibiotics initially and oral antibiotics on discharge. All of the children managed as outpatients received oral antibiotics. Several parenteral and oral antibiotic regimens were used, but cephalexin, administered to 14 children, was the oral antibiotic of choice. Among the 7 children with breast abscesses, 6 underwent incision and drainage procedures, and the seventh child's abscess drained spontaneously. Cultures of the pus yielded *S. aureus* in 4, group A *Streptococcus* in 1 and a Gram-negative bacillus in 1. Four children had drainage from their nipples. These fluids were sent for culture and yielded *S. aureus* in 1, enterococcus in 1, *E. coli* in 1 and an unidentified Gram-negative bacillus in 1. All of the *S. aureus* were susceptible to methicillin. All of the children did well.

DISCUSSION

The age distribution of the cases was remarkable. Nine children were younger than 8 weeks of age, and 11 children were older than the age of 10 years. The reason for this distribution might be related to the presence of enlarged breast tissue in both populations. In the neonate, breast enlargement results from maternal hormones acquired transplacentally. In the case of the female subject older than 10 years, the breast enlargement resulted from hormone production during puberty and the postpubescent period. Among adults, the lactating breast is more susceptible to infection than the nonlactating breast, which is very similar to the neonatal breast. In fact, "witch's milk" produced by neonates of either sex is actually true milk as evidenced by the presence of lactose.[10]

S. aureus was the most common pathogen isolated. Several of antibiotic regimens were used; however, each regimen included an antibiotic with antistaphylococcal activity. Ten of 12 parenteral regimens and 20 of 21 oral regimens were sufficiently broad to include coverage for many Gram-negative bacilli. Regardless of the antibiotic regimen selected, all of the children did well.

The findings on mastitis in older children in the present report suggest that the disease is similar to that observed in neonates. Mastitis and breast abscess in female adolescents may be underreported to pediatricians because female adolescents may seek treatment by gynecologists/surgeons.

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REGIONAL DIFFERENCES IN THE EPIDEMIOLOGY OF INVASIVE PNEUMOCOCCAL DISEASE IN TODDLERS IN GERMANY

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Abstract: In a population-based study, regional differences in incidence, serotype distribution and resistance rates in invasive pneumococcal disease in 1-2-year-old children were related to different day care attendance rates. Day-care attendance appears to be a relevant risk factor in some German states and should be considered for inclusion in the recommendations for pneumococcal vaccination of children at risk.

Key Words: invasive pneumococcal disease, children's day care, incidence, pneumococcal serotypes, antibiotic resistance

Accepted for publication June 9, 2005.

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The hospital surveillance system was supported by grants from the Foundation for Preventive Pediatrics and additional grants from Wyeth Pharma GmbH (Münster, Germany), Aventis Pasteur MSD (Leimen, Germany) and Glaxo SmithKline (Rixensart, Belgium). The National Reference Center for Streptococci is partly funded by the German Ministry of health and social security and receives support among others by Wyeth Pharma GmbH (Münster, Germany). The laboratory surveillance system running at the Robert Koch Institute receives no external funding.

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DOI: 10.1097/01.inf.0000189985.96561.81

In Germany, the use of the 7-valent pneumococcal conjugate vaccine is recommended for children in defined risk groups. Although the influence of day care on pneumococcal carriage and disease has been described in the international literature,[1–3] this risk factor is not included in the recommendations.

There are regional differences in day care attendance between East and West Federal States in Germany, which are greatest in toddlers. Only 4% of all children at 1–2 years of age in West German Federal States (WG) attend day-care facilities as compared with 55% in East German Federal States (EG).[4] Does this affect the regional epidemiology of IPD in toddlers?

METHODS AND RESULTS

Data obtained in a nationwide prospective study from 1997–2002 on invasive pneumococcal disease (IPD) in children in Germany were analyzed by region (WG and EG) regarding age-specific incidence, distribution of serotypes and antibiotic resistance. Active surveillance of IPD cases was performed in hospitals and, independently, in microbiologic laboratories. Cases were included if they were younger than 16 years of age, if they were hospitalized and if *Streptococcus pneumoniae* was isolated from a normally sterile body site.[5]

RESULTS

Although the overall incidence of IPD in children 0–15 years of age was higher in WG than in EG [4.1; 95% confidence interval (CI), 4.0–4.3) versus 3.0 (95% CI 2.7–3.3) per 100,000 of population], the reverse was true for children age 12–35 months of age [WG 11.0 (95% CI 10.3–11.7) and EG 15.8 (95% CI 13.7–18.0) per 100,000, respectively]. In all other age groups, incidence rates in WG were higher than in EG (Table 1). The same observation was made for pneumococcal meningitis; in all age groups except 12–35 months, the incidence was higher in WG than in EG, whereas the reverse was observed for 12- to 35-month-old children.

The proportion of cases based on blood cultures did not differ significantly by region within the age groups but seemed to be generally lower in older children.

Serotype distribution was different in West and East Germany. Pneumococcal serotypes contained in the 7-valent vaccine were less frequently found in isolates from WG than from EG in the total population under surveillance [0–15 years, 58% (95% CI 55–61%) versus 74% (95% CI 66–80%)]. This difference was significant for age 1–2 years, but because of small numbers of cases, significance could not be proved for the other age groups.

Resistance rates to macrolides (erythromycin A) were higher in children from EG with 37% (95% CI 29–46%) than in WG children with 20% (95% CI 17–23%). The greatest difference was observed in the age group 1–2 years.

DISCUSSION

We found an unexpected peak in the incidence of IPD in EG toddlers 1–2 years of age. In children of this age group in EG, the proportion of cases caused by serotypes included in the 7-valent vaccine was highest, mainly because of the higher percentage of serotype 14. Additionally the proportion of resistant strains was highest in this age group and region.

This finding is unlikely to be caused by chance or bias. The number of reported IPD cases remained stable over time in both regions, accounting for a very constant incidence during the study period of 6 years. Ascertainment bias caused by different blood culturing practices is unlikely. The described regional differences were found for both meningitis and nonmeningitis cases.

Response rates in the reporting systems (data not shown) were constant over time and did not differ between WG and EG in the observational period, indicating no reporting bias by region. Because serotyping was done in a single reference laboratory, all strains were stored and typed under standardized conditions.

Differences in incidence, serotype distribution and antibiotic resistance between IPD cases from EG and WG could not be explained by differences in the prevalence of underlying diseases or reported risk factors (data not shown).

The regional differences in attendance of day care facilities are a tempting explanation for the observed differences in incidence, coverage of vaccine serotypes and antibiotic resistance in the age group 1–2 years between EG and WG. This is the age group with the highest difference in day-care attendance between the 2 regions. Day-care attendance is known to have a major impact on pneumococcal carriage[6–8] as well as on the occurrence of IPD in children and their contacts.[1–3]

Some of the vaccine serotypes are more often related to antibiotic resistance than other serotypes.[6] This is particularly valid concerning macrolide resistance of serotype 14. In our data, serotype 14 alone accounted for 65% of the observed erythromycin A resistance. The higher percentage of serotype 14 in cases from EG is likely to account for the higher resistance rates to macrolides (erythromycin) in children from EG than in WG children.

