

CATHETER-RELATED BACTEREMIA CAUSED BY METHICILLIN-RESISTANT COAGULASE NEGATIVE STAPHYLOCOCCI WITH ELEVATED MINIMAL INHIBITORY CONCENTRATION FOR VANCOMYCIN

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Abstract: Treatment of bacteremia caused by methicillin-resistant coagulase-negative staphylococci with vancomycin minimum inhibitory concentration ≥ 2 mg/L frequently requires central venous catheter removal in children with cancer. There are few data supporting efficacy and safety of antibiotic catheter lock or use of daptomycin or linezolid for this indication in children.

Key Words: pediatrics, vancomycin, daptomycin, linezolid, coagulase-negative staphylococci

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Bloodstream infections are the most frequent complications of indwelling central venous catheters (CRBSI) in children with cancer.¹⁻³ The most recent guidelines published by the Infectious Diseases Society of America⁴ indicate the use of vancomycin as systemic or antibiotic lock therapy for CRBSI, because of methicillin-resistant staphylococci, or the use of linezolid or daptomycin for methicillin-resistant *Staphylococcus aureus* with minimum inhibitory concentration (MIC) for vancomycin of >2 mg/L. These treatments are also recommended for children,⁴ without evidence of effectiveness or safety in pediatrics. Moreover, no suggestions are made for methicillin-resistant coagulase-negative staphylococci (MR-CoNS) that represent the most frequent cause CRBSI in cancer patients.²

MATERIALS AND METHODS

We prospectively recorded all the episodes of bacteremias in children receiving antineoplastic chemotherapy or hemopoietic stem cell transplant at G. Gaslini Children's Hospital, Genoa, Italy, from CRBSI; and bacteremia caused by CoNS were classified according to internationally accepted definitions.^{1,5} Failure of antibacterial treatment was defined by the persistence of positive blood cultures after at least 72 hours or after 7 days of catheter lock with an effective drug, according to susceptibility tests. Pathogen identification and antibiotic susceptibility were performed by the automated Phoenix system (Becton Dickinson, Sparks, MD). All MR-CoNS strains with vancomycin MIC ≥ 2 mg/L were also tested for susceptibility to linezolid and daptomycin.

RESULTS

Between January 2007 and December 2009, 24 episodes of bacteremia caused by MR-CoNS were documented. All

TABLE 1. Effect of MIC for Vancomycin on the Success of Treatment of Bacteremia Caused by Methicillin-resistant Coagulase-negative Staphylococci in Children With Cancer

	MIC for Vancomycin (mg/L)		Total
	≤ 1	2-4	
Catheter treated successfully with standard vancomycin therapy	6 (75%)	1 (6%)	7 (29%)
Catheter saved with vancomycin lock	1 (12.5%)	4 (25%)	5 (21%)
Catheter removed	1 (12.5%)	11 (69%)	12 (50%)
Total episodes of MR-CoNS bacteremia	8 (100%)	16 (100%)	24 (100%)

MR-CoNS indicates methicillin-resistant coagulase-negative staphylococci.

strains were defined susceptible to vancomycin by the automated system, but for 16 isolates (67%) the MIC for vancomycin was 2 to 4 mg/mL. These values were confirmed by E-test. All patients were initially treated with 20 mg/kg (maximum 1000 mg) vancomycin, given every 12 hours.² Among the cases of CRBSI because of MR-CoNS with MIC for vancomycin ≤ 1 mg/L, standard intravenous therapy was successful in 6/8 (75%) cases, whereas when the value was 2 to 4 mg/L, the catheter was saved in only 1/16 cases with standard therapy, and in 4/16 cases with antibiotic lock with vancomycin at a concentration of 3 mg/mL. This distribution of MIC values and need for catheter removal is reported in Table 1, and it is statistically significant ($P = 0.002$, χ^2 test). All the strains with MIC for vancomycin >2 mg/L were tested for susceptibility to linezolid and daptomycin by E-test and MIC values were ≤ 1 mg/L, considered susceptible in all cases.

COMMENTS

The most recent recommendations for treatment of CRBSI include catheter lock or systemic linezolid or daptomycin for persistent bacteremia caused by MR-CoNS, also for children.⁴ However, current data regarding efficacy and safety of linezolid and daptomycin in pediatrics for the treatment of bacteremia are scarce, especially for CRBSI caused by CoNS.⁶⁻⁹ Our data suggest that the definition of CoNS with vancomycin MIC 2 to 4 mg/L as susceptible might be inadequate because of the high proportion of treatment failures observed at least with standard treatment. In these cases, the dosage of 20 mg/kg of vancomycin given every 12 hours was inadequate for the treatment of CRBSI due to such strains and a dosage of 15 mg/kg every 8 or even 6 hours (maximum daily dose 60 mg/kg) could possibly be more appropriate.^{10,11} Our limited experience suggests that catheter lock therapy can be useful in some patients, but more experience is needed. In absence of more data about efficacy and safety of linezolid or daptomycin for CRBSI in pediatric, central venous catheter (CVC) antibiotic lock therapy possibly represents the treatment of choice when the device cannot be removed. Comparative studies to demonstrate the efficacy and safety of new antibiotics in pediatrics are difficult to perform because of the low frequency of the disease.¹²

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RESPIRATORY SYNCYTIAL VIRUS AND STAPHYLOCOCCUS AUREUS COINFECTION IN CHILDREN HOSPITALIZED WITH PNEUMONIA

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Abstract: Respiratory syncytial virus (RSV) infection might facilitate bacterial infection. We describe 5 patients with RSV among 30 children admitted to pediatric hospitals in Atlanta between October 1, 2006 and April 30, 2007 with community-onset *Staphylococcus aureus* pneumonia. RSV-*S. aureus* patients were younger and had less medical comorbidity than those without RSV.

Key Words: RSV, respiratory syncytial virus, pediatrics, pneumonia, *Staphylococcus aureus*

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The findings and conclusions in this report 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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Respiratory syncytial virus (RSV) is the leading cause of bronchiolitis and pneumonia hospitalizations in infants and young children, and causes significant disease in the elderly and adults.^{1,2} There is evidence that RSV, like other respiratory viruses, might facilitate pulmonary bacterial coinfection.³ A temporal association between severe RSV and invasive pneumococcal disease has been shown in individuals, at the community level and in the context of a pneumococcal vaccine trial.^{4–6} Mechanisms by which RSV might lead to bacterial infection may be related to the increased bacterial adhesion in RSV-infected respiratory cells seen in studies in animal models and in vitro systems.^{7–9}

Staphylococcus aureus is a common cause of infections in the community and in healthcare settings, and nasal carriage is found in about one-third of children.¹⁰ It is a well-recognized cause of serious lower respiratory disease and death during both seasonal and pandemic influenza infection among children and young adults.¹¹ Whether RSV contributes to *S. aureus* pneumonia similar to that seen with influenza is not known. We aimed to identify and describe cases of RSV-*S. aureus* pneumonia among children as an initial step to determine the role that RSV might have in *S. aureus* disease.

METHODS

Population. Data were collected previously for a larger study of influenza virus-associated *S. aureus* infections in hospitalized children.¹² Patients admitted to 3 pediatric hospitals in Atlanta between October 1, 2006 and April 30, 2007 with a positive culture for *S. aureus* from either a normally sterile site (eg, blood, pleural fluid) or a respiratory specimen (eg, sputum, tracheal aspirate, or bronchoscopy specimen) were identified through a retrospective review of medical records. A case-series of patients classified with community-onset *S. aureus* pneumonia with RSV-coinfection was defined, as follows.

Classification of Disease. Pneumonia was defined as signs or symptoms of a respiratory process (ie, new or worsened cough, new or worsened sputum production, rales, or worsened gas exchange), plus a new or changed infiltrate on chest imaging, plus signs or laboratory evidence of infection (ie, fever, hypothermia, leukocytosis [$>10,000/\text{mm}^3$], or leukopenia [$<4000/\text{mm}^3$]). Cases of pneumonia were further classified into primary pneumonia (pneumonia that did not develop from another preceding or concurrent site of infection, including a primary bacteremia) and community-onset, if onset of symptoms occurred before admission and the patient had not been hospitalized overnight within the preceding 3 calendar days. *S. aureus* was considered the cause of the pneumonia if a specimen from a sterile site (eg, blood, pleural fluid) or a respiratory specimen (eg, tracheal aspirate, bronchoalveolar lavage, sputum) obtained before the end of the third calendar day after admission (when the day of admission is day 1) grew the organism. We excluded patients with cystic fibrosis, because it was difficult to diagnose pneumonia in these patients and the respiratory tract of children with cystic fibrosis is commonly colonized with *S. aureus*.¹³ Those who had a positive test for RSV antigen within 7 days of their *S. aureus* positive culture were defined as having RSV and *S. aureus* coinfection.