Attendance of day care is likely to explain the spread of resistant strains and the higher rate of IPD in children 1–2 years of age in EG. There are data showing that acute otitis media and respiratory tract infections are more common in children in day care[9] and that antibiotic treatment is frequently prescribed to shorten the time off day-care during febrile illness.[10] Previous use of

TABLE 1. Surveillance of IPD in Children in Germany, 1997–2002

	<1 yr		1–2 yr		3–4 yr		5–15 yr	
	WG	EG	WG	EG	WG	EG	WG	EG
No. of cases								
Reported	808	90	758	162	319	25	456	48
% with blood culture	73 (69–76)*	68 (57–77)	81 (77–83)	71 (63–78)	78 (73–83)	80 (59–92)	65 (61–69)	48 (34–63)
Incidence								
All IPD	24.4 (23.1–25.8)	16.7 (18.8–20.1)	11.0 (10.3–11.7)	15.8 (13.7–18.0)	4.8 (4.4–5.3)	2.9 (2.0–4.1)	1.2 (1.1–1.3)	0.6 (0.5–0.8)
Meningitis	10.9 (9.9–11.9)	8.8 (6.7–11.5)	3.2 (2.8–3.6)	6.2 (4.9–7.8)	1.5 (1.2–1.7)	1.0 (0.5–1.8)	0.4 (0.3–0.5)	0.3 (0.2–0.5)
Serotyped cases								
N	329	39	334	89	134	14	172	18
% vaccine serotypes	57 (52–63)	62 (45–76)	72 (67–76)	85 (76–92)	65 (56–73)	86 (56–97)	29 (22–36)	33 (14–59)
Resistance								
Tested for erythromycin A	295	37	312	77	121	11	155	15
% resistant	20 (16–25)	27 (14–44)	26 (21–31)	47 (35–58)	19 (13–27)	36 (12–68)	8 (5–14)	13 (2–42)

*Numbers in parentheses, Fleiss 95% CI.

antibiotics is an important factor for the selection of more resistant strains.[8,11]

Although children's day care may be regarded as a possible explanation for the observed regional differences in IPD epidemiology of the 1- and 2-year-olds, there is no good explanation for a lower rate of IPD in other age groups in EG. Underreporting is unlikely, given that capture recapture analyses showed a higher case ascertainment in EG, where 91% of the estimated number of cases was ascertained in comparison with WG with 81%.

The known impact of day care on pneumococcal carriage and occurrence of IPD and spread of resistant strains provide a plausible explanation for the findings in our study, although our data cannot prove that day-care attendance is the cause of the increased risk for IPD in 1- to 2-year-old children in EG. Day-care attendance was not ascertained in cases with IPD precluding the chance to test this hypothesis in a case control study design. Because the causal relation between day-care attendance and an increased risk for IPD has been established in a number of previous studies and documentation of a >10-fold higher day-care attendance rate in toddlers in EG than in WG, the use of day-care facilities is a very likely explanation of the increased rate of IPD in that age group in EG.

ACKNOWLEDGMENTS

We thank the Erhebungseinheit für seltene pädiatrische Erkrankungen in Deutschland for continuous support for this study and all reporting physicians in hospitals and laboratories for their time and efforts. We thank particularly microbial laboratories for providing the isolates.

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SECONDARY HEMOPHAGOCYTIC LYMPHOHISTIOCYTOSIS IN TURKISH CHILDREN

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Abstract: Between January 1998 and January 2005, a total of 18 children 2 weeks–72 months of age were diagnosed as having secondary hemophagocytic lymphohistiocytosis. The frequency of secondary hemophagocytic lymphohistiocytosis among total hospitalized patients during this period was 0.05% (18 of 34,250). Of the 18 patients, 8 (44.5%) had bacterial infections; cytomegalovirus and Epstein-Barr virus infections were present in 5 (28%) and 1 (5.5%), patient, respectively. Leishmaniasis was diagnosed in 2 patients (11%), and herpes simplex virus was diagnosed in 2 patients (11%). Six patients died during treatment, and 1 patient was lost to follow-up. The survival rate was 61%.

Key Words: secondary hemophagocytic lymphohistiocytosis, frequency of hemophagocytic lymphohistiocytosis, bacterial infection, viral infection, cytomegalovirus infection

Accepted for publication June 16, 2005.

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DOI: 10.1097/01.inf.0000190043.48182.31

Hemophagocytic lymphohistiocytosis (HLH) is characterized by fever, hepatosplenomegaly, cytopenias, coagulopathy, lipid changes, hypercytokinemia and organ infiltration by phagocytosing histiocytes.[1] HLH can be analyzed broadly into 2 groups: familial HLH; and secondary HLH. Secondary HLH, a sporadic syndrome occurring in infancy or childhood as well as in adults, is associated with a systemic viral, bacterial, fungal or parasitic infection in individuals with an underlying metabolic or immunodeficiency disorder.[2–5] Hemophagocytic lymphohistiocytosis, called macrophage-activating syndrome, has also been described in patients with systemic onset juvenile idiopathic arthritis.[6] Sporadic and familial cases of HLH are often precipitated by acute infections.[7] HLH mimics infectious disorders because of high fever and elevated acute phase reactants.

Hemophagocytic lymphohistiocytosis is not rare in our country.[8–11] This study is designed to describe the clinical features, types and frequency of associated disorders of children with secondary hemophagocytic lymphohistiocytosis.

PATIENTS AND METHODS

Between January 1998 and January 2005, 37 children at the Hacettepe University Faculty of Medicine Section of Pediatric Hematology were diagnosed with HLH. Patients with a concurrent malignancy and/or previous immunosuppressive therapy, macrophage-activating syndrome were excluded from the study; 19 of the 37 children were diagnosed with primary HLH (13 familial and 6 sporadic patients). The remaining 18 children, diagnosed with secondary HLH, are the subjects of this study. Primary HLH was excluded on the basis of negative family history and absence of consanguineous marriage.[12] Information about 4 of these 18 patients has been reported previously.[5,10] Routine blood culture, serologic testing for viral infection, polymerase chain reaction for cytomegalovirus (CMV) (3 patients) and herpesvirus (1 patient) were conducted for all patients. Bone marrow sample by needle aspiration was obtained from all of the patients, and routine serologic and hematologic studies were done by standard procedures. Autopsy examination was possible in only 3 of the 7 patients who died of the disease.

Clinical and some laboratory data of the patients with secondary HLH have been compared with a previous study conducted on 13 patients with familial HLH in our center.[9]

Some clinical and laboratory findings, including hepatomegaly, splenomegaly hemoglobin value, absolute neutrophil count, platelet count and ferritin, lactate dehydrogenase and triglyceride concentrations were compared in both the survivors and the deceased.

RESULTS

Frequency of HLH. Between January 1998 and 2005, 34,250 patients were admitted to Ihsan Dogramacı Children's Hospital. The frequency of secondary HLH among these hospitalized patients during the period was 0.052% (18 of 34,250). The frequency of primary HLH was 0.055% (19 of 34,250).

Age and Gender. The age of the HLH patients at diagnosis was between 0.5 and 72 months (mean ± SD, 15.9 ± 22.0 months; median, 5 months); 12 were boys.

Clinical Data. Hepatomegaly and splenomegaly were present in 78% and lymphoadenopathy in 21% of the patients. Fever was present in 78% of the patients, and neurologic involvement was not observed in any patient.

Laboratory Data. Anemia, thrombocytopenia and elevated transaminases were detected in 16 (88%), 17 (94%) and 13 of 14 (92%) patients, respectively. Neutropenia was detected in 11 patients (61%). Hyperferritinemia was noted in 9 of 12 (75%) patients, and elevated LDH was seen in 10 of 12 patients (83%) tested. Hypertriglyceridemia and hypofibrinogenemia with plasma fibrinogen <100 mg/dL were observed in 10 (83%) and 4 (27%) patients, respectively.

Some clinical and laboratory data were compared in both the patients who survived and those who died. The hemoglobin values in deceased patients are statistically lower than those who survived ($P = 0.0079$). There were no differences for other values for those 2 groups.

Associated Disorders. Seven patients had a documented infection in blood culture, and the infective agents included *Pseudomonas aeruginosa* (3 patients), *Staphylococcus hemolyticus* (1 patient), *Staphylococcus epidermidis* associated with tyrosinosis (1 patient), *Escherichia coli* (1 patient) and *Streptococcus constellatus* (1 patient). *Brucella abortus* was detected in 1 patient. Viral serologic studies revealed the presence of CMV in 5 patients, 1 of whom had negative serologic studies antemortem and was diagnosed as having CMV during an autopsy examination. Epstein-Barr virus (EBV) infection was found in 1 patient. Autopsy examination showed the presence of CMV infection in 3 patients, 2 of whom had positive serology before death. Systemic leishmaniasis was diagnosed in 2 patients. Herpes simplex virus was noted in 2 patients; congenital combined immunodeficiency syndrome was present in one of them (Table 1).

Treatment and Outcome. Two patients were treated in a HLH-94 protocol for 1-week.[13] One patient was treated with high dose methylprednisolone before diagnosis of CMV infection. After diagnosis of CMV infection, she was treated with ganciclovir. Bone marrow transplantation was performed in 1 patient with combined immunodeficiency syndrome. However, this patient was lost to follow-up. One patient had no treatment. Seven patients were treated with antibiotics, and 2 patients who had leishmaniasis were treated with liposomal amphotericin B. Six patients (4 with CMV, 1 with *E. coli* septicemia and 1 with tyrosinemia associated with staphylococcal infection) died of their diseases. One patient who had bone marrow transplantation was lost for further evaluation in the fol-

TABLE 1. Infectious Agents in Patients With Secondary HLH Syndrome

Bacterial	n	Viral	n	Parasite	n	Total No.
Pseudomonas aeruginosa	3	CMV	5	*Leishmania donovani*	2	
Staphylococcus hemolyticus	1	EBV	1			
Staphylococcus epidermidis	1	HSV	2			
Escherichia coli	1					
Streptococcus constellatus	1					
Brucella abortus	1					
Total no.	8		8		2	18

HSV indicates herpes simplex virus.

low-up period. Comparison of some clinical and laboratory data in patients with secondary HLH and patients with familial HLH found no statistically significant differences.

DISCUSSION

Secondary HLH has been reported to be associated with viral, bacterial, fungal and parasitic infections as well as with systemic juvenile idiopathic arthritis and metabolic disorders.[2–5,10,11] Although it has been previously reported that secondary HLH usually develops after viral infection, in the present study the numbers of viral and bacterial infections were almost equal (Table 1). It is interesting that, in this study, the number of patients with EBV infection was lower than that with CMV infection. This could be a result of the younger patients in our study.[14] In infants and young children younger than 4 years of age, CMV infection is more common than EBV infection.[14] On the other hand, the mortality rate (33%) in the present study was high, and 4 of 5 patients (80%) with CMV died of their diseases leading to hemorrhage or multiorgan failure.

Veerakul et al[15] analyzed 52 children with secondary HLH. Their study indicated a statistically significant association between poor prognosis and the patients' age being younger than 3 years. The data of the present study supported this assumption because all of the deceased patients were younger than 3 years. Because there are no definite criteria to differentiate primary from secondary HLH, it is possible that some of our patients might have had primary instead of secondary HLH.

In conclusion, although the case-fatality rate is high in patients with secondary HLH, there is also the possibility of full recovery, and early diagnosis of infections and more effective treatment may improve outcome.[16]

ACKNOWLEDGMENTS

We thank Dr. C. Altay for helpful discussions.

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DISCONTINUATION OF SECONDARY PROPHYLAXIS FOR *PNEUMOCYSTIS* PNEUMONIA IN HUMAN IMMUNODEFICIENCY VIRUS-INFECTED CHILDREN TREATED WITH HIGHLY ACTIVE ANTIRETROVIRAL THERAPY

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Abstract: This study shows the long term safety of discontinuing secondary prophylaxis for *Pneumocystis* pneumonia in 5 human immunodeficiency virus-infected children who had recovered from a confirmed episode of *Pneumocystis* pneumonia, had <15% of CD4 cells at the time of starting highly active antiretroviral therapy and whose CD4 cell counts increased to >15% for ≥3 months during highly active antiretroviral therapy.

Key Words: *Pneumocystis* pneumonia, opportunistic infections, human immunodeficiency virus infection, highly active antiretroviral therapy, prophylaxis

Accepted for publication July 12, 2005.

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DOI: 10.1097/01.inf.0000190038.53813.d2

The use of highly active antiretroviral therapy (HAART) for human immunodeficiency virus (HIV) infection has led to improved survival and an increase in the time free of opportunistic

infections as a result of the improvement in the immune system.[1–3] Even in patients with extremely severe immunodeficiency, CD4 cell counts often increase to more than the level considered at risk for the development of opportunistic infections.[3]

Many studies of HIV-infected adults have evaluated whether CD4 cell recovery can protect against various opportunistic infections,[1,2,4,5] and their results have greatly influenced the recommendations for primary and secondary prophylaxis.[3,6] Antiretroviral therapy can restore immune function, and the period of susceptibility to opportunistic processes continues to be accurately indicated by the CD4 cell counts for patients who are receiving HAART.[6] Thus it is now possible to discontinue prophylactic regimens in adults whose CD4 cell counts increase to a level higher than that considered to indicate a risk of developing opportunistic infections before the introduction of HAART. Stopping prophylactic regimens can simplify treatment, reduce toxicity and drug interactions, lower cost of care and potentially facilitate adherence to antiretroviral regimens.[6]

Pneumocystis pneumonia (PCP) has historically been one of the leading causes of serious illness in HIV-infected subjects[7–9]; although its incidence has declined during the HAART era, it remains the most frequent opportunistic illness in adults and children.[9] Unlike their adult counterparts, it is considered that HIV-infected children must undergo lifelong suppression after treatment of PCP to prevent recurrences.[6,10,11]

This study describes our experience on the long term safety of discontinuing secondary PCP prophylaxis in 5 HIV-infected children with a previous PCP diagnosis who show immune reconstitution during HAART.