Analysis. Demographic characteristics, underlying medical conditions, disease severity, and outcomes between patients with *S. aureus*-RSV infection and patients with *S. aureus* without RSV were compared. Fisher exact test and Wilcoxon rank sum tests

were used to evaluate associations of patient characteristics with result of RSV antigen test. A *P* value of less than 0.05 was considered significant.

RESULTS

A total of 169 pediatric hospitalizations for community-onset *S. aureus* infection occurred at the 3 hospitals in Atlanta during October 1, 2006 to April 30, 2007. Of these, 30 hospitalizations (18%) in 30 patients were for primary, community-onset *S. aureus* pneumonia and have been described previously.¹⁴ About 17 (57%) patients had a test for RSV antigen while hospitalized, of whom 5 (29%) were positive. Children with RSV-*S. aureus* coinfection were younger than those without RSV (*P* = 0.0065) and were less likely to have an underlying medical conditions (*P* = 0.0276).

Description of RSV-S. aureus Coinfected Children. Of the 5 children with *S. aureus*-RSV pneumonia, 2 were male. Three children were white, 1 was black, and for 1 race was unknown. Three were of Hispanic or Latino ethnicity. Their median age was 5 months (range, <1 month–42 months). Two children had pre-existing medical conditions: first, age 1 month at admission, had been born prematurely at 36 weeks gestation; and the second, age 36 months at admission, had asthma, metabolic disease, seizure disorder, developmental delay, and neuromuscular disorder.

Clinical features of 5 children with RSV and *S. aureus*-associated primary, community-onset pneumonia are presented in Table, Supplemental Digital Content 1, <http://links.lww.com/INF/A531>. Three were admitted with respiratory failure. Symptoms of cough and difficulty

breathing or rapid, shallow breathing were those most commonly reported at the time of admission. Initial chest radiograph or computed tomography scan was abnormal for each patient (Table 1). All were treated with antibiotics upon hospitalization and cared for in the intensive care unit, where their median length of stay was 5 days with a range of less than 1 day to 66 days. All received mechanical ventilation, the median duration of which was 5 days with a range of 1 to 41 days. Four patients recovered. One preterm, 1-month-old infant with multisystem organ failure received extracorporeal membrane oxygenation and died of septic shock.

The *S. aureus* isolate from the fatal case was methicillin resistant. The other 4 patients had isolates that were methicillin susceptible. Three had positive cultures from a single site: either sputum (n = 2) or endotracheal tube (n = 1); 2 had positive cultures from multiple sites: blood and lung tissue (n = 1), sputum, and endotracheal tube (n = 1). The timing of the *S. aureus* infection relative to the RSV infection could not be determined accurately.

DISCUSSION

This case series describes RSV-*S. aureus* coinfection in 5 patients with community-onset pneumonia. All 5 case-patients had severe illness, as indicated by all requiring intensive care unit admission and mechanical ventilation. RSV-*S. aureus* patients were distinct from the other *S. aureus* positive patients in that they were younger in age and were less likely to have pre-existing medical conditions. The younger age of the RSV-*S. aureus* pa-

TABLE 1. Clinical Features of 5 Children With RSV and *Staphylococcus aureus*-associated Primary, Community-onset Pneumonia*

Case	Symptoms [†]	Age (mo)	Initial White Blood Cell Count, X 10 ³ Cells/mL (% Neutrophils)	Chest Radiograph Findings	Days From Illness Onset to Hospitalization	Days From Onset to Positive RSV Test	Days From Onset to Positive <i>S. aureus</i> Culture	<i>S. aureus</i> Culture Site	Outcome	Discharge Diagnosis or Cause of Death
1	Cough, rhinorrhea, dyspnea, respiratory arrest, wheezing, fever, lethargy, cyanosis	18	8.81 (85)	Single lobar infiltrate	3	3	3	Sputum	Alive	RSV bronchitis with severe respiratory distress, <i>Staphylococcus</i> superinfection, tracheitis
2	Cough, dyspnea, vomiting, tachypnea, diarrhea	42	13.23 (73)	Bilateral lower lobe atelectasis	6	5	7	Sputum, ET tube	Alive	Restrictive lung diseases, asthma, feeding problems, RSV pneumonia and ARDS, cerebral dysgenesis
3	Cough, dyspnea, tachypnea, fever, weak breathing, congestion	5	9.98 (50)	Multiple lobar infiltrate (bilateral)	5	5	6	Sputum	Alive	RSV with pneumonia
4	Cough, dyspnea, respiratory arrest, lethargy, difficulty breathing, weight loss	0.5	6.82 (53)	Perihilar markings bilaterally, no effusion	1	6	2	ET tube	Alive	RSV bronchiolitis, respiratory failure, SA pneumonia, shock, atrial septal defect
5	Wheezing, tachypnea, difficulty breathing, congestion	1	Unknown	Large right pneumothorax	6	Unknown [‡]	6	Blood, lung tissue	Died	RSV with septic shock

*“Primary community-onset pneumonia” is pneumonia that did not develop from another preceding or concurrent site of infection, including a primary bacteremia, onset of symptoms occurred in the community and the patient had not been admitted overnight to a hospital within the preceding 3 calendar days.

[†]At presentation to hospital.

[‡]Date of positive RSV test is not available but positive RSV test and diagnosis of RSV bronchiolitis was made during the course of hospitalization which was within 7 d of the date of positive *S. aureus* culture.

SA indicates *Staphylococcus aureus*; RSV, respiratory syncytial virus; ARDS, acute respiratory distress syndrome; ET, endotracheal.

tients is consistent with the age most likely to be hospitalized with RSV disease, while the inverse association with underlying medical conditions could reflect RSV infection as the key predisposing factor to severe disease in the RSV-*S. aureus* patients.

A role of RSV in *S. aureus* pneumonia is plausible, as RSV causes functional changes in the cells of the respiratory tract that facilitate the initiation of bacterial infections with *S. aureus*,⁷ similar to what has been seen with RSV and the adherence of other pathogenic respiratory bacteria including *Streptococcus pneumoniae*, *Pseudomonas aeruginosa*, and *Bordetella pertussis*.^{8,15} Perhaps the most convincing evidence for an RSV-bacterial association is the reduction in hospitalized RSV-pneumonia in children who received a 9-valent pneumococcal conjugate vaccine in the context of a vaccine trial in South Africa.⁴ Furthermore, Stensballe et al⁵ found that Danish children, especially those with a chronic disease, were significantly more likely to develop invasive pneumococcal disease after RSV infection.

S. aureus has been recognized as a cause of severe community-acquired pneumonia in children during the winter respiratory virus season.^{14,16} *S. aureus* pneumonia has been a cause of pediatric death following influenza infection, and *S. aureus*-influenza coinfection appears to have increased in the United States during 2004 to 2007 seasons.¹² In reviews of invasive or respiratory community-onset *S. aureus* infection during influenza season, the proportion of RSV coinfection in children with *S. aureus* disease is similar to that for influenza coinfection.¹⁴

Our findings are subject to limitations. First, RSV testing was not routinely performed in all patients and cases of RSV-*S. aureus* might have been missed. Additional patients with concomitant RSV infection might have been identified if sensitive reverse-transcriptase polymerase chain reaction testing had been used.¹⁷ Second, our definition of *S. aureus* pneumonia included patients in whom *S. aureus* was isolated from a respiratory specimen. These patients may have been colonized by *S. aureus* and may not have had *S. aureus* pneumonia. However, the presence of chest radiograph changes, clinical findings of lower respiratory tract disease, laboratory evidence of a bacterial infection, and positive culture for *S. aureus* is suggestive of *S. aureus* pneumonia. Finally, the patients included in this investigation were neither studied in a way that made it possible to determine the timing of RSV infection relative to the *S. aureus* infection nor to establish an etiologic link between RSV and *S. aureus* infection and disease. These findings among children with *S. aureus* pneumonia suggest the role of RSV and other respiratory viruses on bacterial pneumonia merits further research.