MATERIALS AND METHODS

We evaluated HIV-infected children who had recovered from a confirmed episode of PCP, had received trimethoprim-sulfamethoxazole or aerosolized pentamidine as secondary PCP prophylaxis and had a <15% of CD4 cells at the time of starting HAART (defined as a combination of ≥3 antiretroviral agents, including nucleoside reverse transcriptase inhibitors plus a protease and/or nonnucleoside reverse transcriptase inhibitor). The percentage of CD4 cells had to have increased to >15% at 2 consecutive measurements obtained ≥3 months apart in the 3 months before enrollment. Virus load was not an eligibility criterion. All patients failing to fulfil the inclusion criteria were excluded from the study, as were those with a previous episode of toxoplasmic encephalitis. The study was approved by the Ethics Committee of the University of Milan, Milan, Italy, and written informed consent was obtained from the parents or legal guardians of the children.

All of the children satisfying the inclusion and exclusion criteria discontinued secondary PCP prophylaxis, and their demographic data, dates of previous PCP episodes, history of PCP prophylaxis and history of antiretroviral therapy were recorded. At least every 3 months, they underwent physical examinations, routine blood checks, CD4 cell counts measured with an Epics XL flow cytometer (Coulter Electronics, Miami Lakes, FL) and plasma HIV RNA determinations with the use of a commercially available quantitative assay (Cobas Amplicor HIV-1 Monitor Test, version 1.5; Roche Diagnostics, Mannheim, Germany). All the patients were enrolled by the Institute of Pediatrics, University of Milan, Milan, Italy.

The primary endpoint was the development of confirmed or presumptive PCP, or PCP-related death, during the study period. The criteria for a confirmed PCP diagnosis were the presence of immunofluorescence-detected *Pneumocystis jiroveci* cysts in specimens of bronchoalveolar lavage fluid or the histologic demonstration of microorganisms in transbronchial or open lung biopsy samples.[1,2] A presumptive diagnosis was based on the presence of exertional dyspnea and a nonproductive cough, bilateral diffuse infiltrates at chest radiography, abnormal arterial blood gas levels and an unequivocal response to anti-*P. jiroveci* therapy in the absence of another diagnosis.[1,2] The secondary endpoints were death unrelated to the primary endpoints, other HIV infection-related events (ie, stage B and C events of the Centers for Disease Control and Prevention HIV classification), a CD4 count of ≤15% and events unrelated to HIV infection.

RESULTS

Table 1 shows the characteristics of the 5 vertically HIV-infected children who fulfilled the inclusion and exclusion criteria and discontinued secondary PCP prophylaxis with trimethoprim-sulfamethoxazole. All of them were treated with 2 nucleoside reverse transcriptase inhibitors plus a protease inhibitor, which significantly improved their CD4 cell counts and HIV RNA levels.

The median duration of secondary prophylaxis before enrollment was 24 months; the median time between the start of HAART and the discontinuation of secondary prophylaxis was 21 months; the median time from PCP diagnosis to the measurement of >15% CD4 cells was 15 months; the median time from the measurement of >15% CD4 cells and the discontinuation of secondary prophylaxis was 6 months; and the median duration of follow-up after discontinuing secondary prophylaxis was 37 months. There was no case of confirmed or presumptive PCP, death, HIV-related events, ≤15% CD4 cells or serious medical event unrelated to HIV infection during the study period.

DISCUSSION

We here demonstrated that in our experience of 5 HIV-infected children with immune reconstitution after the start of HAART, the discontinuation of secondary PCP prophylaxis was safe. The findings suggest that it is possible to discontinue prophylactic regimens in children provided their CD4 cell counts are higher than 15% (ie, that considered to indicate a risk of developing opportunistic infections before the availability of HAART) and can significantly affect the recommendations for secondary PCP prophylaxis in HIV-infected children.

Previous studies have demonstrated the safety of discontinuing secondary PCP prophylaxis in HIV-infected adults with CD4 cell counts of >200 cells/μL after the start of HAART.[1–6] Reports from observational studies and from a randomized trial, as well as a combined analysis of 8 European cohorts being followed prospectively, support this recommendation.[6] In these studies, patients had responded to HAART with an increase in CD4 cell counts to >200 cells/μL for at least 3 months, and the median CD4 cell count at the time prophylaxis was discontinued was >300 cells/μL.[6] The inclusion criteria of our study in children considered >15% CD4 cells because of the age-related variation in CD4 cell counts. Further studies are required to confirm this value as indicating a low risk of opportunistic infections and to identify the threshold CD4 cell counts that can suggest when it is safe to discontinue the secondary PCP prophylaxis in different age groups.

A previous analysis suggested that discontinuation of opportunistic infections and/or PCP prophylaxis for children who demonstrate immune reconstitution does not lead to excessive rates of serious bacterial infections.[12] The serious bacterial infections that occurred were associated with typical pediatric conditions (proven or presumed pneumonia and sepsis) and pathogens (*Haemophilus influenzae*, pneumococci and streptococci).[12] Although the study was not powered to address specifically the risk of discontinuation of opportunistic infections and/or PCP prophylaxis, no events of PCP were observed among all participants.[12] These data, combined with our findings, support the very low risk of a PCP event after

TABLE 1. Characteristics of Children Treated With Highly Active Antiretroviral Therapy (HAART) Who Discontinued Secondary Pneumocystic Pneumonia (PCP) Prophylaxis

Characteristic	Patient 1	Patient 2	Patient 3	Patient 4	Patient 5
Gender	F	M	F	M	F
Age at PCP diagnosis (mo)	110	4	6	7	4
HAART regimen	Zidovudine + lamivudine + ritonavir	Zidovudine + lamivudine + nelfinavir	Zidovudine + lamivudine + nelfinavir	Stavudine + lamivudine + ritonavir	Stavudine + lamivudine + nelfinavir
Secondary PCP prophylactic regimen	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole	Trimethoprim-sulfamethoxazole
Laboratory values at start of HAART					
CD4 cells (%)	11	13	14	10	7
CD4 cell counts (cells/μL)	308	696	760	470	191
HIV RNA (copies/mL)	346,000	248,000	800,000	710,000	357,600
Laboratory values at discontinuation of secondary PCP prophylaxis					
CD4 cells (%)	22	23	22	21	21
CD4 cell counts (cells/μL)	560	930	910	610	510
HIV RNA (copies/mL)	980	9360	1240	7630	3216
Duration of secondary PCP prophylaxis (mo)	24	21	18	24	24
Time between start of HAART and discontinuation of secondary prophylaxis (mo)	23	20	16	22	21
Time from PCP diagnosis to measurement of >15% CD4 cells (mo)	18	12	12	15	18
Time from measurement of >15% CD4 cells and discontinuation of secondary prophylaxis (mo)	6	9	6	9	6
Duration of follow-up after discontinuation of secondary prophylaxis (mo)	70	24	18	54	37
Primary and secondary endpoints					
PCP	No	No	No	No	No
Death	No	No	No	No	No
HIV-related medical events	No	No	No	No	No
≤15% CD4 cells	No	No	No	No	No
Serious non-HIV-related medical event	No	No	No	No	No

discontinuation of PCP prophylaxis for children who have achieved immune reconstitution.

Previous studies of adults discontinuing secondary PCP prophylaxis have demonstrated that a recurrent episode of PCP is possible (albeit with an extremely low incidence) even when the CD4 cell count is >200 cells/μL.[1–4] This means that clinicians treating both adults and children should consider their patients' history of opportunistic infections, nadir CD4 cell counts and the discontinuation of prophylactic regimens. The patients presenting respiratory symptoms after discontinuing secondary prophylaxis should in any case be closely evaluated for PCP even in the presence of high CD4 cell counts/percentages and complete virus load suppression.

In conclusion, although definitive studies in children are required, our preliminary results suggest that the risk of opportunistic infections or other serious medical events seems to be as minimal in childhood patients as in adult patients whose CD4 cell counts have significantly increased with HAART and who discontinue secondary PCP prophylaxis. Nevertheless all such patients should be carefully monitored for a sudden decrease in the number of CD4 cells, which would require the resumption of prophylaxis.

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INTESTINAL OBSTRUCTION COMPLICATING *YERSINIA ENTEROCOLITICA* SEROTYPE O:21 INFECTION IN AN INFANT

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Abstract: Intestinal obstruction is an uncommon complication of *Yersinia enterocolitica* infection. We report a case of enterocolitis in an 11-month-old infant, complicated by intestinal obstruction. *Y. entercolitica* serotype O:21, previously reported to cause severe disease, was isolated from the patient's stool. Unusual or complicated presentations of yersiniosis may be associated with more pathogenic strains of *Y. enterocolitica*.

Key Words: *Yersinia enterocolitica*, yersiniosis, serotype O:21, intestinal obstruction

Accepted for publication June 16, 2005.

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DOI: 10.1097/01.inf.0000190036.20256.ea

Enteral yersiniosis is an uncommon human disease caused by *Yersinia enterocolitica* (YE) or *Yersinia pseudotuberculosis*. YE can cause illness ranging from self-limited enteritis to life-threatening systemic infection. We describe a case of YE ileocolitis with intestinal obstruction.

CASE REPORT

A previously healthy 11-month-old white girl was hospitalized in November 2004 with a 5-day history of nonbloody diarrhea, fever, vomiting and new onset abdominal distension. Her mother had recently been ill with self-limited diarrhea. Her family lived in a rural community in eastern Ontario and used well water for drinking. There was no history of animal contact or travel.

Examination revealed a febrile (37.9°C axilla), irritable, moderately dehydrated child. Findings included mild generalized abdominal tenderness without peritoneal signs, abdominal distension and mild peripheral edema. She was anemic (hemoglobin, 10.1 g/L). Leukocytosis (36.50×10^9/L) with a neutrophilic predominance (26.60×10^9/L) was present. Blood culture was sterile.

Shortly after admission, abdominal distension increased, accompanied by increased right lower quadrant tenderness. Cefotaxime and metronidazole therapy was started. Abdominal ultrasound examination revealed thickening of the ascending colon, cecum, terminal ileum and ileocecal valve; intussusception was suspected but was ruled out by barium enema. An abdominal computed tomography scan demonstrated inflammation and edema of the ileocecal valves and cecum, with small bowel distension; the right colon was severely inflamed, with almost complete obliteration of the colonic lumen. Laparotomy was performed because of localized right lower quadrant peritonitis and distal intestinal obstruction. Ascites was present, and the terminal ileum, ileocecal valve region, cecum and right colon were thickened and acutely inflamed; the associated mesentery was edematous without fibrosis or creeping fat. An edematous appendix was resected. Localized ileocecal mesenteric lymphadenopathy was noted and biopsied. Pathologic examination revealed reactive lymphoid follicular hyperplasia of the lymph node; the appendix was edematous with fibrinous exudate. Microbiologic cultures were not performed on ascites fluid or tissue.

One day postoperatively, stool obtained on admission was reported to be growing *Y. enterocolitica*, which was eventually identified as serotype O:21, biotype 1B. Metronidazole was replaced by gentamicin; this was later changed to ciprofloxacin. Fever, small bowel obstruction and ascites persisted for 9 days, with no radiologic change. Bowel function then began to improve; by discharge, almost 3 weeks after admission, she was tolerating oral feedings without difficulty. She received a total of 3 weeks of antimicrobial therapy, mainly cefotaxime and ciprofloxacin. Since discharge, the patient has remained afebrile, with good weight gain and no recurrence of gastrointestinal symptoms. Radiologic follow-up has demonstrated thickening of the ileocecal region without evidence of obstruction.

DISCUSSION

YE is a zoonotic bacterium with a wide range of natural reservoirs including rodents, rabbits, farm animals (in particular pigs) and domestic pets. Humans, who are accidental hosts, usually acquire the infection by the foodborne route. YE was first identified as a cause of human disease in 1939. Until 1975, fewer than 100 cases had been reported in the United States. It remains an uncommon cause of foodborne illness in humans, accounting for an estimated 0.6% of all foodborne disease in the United States[1] and 1.5% in Canada (B. Ciebin, personal communication) each year. Recent surveillance suggests that 1 culture-confirmed case occurs per 100,000 persons per year.[2] Outbreaks caused by YE are also rare; between 1990 and 2003, YE caused only 11 (0.4%) of 2631 bacterial foodborne outbreaks (and $<0.1\%$ of all foodborne outbreaks) reported to the Centers for Disease Control and Prevention.[3] From 80 to 85% of cases occur in children younger than 5 years of age. There is no seasonal variation, except in northern Europe and in African-Americans in the United States, where disease is more common in winter.[2,4]