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IMPACT OF ROUTINE FLUCONAZOLE PROPHYLAXIS FOR PREMATURE INFANTS WITH BIRTH WEIGHTS OF LESS THAN 1250 GRAMS IN A DEVELOPING COUNTRY

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Abstract: Systemic fungal infections are associated with substantial case-morbidity and fatality rates in premature infants. Considerable evidence indicates that prophylaxis with fluconazole given to premature infants reduces the risk of invasive fungal infection. There is scant information from developing countries. A comparative study of 2 years, one with fluconazole prophylaxis and the other without was conducted in all premature babies weighing less than 1250 g at birth. Fluconazole was administered in 3 mg/kg doses, given every 48 hours, starting on day 3 of life, for a period of 6 weeks. Documented systemic *Candida* infection was the primary outcome. A total of 271 and 252 patients, respectively, were evaluated during the year before (control group) and after (treatment group) routine fluconazole prophylaxis. The control group developed 21 *Candida* infections (7.7%) while the treatment group had only 3 *Candida* infections (1.1%). This difference was statistically significant ($P = 0.007$; odds ratio, 0.13; 95% confidence interval, 0.03–0.47). The number needed to treat to prevent one case was 7. Although case-fatality rates for documented *Candida* infection were similar in both periods (76% vs. 67%), fewer deaths attributed to the fungal infection were noted in the prophylaxis year (6% vs. 1%, $P = 0.003$). Routine fluconazole prophylaxis given to premature infants of less than 1250 g at birth is associated with a significant impact on frequency of documented systemic *Candida* infections.

Key Words: fluconazole prophylaxis, neonatal prophylaxis, neonatal fungal infection, neonatal candidiasis

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All neonates managed in the neonatal intensive care unit (NICU) are at risk for systemic fungal infections due to invasive vascular procedures, broad spectrum antimicrobial treatments and prolonged hospital stays.¹⁻³ The incidence of *Candida* sepsis has recently increased and it is an important cause of mortality. It is estimated that 4% to 15% of premature infants with very low birth weight (VLBW) develop candidiasis and 30% to 75% die.⁴⁻⁷ *Candida* colonization of NICU infants can be as high as 60% during their first month of life.

Several studies conducted in developed countries have demonstrated that the fluconazole prophylaxis given to VLBW infants significantly reduces the risk of invasive fungal infection.⁸⁻¹⁸ Scarce information has been generated from developing areas of the world.

In our tropical Latin American pediatric hospital, systemic candidiasis has been documented in roughly 7% to 10% of VLBW infants annually during the past years.¹⁹ Therefore, in 2009 we decided to introduce a universal policy of fluconazole prophylaxis to all infants of less than 1250 g. A historic comparison with the previous year, before prophylaxis, was performed.

METHODOLOGY

A comparative study of 2 years, before (2008) and after (2009) the introduction of a routine policy of fluconazole prophylaxis given to all VLBW infants of less than 1250 g admitted to the Neonatology Unit of Hospital del Niño at Panama was performed. The only excluded patients were those who died before the third postnatal day of life when fluconazole was routinely started.

Sample Size. In year 2007, our unit managed 247 infants born with less than 1250 g, and 24 (~10%) cases of candidiasis were documented. For an alpha value of 0.05, a beta value of 80%, and an estimation of reduction to 2% with prophylaxis, we had to recruit at least 130 premature newborns each study year to reach statistical power.

Management of Candidiasis. All infants with suspected or documented fungal infection were treated with amphotericin B deoxycholate at 1 mg/kg daily doses, either for 10 to 14 days (candidemia only) or for a minimum of 30 days (disseminated disease). Definition of invasive candidiasis required isolation of the organism from normally sterile body fluids (primarily blood and CSF). To avoid misinterpretation of fungal colonization or contamination, positive urine cultures were not considered as documentation of infection. Lumbar punctures, eye examinations, liver, spleen, and renal ultrasounds, echocardiography, and bone radiographs were routinely done in all cases. When candidemia was confirmed by blood culture, an immediate removal of central venous catheters was performed.

Fluconazole Prophylaxis. Fluconazole, in a dosage of 3 mg/kg, given every 2 days, started at the third day of life, was administered for a total of 6 weeks, either parentally or orally (gastric tubes where inserted when indicated).

Statistical Analysis. Culture records of all infants managed at out Neonatology Unit during the years of 2008 and 2009 were obtained from the Microbiology Department database. All positive cultures for fungal isolates were then matched with patient hospital charts to determine fulfillment of inclusion criteria. To compare results of both periods, proportions were analyzed by 2-tailed Fisher exact test or

Yates-corrected χ^2 method according to sample sizes. Odd ratios were also calculated. A $P < 0.05$ was considered significant.

RESULTS

A total of 655 infants with birth weights less than 1250 g were identified in both years, of whom 138 were excluded. Reasons for exclusion in year 2008 were death before the third day of life (52 subjects) and admissions after the third day of life (9 subjects). In year 2009, exclusions were related to death before the third day of life (43 subjects), admitted after the third day of life (15 subjects), and amphotericin B treatment for suspected fungal infection with discontinuation of fluconazole prophylaxis (11 subjects). None of the excluded infants who lived after 3 postnatal days had documented *Candida* infection. In total, 271 infants were studied in 2008 and 252 in 2009.

Epidemiological and clinical characteristics of evaluated infants for both years are shown in Table, Supplemental Digital Content 1, <http://links.lww.com/INF/A528>. Sex, gestational age, birth weight, and maternal risk factors for neonatal infection were similar in the 2 groups. Infant conditions implicated in increasing the risk of microbial infection (durations of tracheal intubation, vascular catheterization, parenteral nutrition, antibiotic therapy, and hospital stay) were similar in control and fluconazole-treated subjects. We observed an equal proportion of cases in which clinical sepsis was suspected. Documented sepsis of any etiology, however, was significantly less common in the fluconazole group (12% vs. 23%, $P < 0.05$), mostly as a result of the substantial decrease in *Candida* invasive infections.

In year 2008 (control group), 21 (7.7%) infants developed documented *Candida* infection (positive blood cultures). Fungal isolations were attributed to *Candida albicans* (n = 9), *C. parapsilosis*,⁹ *C. tropicalis*,² and *C. fumata*.¹ In year 2009 (prophylaxis group), only 3 (1.1%) infants developed infection, 1 by *C. albicans*, 1 by *C. tropicalis*, and 1 by *C. parapsilosis*. The difference in rates was statistically significant ($P < 0.001$; odds ratio, 0.13; 95% confidence interval, 0.03–0.47). The number of infants needed to treat to avoid 1 infection was 7. Although case-fatality rates for documented *Candida* infection were similar in both periods (76% vs. 67%), fewer fungal associated deaths in relation to the all-cause mortality were noted in the prophylaxis year (6% vs. 1%, $P < 0.05$). Most deaths occurred in infants of less than 1000 g and 31 gestational weeks at birth (Table 1).

Table 2 shows the number of antifungal drugs used in both periods and their related costs. A net reduction of \$31,428.61 USD during the year of fluconazole prophylaxis was observed.

TABLE 1. Deaths Associated With *Candida* Infection During Both Study Periods According to Selected Demographic Characteristics

	2008 (Control)		2009 (Prophylaxis)	
	N = 21 Cases	16 Deaths	N = 3 Cases	2 Deaths
Sex				
Female	10 (47)	7	0 (0)	0
Male	11 (52)	9	3 (100)	2
Gestational age (wk)				
<27	3 (14)	3	2 (67)	2
27–30	14 (67)	12	1 (33)	0
31–34	4 (19)	1	0 (0)	0
Birth weight (g)				
<750	2 (9)	2	2 (67)	2
750–1000	13 (62)	11	1 (33)	0
1001–1250	6 (28)	3	0 (0)	0

No. cases (percentage).

TABLE 2. Expenses (US Dollars) in Antifungal Therapies Used in 2008 (Before Prophylaxis) and 2009 (During Prophylaxis) for Premature Infants With Birth Weights of 1250 g or Less

Drugs	2008	2009	Savings
Caspofungin	48,793.09	17,766.12	31,026.97
Amphotericin B	7044.10	2221.56	4822.54
Fluconazole	989.00	5409.90	-4420.90
Total	56,826.19	25,397.58	31,428.61

Source: hospital pharmacy.

DISCUSSION

Many publications have determined that *Candida* species constitute an important cause (9%–12%) of late-onset infections in VLBW infants in developed countries and are associated with case-fatality rates of 30% or greater in this birth weight group.^{1–7} Although several studies have demonstrated the efficacy of fluconazole prophylaxis to reduce *Candida* infections in VLBW neonates, its use has not been widely accepted^{20,21} and scant information is available from developing countries.

In 2007, we noted that 10% of our NICU infants weighing less than 1250 g developed fungal invasive infections, and roughly half of them died.¹⁹ We implemented routine prophylaxis with fluconazole for this selected population, starting in January 2009 and compared results with the figures of the precedent year.

Similar to many other studies,^{8–18} we found a significant reduction of invasive *Candida* infection in treated infants, from 7.7% in 2008 to 1.1% in 2009. We determined that 7 VLBW infants needed to be treated to avoid one systemic fungal infection. Although the case-fatality rate of documented candidiasis was similar for both years, fewer infants died of *Candida*-associated infections. Without a thorough histopathologic examination of all deaths (very few autopsies were performed), with or without isolation of *Candida* in blood cultures, it is impossible to conclude, however, that fluconazole prophylaxis decreased specific mortality. Important savings in administration of antifungal drugs were noted in our unit. Additionally, the costs of diagnostic procedures and medical examinations evaluating dissemination in the documented cases of invasive fungal infections would have resulted in even more hospital savings.

In conclusion, we believe that there is adequate information of the benefits of fluconazole prophylaxis for VLBW infants managed in units where *Candida* infections are commonly documented. Once this policy is started, a continuous evaluation of efficacy should be performed to anticipate future shifts in the species of *Candida* isolated and in potential resistance to antifungal drugs.

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SEVERE COINFECTIONS OF DENGUE AND PANDEMIC INFLUENZA A H1N1 VIRUSES

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Abstract: Here we report on 4 hospitalized patients with dengue-influenza virus coinfections. All patients were RT-PCR positive for dengue virus and pandemic influenza A H1N1. Clinical findings at presentation ranged from

influenza-like illness to severe dengue. Clinical progression of the infections varied, but all developed dengue symptoms and had interstitial infiltrates. Three cases required intensive care management and 1 case was fatal.

Key Words: dengue, influenza, coinfection, Nicaragua, children

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In April 2009, a new influenza A virus, pandemic H1N1, caused a severe outbreak in Mexico.¹ The virus quickly spread throughout the world, and the World Health Organization declared a pandemic in June 2009.² In Nicaragua, pandemic H1N1 was first detected on June 1, and high levels of influenza transmission occurred from June to October. The influenza and dengue seasons in Nicaragua do not normally overlap, with epidemics of influenza in May to July and dengue epidemics in August to December.³ High transmission of pandemic influenza outside the normal season resulted in an overlap of influenza and dengue transmission in Nicaragua.

Dengue virus infection can be asymptomatic or produce a range of clinical presentations, from undifferentiated fever to dengue

fever, characterized by abrupt-onset fever with headache, malaise, retro-orbital pain, arthralgias, and/or myalgias, to severe dengue, characterized by plasma leakage that may lead to shock and death. Currently, there is no antiviral therapy for dengue; thus, treatment relies on supportive care, primarily fluid and electrolyte management.⁴

Here we report on the clinical and epidemiological characteristics and laboratory findings from 4 patients with dengue virus serotype 3 (DENV-3) and influenza A H1N1 coinfections. All patients were hospitalized at the National Pediatric Reference Hospital, Hospital Infantil Manuel Jesús Rivera (HIMJR), in Managua, Nicaragua. A summary of clinical characteristics is presented in Table 1.

CASE REPORTS

Case 1

A 5-year-old boy with a history of asthma and recent household exposure to H1N1 presented at a local health center in Boaco, Nicaragua, on September 2 with fever >40°C and pain on swallowing of 2 days duration, headache, arthralgias, myalgias, prostration, and loss of appetite. He was hospitalized 1 day later with a diagnosis of probable influenza in a local hospital and treated with Oseltamivir (75 mg BID) for 2 days. The patient was transferred to HIMJR in critical condition 5 days postsymptom onset, and respiratory and blood samples were collected for RT-PCR, which subsequently revealed H1N1 and DENV-3 infection, respectively. Despite treatment, the patient's condition deteriorated and he was reclassified as a suspected severe dengue case because of decreasing platelet counts and increasing hematocrit, radiologic evidence of bilateral pleural effusions, and hepatomegaly, ascites, and enlarged gallbladder as evidenced by abdominal ultrasound. The patient developed shock and was transferred to the

TABLE 1. Clinical Characteristics of 4 Children With Dengue-influenza Virus Coinfections*

	Case 1	Case 2	Case 3	Case 4
Underlying conditions	Asthma	None	Asthma	Asthma, obesity
Clinical findings				
Maximum temperature (°C)	40.0	39.5	38.5	38.4
Headache	Yes	No	Yes	Yes
Myalgias	Yes	Yes	Yes	Yes
Arthralgias	Yes	Yes	Yes	Yes
Cough	No	Yes	Yes	No
Sore throat	Yes	Yes	No	Yes
Rhinorrhea	No	No	Yes	No
Abdominal pain or tenderness	Yes	No	No	Yes
Vomiting	Yes	No	No	No
Diarrhea	No	No	No	No
Rash	Yes	Yes	Yes	No
Loss of consciousness	No	No	Yes	Yes
Cold extremities	Yes	No	Yes	Yes
Weak pulse	No	No	Yes	Yes
Hypotension	Yes	No	Yes	Yes
Tourniquet test	Positive	Positive	Positive	Positive
Laboratory results				
Minimum leukocytes (×10,000 cells/mm ³)	11.3	3.2	2.2	6
Maximum lymphocytes (%)	50	82	73	10
Maximum hematocrit (%)	45.0	36.2	35.0	31.9
Minimum platelets (×10,000 cells/mm ³)	75	92	129	211
Maximum creatinine (mg/dL)	1.96	NP	0.50	0.75
Minimum albumin (mg/dL)	2.95	NP	4.39	4.43
Maximum AST (IU)	278	92	60	38
Maximum ALT (IU)	62	32	26	22
Hospitalization data				
Days hospitalized	21	4	21	1 (11 h)
Mechanical ventilation	Yes	No	Yes	Yes
Intensive care unit	Yes	No	Yes	Yes
Outcome	Full recovery	Full recovery	Full recovery	Death

*NP indicates not performed; AST, aspartate amino transferase; ALT, alanine amino transferase; IU, international units.

intensive care unit (ICU), where he was given IV saline solution followed by dextran, albumin, and then norepinephrine. Mechanical ventilation was begun, and norepinephrine and oseltamivir (150 mg BID) were administered. A chest radiograph showed bilateral interstitial infiltrates. After 3 days in the ICU, the patient continued to be febrile and present leukocytosis, a predominance of segmented neutrophils, and clinical evidence of severe acute respiratory distress, together with consistent radiographic changes. Antibiotic treatment was changed to imipenem and vancomycin. Over the course of the following days, bronchospasms were treated with bronchodilators and corticosteroids. The patient improved clinically and radiographically. He was extubated after 12 days of mechanical ventilation, and vasopressure therapy was discontinued; he received a total of 14 days of double antibiotic therapy and 18 days of oseltamivir. Following 21 days of hospitalization (14 days ICU), the patient was discharged in stable condition.