Symptoms of YE infection generally occur within 1 week of exposure and may last up to 4 weeks. A protracted course is more likely in children, of whom about one-third require hospitalization. Uncomplicated enterocolitis, characterized by fever, diarrhea, vomiting and bloody stools, is the most common presentation (two-thirds of cases), and occurs predominantly in children younger than 5 years. In older children, adolescents and adults, illness tends to be shorter (1–2 weeks), and terminal ileitis and mesenteric adenitis are more common. Bacteremia occurs more commonly in infants, immunocompromised individuals and iron-overloaded states but can also develop in normal hosts. Intraabdominal complications (intussusception, intestinal perforation, peritonitis, hepatic or splenic abscesses, cholangitis, toxic megacolon) can occasionally develop and might necessitate surgical intervention. Extraintestinal manifesta-

tions are rare and diverse and include cervical lymphadenopathy, pneumonia, endocarditis, meningitis and osteomyelitis.[4] Postinfectious reactive arthritis, uveitis, erythema nodosum and glomerulonephritis have also been reported.[4]

YE is a non-lactose-fermenting, pleomorphic, Gram-negative bacillus, motile at 25°C but not at 37°C. Recovery of YE from stool is hampered by its slow growth and overgrowth of the organism by normal colonic flora but can be facilitated by use of selective media such as cefsulodin-irgasan-novobiocin agar. Isolates are classified according to biotype and serotype. Based on biochemical reactions, strains are subdivided into 6 biotypes (1A, 1B, 2–5); only biotype 1A is unassociated with human disease.[4] Strains may also be typed according to serologic reactions; more than 50 serotypes of YE have been described, but most pathogenic isolates belong to a limited number of serotypes.[4] Serotypes O:3, O:5,27, O:8 and O:9 cause the majority of human cases worldwide.[4,5] In Europe and Canada, serotype O:3 accounts for ~80% of clinical isolates.[2,6] In contrast, the most commonly identified serotype in the United States is O:8, although serotype O:3 has recently become more prevalent.[2] Virulence factors of YE include an outer membrane protein that enhances attachment to the intestinal lumen, invasins, a heat-stable enterotoxin, a cytotoxin, heme receptors and additional factors that confer resistance to phagocytosis and other host immune responses.[4]

Clinical disease from serotype O:21 has rarely been reported. In 2 studies of YE disease in Canada,[6,7] O:21 accounted for only 0.4 and 0.6% of clinical isolates and 2% of all environmental YE isolates (from water sources and food products).[6] Between 1999 and 2003, only 2 of 2820 clinical isolates (0.07%) in Canada were serotype O:21 (B. Ciebin, personal communication). We found detailed descriptions of only 4 cases of gastrointestinal O:21 infection, all from Canada;[5,8] 2 patients who had surgery had significant inflammation of the terminal ileum, and 1 died. Unique virulence factors have not been described for O:21, but like O:8 strains they are highly pathogenic in mouse models and possess a similar siderophore (yersiniophore) which may enhance pathogenicity.[4,9]

Enterocolitis in healthy hosts is self-limited and may not require therapy. Treatment is warranted in immunocompromised patients with enterocolitis and in those with disseminated infection. In vitro testing usually demonstrates susceptibility to trimethoprim-sulfamethoxazole, piperacillin, aminoglycosides, extended spectrum cephalosporins and ciprofloxacin. Treatment with a third generation cephalosporin coupled with an aminoglycoside, or a fluoroquinolone, is effective in treating complicated disease.[10] Although our patient was immunologically normal and had localized gastrointestinal disease, antimicrobial therapy was administered based on the severity of illness and degree of inflammation at laparotomy; whether this had significant clinical benefit is unclear. The addition of a systemic steroid for its antiinflammatory effect was considered, but none was administered given the lack of evidence for its use and the potential adverse effects.

In our patient, infection with YE serotype O:21 was associated with a severe course of illness. The source of infection is unknown; well water did not yield YE. The patient's mother had a self-limited diarrheal illness; it is possible that she had yersiniosis and that person-to-person transmission occurred. Symptoms at presentation were characteristic of yersiniosis in this age group. The most striking features were the degree of intestinal inflammation observed radiologically and surgically and the prolonged period of intestinal obstruction. Intestinal obstruction has not specifically been reported with YE infection, and in this case was attributed to almost complete obliteration of the colonic lumen by the inflammatory reaction in the bowel wall and ileocecal valves. By computed tomography scan, obstruction was present preoperatively; the fact that surgery was exploratory only and that radiologically, the ascending colonic obstructive changes persisted without evidence of a postoperative dilated small intestine, suggests that the prolonged course was a result of the disease itself and was not a consequence of the surgery. Another notable feature is that the radiologic findings have persisted for several months after the initial presentation. Whether or not a stricture will develop remains to be seen.

ACKNOWLEDGMENTS

We gratefully acknowledge the assistance of the following individuals: Mr. Bruce Ciebin, Head of Enteric and Environmental Microbiology, Ontario Ministry of Health Laboratories, Toronto; Dr. Julie Hurteau-Miller, Department of Radiology and Dr. Frank Chan, Clinical Microbiology Laboratory, Children's Hospital of Eastern Ontario; and Drs. Nicole Le Saux and Tim Karnauchow for reviewing the manuscript.

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SUBPECTORAL ABSCESS A RARE GROUP A β-HEMOLYTIC *STREPTOCOCCUS* INFECTION

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Abstract: Suppurative complications of group A β-hemolytic *Streptococcus* infection can present acutely and progress rapidly if prompt treatment is not initiated. Awareness of uncommon presentations is essential. We report a patient with a rare form of group A β-hemolytic *Streptococcus* disease, subpectoral abscess.

Key Words: subpectoral abscess, group A β-hemolytic *Streptococcus*, *Streptococcus pyogenes*

Accepted for publication June 22, 2005.

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DOI: 10.1097/01.inf.0000190037.79533.34

The incidence of invasive group A β-hemolytic *Streptococcus* (GAβHS) appears to have increased in the recent years.[1,2] Some presentations remain uncommon and unmentioned in the medical literature. Therefore a high index of suspicion is essential for prompt management to be initiated rapidly.

CASE REPORT

A 12-year-old otherwise healthy white boy presented with a chief complaint of right axillary pain that progressed to the right pectoral regions. During the next 5 days, he developed swelling over the right pectoral area. Two days after the onset of right axillary pain, he developed fever. He had no history of injury to the pectoral or clavicular area or the right shoulder but had been more active recently (including tree climbing). There was no history of skin color changes, recent infection to the hand or arm, animal exposure or recent travel.

On physical examination he had a temperature of 38.7°C orally and was a well-nourished boy in moderate pain. The right pectoral area was swollen, tender, firm and not fluctuant. There was no erythema, bruises or other lesions of the overlying skin. The right shoulder had significant limitation in the range of motion in all directions but more on abduction and with active movement than with passive movement.