Case 2

A 10-year-old girl presented at a primary care health center on September 3, the day of symptom onset, with a temperature of 39.5°C, headache, sore throat, arthralgias, and myalgias. She was suspected of having influenza, and a respiratory sample was collected. Additionally, a blood sample was collected for dengue RT-PCR testing and complete blood count. The patient was instructed to return for follow-up visits for each of the following 4 days, during which time she complained of pain on swallowing and loss of appetite, and laboratory results confirmed a dengue infection. On the fifth day, she had a capillary refill ≥ 3 seconds, was confirmed as a case of H1N1, given an IV of saline solution (0.9%), and transferred to the HIMJR. She was admitted to HIMJR in hemodynamically stable condition with a positive tourniquet test, skin rash, and crepitations at both lung bases. Treatment was initiated with oral fluids, acetaminophen, oseltamivir (75 mg BID), and continual monitoring of hemodynamic state. The following day, a complete blood count revealed thrombocytopenia and leukocytopenia, and a chest radiograph showed bilateral interstitial infiltrates. The patient continued in a stable state and was discharged after 4 days of hospitalization.

Case 3

A 5-year-old girl with a history of asthma presented with a temperature of 39.2°C, sore throat, cough, runny nose, swollen cervical lymph nodes, vomiting, and a positive tourniquet test, on September 15, 2 days postonset of illness. Specimens were collected for influenza and dengue testing. The family was provided oseltamivir; however, the parents chose not to administer it to the child. The patient returned the following day with continuing symptoms and loss of appetite, retro-orbital pain, headache, myalgias, arthralgias, and back pain. On the fifth day postonset, she was given IV saline solution and transferred to HIMJR. Upon admission, the patient was well-hydrated with the following signs, symptoms, and findings on physical examination: malaise, somnolence, hepatomegaly, cold extremities, diaphoresis, capillary refill >3 seconds, and a weak pulse. She was given IV saline solution and admitted to the ICU. Chest radiograph revealed bilateral interstitial infiltrates. Laboratory results confirmed H1N1 infection, and oseltamivir treatment (120 mg BID) was started. Ampicillin was initiated for a possible bacterial coinfection.

The patient continued in stable condition, and was confirmed to have DENV-3 infection by RT-PCR. On the eighth day of illness, she developed hypotension, which initially responded to administration of saline solution; however, 6 hours later, her diastolic blood pressure (BP) decreased rapidly;

dextran and dopamine were administered, and a central venous catheter was inserted. BP improved and dopamine was reduced, but then somnolence and hypotension were noted, and endotracheal intubation was performed. On the ninth day of illness, norepinephrine was substituted for dopamine, which stabilized BP. A second chest radiograph revealed increased bilateral infiltrates, and antibiotic therapy was changed to ceftriaxone. Over the following 24 hours, the patient's hemodynamic indices and respiration status improved, which was reflected in a third chest radiograph. Norepinephrine was decreased, and the patient was extubated after 65 hours of assisted ventilation in stable condition. She recovered fully and was discharged after 21 days in the hospital.

Case 4

An 11-year-old girl with obesity and asthma presented at the emergency room of HIMJR on September 27 with a history of pain on swallowing for 1 day. The clinical examination was unremarkable and the patient was released with instructions to return if symptoms worsened. The following morning she presented with the development of high fever, headache, arthralgias, and myalgias in the previous 12 hours. On examination, she was afebrile, breathing regularly, pale, had no hepatomegaly, and had pharyngeal hyperemia, tonsillar hypertrophy, abdominal tenderness, cold extremities, poor capillary refill, and a weak pulse. She was transferred immediately to the critical care area with a presumptive diagnosis of severe dengue. Saline solution was administered intravenously. Afterward, her pulse was 110 and BP was 130/50 mm Hg; she continued to have cold extremities, capillary refill of 4 seconds, and a weak pulse. Two additional boluses of saline solution and then dextran were administered, but the patient continued to have low diastolic BP (110/55). She was treated with dopamine, oxygen, and IV saline solution. Chest radiograph revealed bilateral interstitial and alveolar infiltrates. A diagnosis of pneumonia was made and cefotaxime treatment initiated. Ultrasound revealed a distended gallbladder with thin walls (2 mm), and an echocardiogram was normal. The patient continued in unstable condition with low diastolic pressure, so sepsis was suspected. Norepinephrine was substituted for dopamine. The patient was intubated and the dosing of norepinephrine was increased. Abundant blood-tinged mucous was removed through the endotracheal tube. Eleven hours after presenting at the hospital, the patient developed cardiac arrest and efforts to resuscitate her were unsuccessful. The cause of death was recorded as dengue shock syndrome. A respiratory sample collected immediately post mortem and a blood sample collected at admission were positive for influenza A H1N1 and DENV-3, respectively.

METHODS

A blood sample and nasal and throat swabs were collected for dengue and influenza testing, respectively. Cases were tested for DENV-1–4 by RT-PCR targeting the capsid gene.⁵ The Centers for Disease Control and Prevention's qRT-PCR protocol was followed for the detection of pandemic influenza A H1N1.⁶ Laboratory tests were performed at the Nicaraguan National Virology Laboratory, Ministry of Health.

Comment. We present 4 documented DENV-influenza A H1N1 coinfections in children; all 4 were RT-PCR positive for both viruses. In 3 cases, samples for influenza and dengue RT-PCR testing were taken on the same day; in case 4, the influenza sample was taken postmortem, 1 day after the sample for dengue. While bilateral interstitial and/or alveolar infiltrates were present in all 4 cases, the clinical presentation of the 4 cases varied and a single

pattern was not observed. Three patients had a history of asthma, a known risk factor both for severe dengue and influenza.^{7–10}

In case 1, respiratory symptoms were absent. Due to asthma and close contact with a confirmed H1N1 case, the patient was given oseltamivir very early, which may have prevented respiratory symptoms and resulted in a predominately dengue-like clinical presentation, which evolved into shock, possibly aggravated by a bacterial infection. In contrast, case 2 presented with classic flu-like symptoms, including cough, as well as classic dengue symptoms. This patient, though hospitalized, had an illness of only mild-to-moderate severity, possibly due to early treatment with IV fluids when the patient began to display signs of hemodynamic instability. This patient is the only 1 of the 4 cases with no prior history of underlying conditions predisposing to severe influenza and dengue. In the third case, respiratory symptoms preceded the development of dengue symptoms. Oseltamivir treatment was not started until after the child was hospitalized and developing hemodynamic instability. Shock in this patient was different from case 1 and was atypical for dengue, in which the observed hypotension is usually limited to the systolic component. Initially, the patient was diagnosed with viral pneumonia, followed by bacterial pneumonia. The fourth case presented with classic dengue symptoms and rapidly went into shock. Onset of shock 2 days rather than 4 to 6 days postsymptom onset is unusual for dengue, as is a markedly reduced diastolic BP (as in case 3).

In conclusion, we present 4 children with laboratory-confirmed dengue-influenza virus coinfections with varying clinical presentations, and based on this experience, find that coinfections may be a risk factor for severe disease. Due to the range of clinical presentation and difficulties differentiating DENV-influenza coinfections from single infections, especially early on, it is advisable that testing for both viruses be performed when they are cocirculating.

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A HUMAN IMMUNODEFICIENCY VIRUS-POSITIVE INFANT WITH PROBABLE CONGENITAL HISTOPLASMOSES IN A NONENDEMIC AREA

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Abstract: A 5-week-old infant presented with a fever, and was diagnosed with congenital human immunodeficiency virus and histoplasmosis. Both infections were likely transmitted vertically. The child was effectively treated with antifungal medications and highly active antiretroviral therapy. This represents the first case of delayed presentation of vertically transmitted histoplasmosis, and the first case in a nonendemic area.

Key Words: HIV, histoplasmosis, disseminated histoplasmosis, infant

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Histoplasmosis is common in many parts of the world. A majority of cases are mild and self-limited; however, infants often present with disseminated disease. Disseminated histoplasmosis in infants carries a mortality between 83% and 93%, rendering rapid definitive diagnosis critical.¹ Where the spores are not endemic, consideration of this disease is only in recent immigrants from endemic areas. Rates of disease in nonendemic areas are exceptionally rare, and difficult to quantify.² There has not been a report of a case reported in an infant who has not traveled to or who was not born in an endemic area. As a cause of infantile fever, histoplasmosis can be challenging to diagnose quickly, and is unlikely to be considered in nonendemic areas.

PATIENT PRESENTATION

A 4-week-old girl was admitted to the hospital with fever and a nonspecific maculopapular generalized rash. Standard urine, blood, and cerebrospinal fluid (CSF) studies were obtained and were unremarkable. The infant was treated with ampicillin and gentamicin for 48 hours, defervesced, and was then discharged, with a diagnosis of viral syndrome. The infant presented back to the hospital 1 week later, with continued fever and rash, and with increased irritability for 1 day. The patient had tachycardia and a rectal temperature of 101.3°. Her lungs were clear to auscultation bilaterally; however, she had multiple episodes of desaturations to the low 90s. A chest radiograph demonstrated hyperinflation and increased perihilar interstitial markings consistent with bronchiolitis. She was again given ampicillin and gentamicin after urine, blood, and CSF studies were obtained. During this second workup, a nurse in the emergency room incurred a needle-stick, and Western blot human immunodeficiency virus (HIV) test was per-

formed on the infant, which was positive. Of note, the mother was an undocumented Guatemalan immigrant who moved to Rhode Island 2 years prior and had not traveled since. She was recently diagnosed with Hodgkin lymphoma and had refused HIV testing during her pregnancy. *Histoplasma capsulatum* is endemic in many parts of Guatemala, and up to 7% of HIV patients in this country presented with progressive disseminated histoplasmosis (PDH).³ The child was born and raised in a nonendemic area, with no history of travel. Laboratory values for the infant are summarized in Table, Supplemental Digital Content 1, <http://links.lww.com/INF/A512>.

On day 2, the patient was noted to have oral candidiasis and a liver 2 cm below the costal margin. By day 3, she had developed a mild thrombocytopenia with a platelet count of 87,000 per μL . An abdominal ultrasound noted a small density suggestive of a hepatic hemangioma with a thickened gall bladder wall. By day 5, her fever persisted and her respiratory status worsened. A new chest radiograph showed increased haziness with hyperinflated lungs. On day 7, she had a 7-minute seizure that resolved with a dose of lorazepam. As a result of persistent fevers and new-onset seizure, a repeat sepsis work-up of blood, urine, and spinal fluid were performed, followed by a bronchial alveolar lavage. These studies led to the diagnosis of histoplasmosis. At this time, significant thrombocytopenia also developed, falling to a nadir of 12,000 per μL .

A Wright stain of the peripheral blood (Fig. 1) smear demonstrated an encapsulated organism within a neutrophil. Consistent with the forms seen on the peripheral blood smear, a Wright-stained preparation of CSF also showed intracellular organisms within the monocytes. Standard laboratory techniques allowed the definitive identification of *H. capsulatum*. Spinal fluid fungal cultures also confirmed growth of *H. capsulatum*. A bronchial alveolar lavage specimen was obtained in liquid cytology medium, and small budding yeast cells were demonstrated with Papanicolaou and Gomori methenamine silver stains.

After examination of the peripheral smear, urine was obtained for enzyme immunoassay (EIA) of *H. capsulatum* polysaccharide antigen to affirm the presumptive diagnosis (Mira Vista Diagnostics, Indianapolis, IN). A value of 82.92 EIA units was reported (<1.0 negative; 4.1–10.0 moderate positive; and >10.0 highly positive). Additionally, the mother of the patient had an elevated urine Histoplasma antigen (5.24 EIA units), confirming moderate positivity.

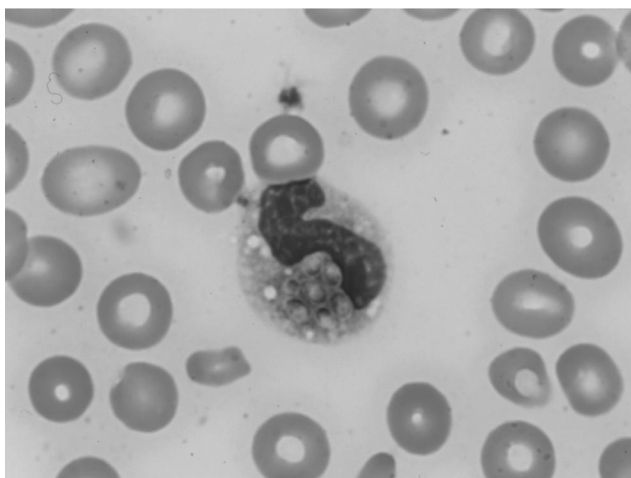


FIGURE 1. A Wright stain of the peripheral blood smear (an encapsulated organism within a neutrophil).

Lipid complex amphotericin B was initiated. Because of poor response, itraconazole was added. Her treatment was changed from lipid complex amphotericin B to a nonlipid form by week 7 of antifungal treatment. The patient was treated with amphotericin B therapy for a total duration of 14 weeks.⁴

Highly active antiretroviral therapy was initiated after confirmation of HIV infection on day 12 with stavudine, lamivudine, and lopinavir/ritonavir. Her CD4% decreased from 46% on admission to 28% 3 months later; but the HIV plasma viral load improved from >500,000 to 230 copies per milliliter plasma.

After more than 3 months, she was discharged home receiving itraconazole suppressive therapy. Her urine histoplasma antigen result decreased from 90 EIA units to 4 EIA units, and was eventually undetectable. Pneumocystis carinii prophylaxis was provided with trimethoprim-sulfamethoxazole. A follow-up spinal tap was performed after 6 weeks of antifungal therapy, and fungus failed to grow on culture, confirming clearance of the organism from the CSF.

The mother was initially diagnosed with HIV as a result of her infant testing positive. At the time of her diagnosis, her CD4 count was 27 with a plasma viral load of 51,600. Her moderately elevated urine histoplasma antigen gradually decreased after receiving itraconazole therapy. Additionally, the supraclavicular biopsy which had been done previously to diagnose her Hodgkin lymphoma was reexamined and stained negative for fungus. Her fungal blood culture showed negative results.

DISCUSSION

This child developed disseminated histoplasmosis within weeks of her birth in Rhode Island, an area where histoplasma is rare. Because person-to-person spread of histoplasmosis is exceptionally rare, the documented HIV and histoplasmosis infection in the patient's mother led to the patient's congenital acquisition of both diseases. These progressed to severe illness by 4 weeks of age. This is likely the first case of congenitally acquired disseminated histoplasmosis and HIV in a nonendemic area.

Histoplasma lives in soil and infection occurs after inhalation from spores in dust, soil, bird/bat guano, or decaying vegetation. Although extremely rare in the northeast, disseminated histoplasmosis is not uncommon in endemic areas of the United States, such as the Ohio, Missouri, and Mississippi river valleys. Endemic fungal infections of Central America include histoplasmosis, coccidioidomycosis, and cryptococcosis. Studies in Central America and parts of South America demonstrate some regions, where up to 80% of people have been infected with Histoplasma by their early 20s.⁵ A majority of individuals with hematogenous disseminated disease are asymptomatic, and the disease usually goes undiagnosed.⁶ PDH of infancy is defined as an illness that persists longer than 3 weeks, and where extrapulmonary evidence of infection is present.⁶ The largest study of PDH of infancy occurred between 1983 and 1996 in a Costa Rican hospital, where there were 40 cases of confirmed infantile histoplasmosis. In that facility, the average age of diagnosis was 15 months with the youngest being 6-weeks old.⁷

Human-to-human transcutaneous transmission has been described only twice in the literature, both of which required open skin lesions on the index case and intimate contact.^{8,9} Peripartum transmission of the organism in this case is possible. We cannot rule out postbirth acquisition of histoplasmosis in this child; however, person-to-person transmission is extremely rare. This infant's mother did not have open lesions on her skin. Histoplasmosis of the breast has been reported as a granulomatous disease; however, we are unaware of a report in humans or animals of histoplasmosis being spread through breast-feeding.¹⁰ This baby was bottle fed; however, early attempts at breast-feeding were tried.