The white blood cell count was 27,100/mm^3 with 76% neutrophils, 2% band forms, 12% lymphocytes, 8% monocytes, 1% eosinophils and 1% basophils. The erythrocyte sedimentation rate was 70 mm/h, C-reactive protein was 14.4 mg/dL and creatine kinase was 344 IU/L. Blood and urine cultures were obtained. A chest roentgenogram showed soft tissue swelling over the right pectoral area and normal bone, heart and lungs. Radiographs of the right shoulder and humerus showed soft tissue swelling in the axilla with normal bone. A computed tomography scan with contrast enhancement of the pectoral area showed a large abscess measuring 7 × 7 × 5 cm extending from the level of right axillary vein inferiorly almost to the level of the nipple underneath the right pectoralis muscle groups with enlarged axillary lymph nodes measuring 1.5–2 cm.

Computed tomography-guided percutaneous aspiration of the abscess returned 2–3 mL of tan to light brown purulent material. A Gram-stained smear of the fluid showed many leukocytes and Gram-positive cocci in chains. Group A β-hemolytic streptococci were isolated from the fluid. Blood and urine cultures were sterile.

Therapy was started with intravenous nafcillin on admission. When the results of the abscess fluid cultures were available, the antibiotic was changed to intravenous penicillin G and clindamycin. He received a 10-day course of intravenous antibiotics and was discharged home to receive oral clindamycin. At the time of discharge, he still had limitation of movement of the right shoulder joint with some swelling and pain in the right pectoral area. These symptoms persisted for 2 weeks after discharge from the hospital. He received clindamycin for an additional 18 days to complete a 4-week course of antibiotic therapy. The patient completely recovered at the 2-week follow-up after the antibiotics were stopped.

DISCUSSION

Although rarely reported, subpectoral GAβHS abscess has been well described.[3,4] The referring physician in this patient was concerned about osteomyelitis or septic arthritis of the shoulder joint. The patient did not look acutely ill; however, he had very significant localized signs on physical examination. This condition is reportedly associated with previous injury to or infection of the thumb and the index finger, in our patient there was no history of either direct trauma to the pectoral area or infection of the hand. The trauma or infection can be minor and resolve before pectoral signs and symptoms develop.[3]

The pathogenesis of pyomyositis is speculated to include seeding of the muscle from bacteremia after minor trauma to the muscle eventually progressing to suppuration. Bacteremia is rarely confirmed. The pathogenesis of subpectoral abscess is different. The lymphatic drainage from the thumb and the lateral half of the index finger bypasses the epitrochlear lymph nodes and goes directly into the deltoideopectoral group of lymph nodes rather than the axillary lymph nodes.[5] The deltoideopectoral group of lymph nodes lies between the pectoral muscles and the deltoid. Infection of the deltoideopectoral lymph node group causes suppurative lymphadenitis that subsequently develops into a subpectoral abscess.

The review of the literature for similar cases was done using key words GAβHS, GAS, *Streptococcus pyogenes*, pyomyositis, pectoral myositis, pectoral and subpectoral abscess. The results of this search returned only the article by one of us (M.H.R.) that reports a similar case of subpectoral abscess caused by Gaβ HS,[3] Amren[4] in Wannamaker's treatise on GAβHS describes this condition.

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TRANSMISSION OF COMMUNITY-ASSOCIATED METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* FROM BREAST MILK IN THE NEONATAL INTENSIVE CARE UNIT

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Abstract: Methicillin-resistant *Staphylococcus aureus* (MRSA) has become increasingly common in neonatal intensive care units and can lead to severe outcomes. Baby C, of a set of quadruplets, died of MRSA sepsis. The surviving siblings were colonized with MRSA. Expressed breast milk was fed to all infants; tested breast milk samples were all MRSA-positive. Pulsed field gel electrophoresis results of isolates from the infants and breast milk were indistinguishable.

Key Words: community-associated methicillin resistance, *Staphylococcus aureus*, breast milk, intensive care units, neonatal
Accepted for publication July 12, 2005.
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DOI: 10.1097/01.inf.0000189983.71585.30

Increasing prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) is a worldwide problem. MRSA infections became a large problem in neonatal intensive care units (NICUs) in the

1990s. Recent reports revealed that 87% of major NICUs in Japan suffered from MRSA infections. This study of 60 NICUs in Japan showed that infant admissions had an average MRSA infection rate of 5.1%, with the most prevalent causative bacterium of hospital-acquired infection being MRSA (41.9%).[1] Los Angeles County, California, has documented multiple MRSA outbreaks in NICUs.[2] The outbreaks in Japanese NICUs have been caused by health care-associated MRSA, whereas many of the Los Angeles outbreaks have been attributed to community-associated MRSA (CA-MRSA).

CASE REPORT

On January 6, 2004, a 35-year-old (G2P1) mother was admitted to the hospital and delivered premature quadruplets (gestational week 29) by cesarean section; she had undergone in vitro fertilization to become pregnant. Starting on January 7, the mother collected milk in the hospital by electric breast pump (single use disposable collection equipment and common motor). On January 8, the mother reported a rash and inflammation of her breasts 1 day after beginning milk production; the inflammation reportedly progressed to a lump in the left breast, but no diagnosis was made at that time. After she was discharged home on January 10, she continued to pump with her own equipment. Collected milk was frozen for hospital storage. Infants A, C and D were fed breast milk via nasogastric tube starting on January 12. Infant B started breast milk on January 13. Instructions on breast milk collection, storage and utilization were provided by the hospital. All 4 siblings were located in the NICU nursery A, which contains 12 beds. Both parents had skin-to-skin contact with the infants through the Isolette ports but were unable to hold their children. According to hospital policy, parents are instructed to wash their hands in a designated sink for a specified amount of time before contact with the infants.

On January 22, 2004, Baby Girl C, birth weight 1180 g, died of MRSA sepsis after onset of symptoms and positive blood cultures on January 21. On January 23, all 3 siblings were cultured and found to be colonized with MRSA detected on both nares and tracheal cultures. Infant A also had rectal and stool cultures positive for MRSA. Blood cultures of the surviving siblings were negative. Frozen breast milk specimens were cultured, including one collected in the hospital on the day of discharge (January 10) and 3 collected at home (January 12, January 15 and January 22, 2004); all were MRSA culture-positive. The mother's nares were also colonized with MRSA. Antibiotic susceptibility patterns for all available isolates were the same with susceptibility in vitro to clindamycin, trimethoprim-sulfamethoxazole and erythromycin.

The mother's symptoms of rash and breast inflammation that started on January 8 persisted, and the mother was diagnosed with mastitis by her physician on January 30. The infection was treated with dicloxicillin. No cultures were performed. On February 2, the mother was diagnosed with a cesarean section site infection caused by methicillin-sensitive *S. aureus*; the wound was cleaned; no antibiotics were given.

The mother had in vitro fertilization with her prior pregnancy, which was delivered in 2001, as well as the current pregnancy. For the current pregnancy, she was admitted to the hospital on December 8, 2003 and discharged on December 9, 2003 to rule out preterm labor. The quadruplets' 2-year-old sibling had a history of "pimples" in December 2003. The child was not seen by a physician nor were cultures obtained.