Confirmed vertical transmission of neonatal histoplasmosis has been reported only once in the literature. This case took place in Missouri, where a 23-year-old woman with AIDS presented at 22-weeks gestation with fever, night sweats, diarrhea, and abdominal pain. The neonate was delivered in emergent cesarean section at 25-weeks gestation due to maternal respiratory distress with shock. Histoplasmosis was identified in the infant's serum, CSF, and peritoneal fluid. The placental pathology showed evidence of histoplasmosis as well.¹¹

Itraconazole and fluconazole are teratogenic. If necessary, pregnant patients are treated with amphotericinB, and prophylaxis is not indicated. Therapy for disseminated histoplasmosis in an immunocompromised infant includes amphotericin, fluconazole, or itraconazole.¹² The urine antigen test is a good marker for clinical improvement.¹³

In children with HIV infection who present with PDH, life-long therapy has been recommended. However, recent guidelines have considered the possibility of discontinuing therapy in these patients when demonstrating 12 months of continuously undetectable urine antigen values.¹⁴

This case is unique for several reasons. It is only the second case of suspected vertical transmission of *H. capsulatum* reported in the literature, and the first where delivery occurred in a nonendemic area. Even in the low likelihood that the fungal infection was acquired after birth through person-to-person contact, this would be the first example of such acquisition of this disease in infants. Given the mother's documented infection, and given the extraordinary rarity of endemic acquisition of the disease in Rhode Island, it is improbable that this was an autochthonous infection. In nonendemic areas of the United States, histoplasmosis is primarily an activation of an old infection or a result of travel to endemic areas.² Despite the concomitant vertical transmission of HIV, the patient responded well to antifungal therapy and is currently thriving. It is our belief that in a nonendemic area, vertical transmission is the most likely explanation for transmission of this child's fungal disease.

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NOROVIRUS ENCEPHALOPATHY IN A PREVIOUSLY HEALTHY CHILD

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Abstract: Norovirus causes acute gastroenteritis in all age groups. Afebrile convulsion is an occasional neurologic complication in norovirus infection, but encephalitis is rare. We report the case of a previously healthy 15-month-old girl with norovirus encephalopathy who had a poor neurologic outcome. Norovirus (genogroup II) was detected in plasma and stool by real-time reverse transcription polymerase chain reaction, but the cerebrospinal fluid showed negative result for genome. Elevated concentrations of cerebrospinal fluid interleukin-6, interleukin-10, interferon- γ , and tumor necrosis factor- α were observed on the third day of illness. The encephalopathy in our patient may be related to hypercytokinemia rather than to direct viral invasion.

Key Words: norovirus, encephalopathy, neurologic involvement

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Norovirus and rotavirus are the major causes of acute gastroenteritis. The clinical course is usually self-limited, but both viral infections can have associated neurologic complications. Norovirus is genetically divided into 5 genogroups (GG I–GG V), and GG II was the cause of a recent global acute gastroenteritis epidemic.¹ A significantly higher incidence of convulsion has been reported with norovirus infection compared with rotavirus infection,² but there has been only one report of encephalopathy that may have been caused by norovirus.³ Here, we describe a female infant with norovirus infection who developed encephalopathy with severe neurologic sequelae.

CASE REPORT

A previously healthy 15-month-old girl was brought to the emergency department of Koshigaya Municipal Hospital with a 2-day history of frequent vomiting and mild dehydration. She was alert, and her blood tests showed unremarkable findings. The next morning, she was brought into the emergency room again because of recurrent generalized convulsions, and her temperature was 39.8°C. Her vital signs showed a heart rate of 223 beats/min, blood pressure of 81/46 mm Hg, and a respiratory rate of 62 breaths/min. She did not respond to painful stimuli and her light reflex was sluggish. The muscle tone of her extremities was increased. Laboratory findings showed a white blood cell count of 18,700/ μ L, hemoglobin 13.98 g/dL, platelet count 41.5×10^4 / μ L, C-reactive protein 6.31 mg/dL, creatine kinase 134 IU/L, urea nitrogen 26.7 mg/dL, creatinine 0.37 mg/dL, glucose 29 mg/dL, insulin 1.1 μ mol/dL, total ketones 5747 μ mol/L, and lactate 17.4 IU/L. Serum electrolytes were within the normal. Liver enzymes were moderately elevated, and lactate dehydrogenase was 423 IU/L. Rapid tests showed negative results for influenza virus (immunochromatography Gold Colloid particle) and rotavirus (latex agglutination), but positive for norovirus (immunochromatography). The cerebrospinal fluid (CSF) had a normal protein value (18 mg/dL) and 2 cells/ μ L leukocytes. Bacterial cultures in the blood, stool, and CSF were negative.

The blood glucose value normalized after an injection of glucose and did not decrease again. After a 2-day episode of vomiting, watery diarrhea started and lasted for 2 days. Fever had resolved on the third day of admission. Dehydration was improved by administration of an isotonic crystalloid solution. Her convulsions were not controlled by intravenous administration of diazepam, midazolam, and phenytoin, but subsided with thiopental given with mechanical ventilation.

Diffusion-weighted magnetic resonance (MR) images of the brain on admission (the third day of illness) showed high intensities in the right occipital cortex. On the second hospital day, the high intensity areas had expanded to the subcortical white matter of frontal, temporal, and parietal lobes (Fig., Supplemental Digital Content 1, <http://links.lww.com/INF/A522>). An electroencephalogram showed marked generalized slowing without paroxysmal discharges, and she had severe disturbance of consciousness. Therefore, the patient was diagnosed with norovirus-associated encephalopathy and was treated with gamma-globulin, a single course of steroid pulse therapy, and brain hypothermia induced by selective head cooling.

The norovirus genome (GG II) was detected in plasma and stool by real-time reverse transcription polymerase chain reaction, but was not detected in the CSF on the third day of illness. CSF and serum showed negative results for herpes simplex virus, human herpes virus 6, and human herpes virus 7 by real-time polymerase chain reaction. No enterovirus was detected in cultures of the stool and CSF.

Cytokine profiles in the CSF on the third day of illness showed marked elevation of interleukin (IL)-6 (880.82 pg/mL; cut-off 10.9 pg/mL), IL-10 (10.0 pg/mL; 0.97 pg/mL), interferon (IFN)- γ (179.0 pg/mL; 7.02 pg/mL), tumor necrosis factor (TNF)- α (50.4 pg/mL; <0.06 pg/mL), and mild elevation of IL-8 (568.7 pg/mL; 118.47 pg/mL), IL-2 (0.73 pg/mL; <0.5 pg/mL), and IL-4 (0.8 pg/mL; 0.12 pg/mL).

Diffusion-weighted MR images on the 11th day of illness showed reduced diffusivity in the bilateral cerebral hemispheres. On the 101st day, severe atrophic changes were recognized on MRI (Fig., Supplemental Digital Content 1, <http://links.lww.com/INF/A522>).

At the last follow-up at 2 years of age, severe neurologic sequelae were observed. The patient had severe delays in mental

and motor development. Head control, sitting and verbal communication had not been achieved. She also had tonic seizures that were controlled by carbamazepine.

DISCUSSION

Enterotropic viruses can cause encephalopathy and encephalitis. Acute gastroenteritis with rotavirus is occasionally associated with nonfebrile seizure in children, but central nervous system (CNS) involvement rarely occurs, with only 24 reported cases of CNS diseases attributed to rotavirus.⁴ Detection of the rotavirus genome in the CSF can result from contamination with diarrheal stool at the time of lumbar puncture or to another artifact,⁵ and it remains unclear whether detection of the genome in the CSF indicates rotavirus infection in the CNS.

Norovirus infection can be also associated with convulsion, but there has been only one published case of norovirus-associated encephalitis. Ito et al³ detected the norovirus genome in a CSF sample from a 23-month-old girl with encephalopathy. However, the patient had no abnormal findings on brain MRI or CT, and recovered without neurologic sequelae.³ Direct viral invasion into the brain was suggested to have caused the encephalopathy,³ but it is also possible that contamination of the CSF occurred.

In contrast, our patient developed severe encephalopathy in association with norovirus infection. The norovirus genome (GG II) was detected in the stool and the serum, but not in the CSF. MRI findings showed diffuse high intensity lesions suggesting acute encephalopathy with late reduced diffusion.⁶ On the third day of illness, marked elevation of IL-6, IL-10, IFN- γ , and TNF- α and mild elevation of IL-2, IL-4, and IL-8 were observed in the CSF. A study of cytokine production in gnotobiotic pigs after infection with the human norovirus GG II/4 group showed a high concentration of IL-12, low transient IFN- γ and IL-6, and low IL-4 and IL-10 values in serum.⁷ The CSF cytokine profile in the early stage of our case differed from this cytokine pattern, but the clinical course suggested severe extraintestinal involvement of norovirus. There have been several studies of CSF cytokines in children with acute encephalopathy. Ichiyama et al⁸ reported that IL-6 and TNF- α values were elevated in the CSF of some patients with influenza-associated encephalopathy, and that elevation of these cytokines in the CSF was related to a poor neurologic outcome. Otake et al⁹ reported a patient with respiratory syncytial virus encephalopathy associated with elevated CSF IL-6, and suggested that elevation of cytokine concentrations in the CSF is involved in the pathogenesis of acute encephalopathy. Norovirus binds to histo-blood ABO antigen expressed in gastroepithelial cells, which means that susceptibility and resistance to norovirus depend on blood type.¹⁰ It is unknown whether human norovirus replicates in the CNS. We suggest that noroviral antigenemia and increased cytokines in the CSF can possibly lead to immune-mediated brain injury and severe neurologic damage.