In response to the MRSA associated death, hospital staff cultured the remaining 5 infants in NICU nursery A at the time of Baby C's illness onset on January 21. These 5 infants were negative for MRSA. The 3 colonized siblings were cohorted by location and nursing/respiratory staff, placed on contact precautions and treated with systemic and topical antibiotics.

On January 30, 2004, a 19-day-old NICU male infant was found to have a MRSA positive nasopharyngeal culture and coagulase-negative *Staphylococcus* blood culture obtained during a sepsis workup. This infant had shared a room with the quadruplets in NICU nursery A from his day of birth January 11, 2004 until January 22, when he was transferred to NICU nursery D. Surveillance nasopharyngeal cultures were performed on a total of 18 other NICU infants in the nursery exposed to cases in nurseries A and D. No other positive cultures were identified.

A total of 5 confirmed cases colonized or infected with MRSA were identified among neonates. All cases were treated with intravenous antibiotics including vancomycin, cefotaxime and clindamycin. All concurrently received intranasal mupirocin. Except for the quadruplets' mother, no family members were tested; however, the mother, father, 2-year-old sibling and father's nephew who lived with the family completed decolonization procedures including intranasal mupirocin twice a day for 5 days and chlorhexidine showers once a day for 7 days (adults only). After treatment, no follow-up cultures were obtained.

Pulsed field gel electrophoresis (PFGE) was performed on MRSA isolates from the quadruplets, the fifth NICU case, the mother's breast milk and the mother's nares (Fig. 1). Individual DNA fingerprint patterns were produced for isolates with the restriction enzymes *Sma*I *and Eag*I. PFGE patterns of isolates from the 5 infants and breast milk were indistinguishable (0 band differences), compatible with a common source; the maternal nasopharyngeal isolate differed by >7 bands, indicating a different source. Our set of MRSA isolates could not be matched to pulsed field types USA 100-800,[3] including the prevailing Los Angeles County CA-MRSA strain USA 300. Further subtyping at the Centers for Disease Control and Prevention showed isolates contain the staphylococcal cassette chromosome *mec* IVc (SCC*mec* IV) and Panton-Valentine leukocidin (PVL) toxin genes.

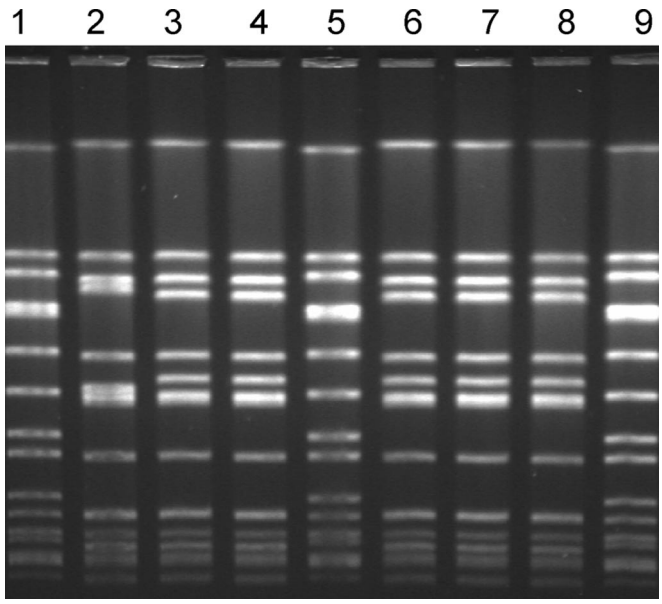

FIGURE 1. PFGE gel case report 1. Lanes 1, 5 and 9, NCTC 8325 standard; lane 2, mother's nares; lane 3, mother's milk; lane 4, infant B trachea; lane 6, infant C blood; lane 7, infant D nasopharyngeal; lane 8, infant A nasopharyngeal.

DISCUSSION

We believe that the index case infant acquired MRSA from her mother's expressed breast milk. Identical strains of MRSA were identified in multiple samples of breast milk and all 5 infant cases. The sibling cases were exposed to the breast milk before infection or colonization with MRSA. None of the infants was breast-fed. The first case occurred after an undiagnosed and untreated case of maternal mastitis. Transmission could have occurred by spread of the infection in milk from the mammary ducts or glands.

Mastitis occurs in 2–33% of breast-feeding mothers; yet there is a consensus to continue lactation despite a history of staphylococcal infection, and women with mastitis are often are encouraged to continue breast-feeding.[4] *S. aureus* is the most frequently isolated pathogenic bacteria in breast milk[4] and is strongly associated with a poor outcome such as maternal abscess or septic fever.[5] The same study also identified sore nipples as being strongly associated with the presence of potentially pathogenic bacterium in breast milk. Studies of bovine mammary gland epithelia demonstrate that *S. aureus* adheres well to these epithelial cells and fat globules in milk, which is the proposed mechanism of dissemination of the bacteria throughout the gland.[6]

Even in healthy women, breast milk is not a sterile fluid. Breast milk from healthy mothers usually contains bacteria representative of normal skin flora. The macerated nipples of a lactating woman can easily be colonized with *S. aureus*.[7] MRSA contamination was found in 11% of 500 expressed frozen breast milk samples at a Brazilian milk bank.[8]

Breast-feeding, maternal carriage and number of siblings each has been shown to be a risk factor for infant carriage of MRSA.[9] Another study specifically linked *S. aureus* and MRSA transmission between healthy, lactating mothers without mastitis and their infants by breast-feeding.[10] However, there is little in the literature describing transmission from MRSA culture-positive breast milk in infants.[11]

We do not know conclusively that breast milk was the mode of transmission. It is possible that the infants became colonized with MRSA during birth or through contact with the parents or health care workers after birth. We also cannot rule out that the breast milk did not become contaminated from a source other than the mother during storage and handling.

All MRSA isolates were susceptible in vitro to clindamycin, trimethoprim-sulfamethoxazole and erythromycin. These isolates contained the SCC*mec* IV and PVL toxin genes; together these characteristics are found in CA-MRSA strains seen in the United States.[12] Additionally the mother's mastitis was consistent with the most common clinical presentation of CA-MRSA of soft tissue infections. Thus although it was hospital-acquired, we believe this outbreak was caused by a CA-MRSA strain. A different strain of MRSA was detected in the mother's nares compared with the mother's breast milk. It has been reported that more than 1 strain of MRSA may colonize or infect a single person.[13]

ACKNOWLEDGMENTS

We thank Judith M. Lanson, RN, CIC, Lisa Wilkins, BS and the Los Angeles County Public Health Laboratory, and Los Angeles County, Acute Communicable Disease Control, especially, Dao Nguyen, MD, Elizabeth Bancroft, MD, SM, Melba Veza, RN, and the public health nurses.

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