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ACUTE DISSEMINATED FATAL TOXOPLASMOSIS AFTER HAPLOIDENTICAL STEM CELL TRANSPLANTATION DESPITE ATOVAQUONE PROPHYLAXIS IN A YOUNG MAN

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Abstract: We describe a case of fatal acute disseminated breakthrough toxoplasmosis in a 19-year-old adolescent after haploidentical hematopoietic stem cell transplantation for acute lymphoblastic leukemia despite continued atovaquone prophylaxis. Diagnosis was at necropsy, and confirmed by postmortem polymerase chain reaction analysis in plasma. This report illustrates the need for protozoal monitoring despite atovaquone prophylaxis, in severely immunocompromised patients with intolerance to standard treatment.

Key Words: disseminated, toxoplasmosis, atovaquone, stem cell transplantation, prophylaxis

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Toxoplasma gondii is a ubiquitous protozoan parasite causing latent and benign infection in 10% to 50% of people in western countries. In fetuses and immunocompromised individuals, especially those with deficient cellular immunity, *Toxoplasma* infection can cause significant morbidity and mortality. Reactivation of toxoplasmosis after hematopoietic stem cell transplantation (HSCT) is rare with poor prognosis and high mortality resulting from delayed diagnosis.¹ Toxoplasmosis reactivation is usually prevented with trimethoprim-sulfamethoxazole (TMP-SMX) prophylaxis in immunocompromised patients, such as HSCT recipients. Atovaquone is considered an alternative agent according to Centre for Disease Control and Prevention recommendations² and is used when TMP-SMX is not tolerated or causes bone marrow suppression.

We report a fatal case of disseminated toxoplasmosis in a young man 4.5 months after haploidentical HSCT for acute lymphoid leukemia, despite atovaquone prophylaxis.

CASE REPORT

A 19-year-old male adolescent was admitted to our pediatric hospital on day 144 post haploidentical HSCT for pre-B acute lymphoblastic leukemia. He disclosed a history of unexplained fainting in his bathroom, fever at 39°C, and significant weight loss (7 kg in 1 month). He reported no diarrhea, or cough.

The patient had a history of high-risk pre-B acute lymphoblastic leukemia refractory to induction chemotherapy. He received T cell depleted haploidentical HSCT from his mother. He was refractory to standard induction treatment, and received salvage chemotherapy with high dose of cytarabine and clofarabine. Conditioning regimen for HCST contained cyclophosphamide, antithymocyte globulin, etoposide, and total body irradiation. The graft was T-cell depleted by Campath-1H. The patient presented digestive and cutaneous stage I graft-versus-host disease on day 9 post-HSCT, treated with steroids. The bone marrow was reconstituted on day 12 post-HCST, and remission was confirmed on day 42. Complications after the conditioning chemotherapy included pulmonary aspergillosis, and cytomegalovirus (CMV) pneumonia (3 months before current admission) successfully treated with surgical removal of lung tissue, liposomal amphotericin B, voriconazole, and ganciclovir. TMP-SMX was initially administered for *Pneumocystis jirovecii* pneumonia (PCP) prophylaxis, but was switched to atovaquone 2^{1/2} months before current admission (on day 69 after HSCT) because of severe pancytopenia (white cell count: 0.7 G/L, neutrophils: 0.6 G/L, lymphocytes 0.1 G/L, hemoglobin 95 g/L, platelets: 20 g/L).

At admission, the patient was fully compliant and treated with valganciclovir for CMV prophylaxis, atovaquone for PCP prophylaxis, liposomal amphotericin B, and voriconazole for aspergillosis. He was febrile and ill, and had a nonspecific maculopapular abdominal rash. The source for his fever could not be identified on clinical examination. The laboratory work-up showed a white blood count at 4.3 G/L and a C-reactive protein >200 mg/L. His chest radiograph showed no evidence of pulmonary infection. Blood and urine cultures remained sterile for both bacteria and fungi. *Legionella* urinary antigen was negative, and no active infection was found on the viral work-up: Epstein Barr virus (EBV) was undetectable by polymerase chain reaction (PCR); he was seronegative for parvovirus B19; he had a negative CMV early antigen, and viral copies by PCR were in regression compared with prior controls. The patient was empirically treated with vancomycin, meropenem, and liposomal amphotericin B.

Two days after admission, the patient had persisting fever, developed acute respiratory distress syndrome, and cardiac failure with pericardial effusion. He was admitted to the intensive care unit for mechanical ventilation and inotropic support. All bacterial and fungal cultures (blood, pericardial effusion, and tracheal aspirate) were negative. Bone marrow biopsy showed no leukemia. He rapidly developed refractory hypotension, multiple organ failure, including renal insufficiency, and severe acidosis. The patient died 3 days after admission.

Necropsy revealed acute disseminated toxoplasmosis with free forms (tachyzoites) and cysts containing large numbers of bradyzoites in the myocardium, liver, kidney medulla, and bone marrow. Parasites were associated with necrotic foci in these organs. In the lungs, the parasites were mixed with intra-alveolar fibrin deposition. An immunohistochemical evaluation confirmed toxoplasmosis. Brain examination was declined. No graft-versus-host disease was observed.

Real-time PCR (RT-PCR) performed from frozen plasma from the day before the death was highly positive for toxoplasmosis (13,000 copies/mL). *Toxoplasma* serology was available before the bone marrow transplant (BMT) and immunoglobulin treatment, and showed latent infection (positive IgG (18.4 UI/mL), negative IgM and IgA). Toxoplasmosis RT-PCR performed on frozen plasma obtained 2 days before TMP-SMXs was changed to atovaquone (on day 67 after HSCT) was negative (<100 copies/mL). One month before death, a bronchoalveolar lavage was performed because of CMV reactivation. Immunohistochemical staining for CMV and toxoplasmosis was negative. Toxoplasmosis serologies of the graft donor (mother) showed latent infection with positive IgG and negative IgM; she was asymptomatic.

DISCUSSION

We report a case of disseminated and fatal toxoplasmosis breakthrough reactivation despite receiving atovaquone prophylaxis after allogeneic BMT. Acute toxoplasmosis is an uncommon but severe and often fatal complication of BMT and usually occurs 4 to 6 months after transplantation. Prevalence is probably underestimated and influenced by endemicity, diagnostic techniques, and the availability of necropsy results. Risk factors include *Toxoplasma* seropositivity before BMT, allogeneic BMT, graft-versus-host disease, and immunosuppression.¹

Thus, our patient was at high risk for toxoplasmosis reactivation: he presented all the risk factors and a particularly immunosuppressive graft (haploidentical, T depleted, and a highly immunosuppressive preconditioning chemotherapy).³ In solid organ transplant recipients, toxoplasmosis results mainly via parasite transmission through the transplanted organ from a *Toxoplasma*-positive donor to a *Toxoplasma*-negative recipient. By contrast, in HSCT recipients, the major risk factor for toxoplasmosis results from the reactivation of a pretransplant latent infection in seropositive recipients.³ For this reason, even if our patient's donor's serologic status was compatible with latent infection, it seems unlikely that she was the source of transmission. Diagnosis is challenging because of the nonspecific clinical manifestation in complicated patients leading to delayed or even postmortem diagnosis. Diagnosis of the infection is usually performed by direct demonstration of parasites on biopsy specimens; however, this may be a concern in thrombopenic BMT patients. Indirect serologic methods, widely used in immunocompetent individuals, may be unreliable in immunodeficient individuals especially in BMT patients who fail to produce significant antibody titers.⁴ Furthermore, these patients are often treated with immunoglobulins, which make serology testing impossible. IgM antibodies are usually not detected during reactivation, and IgG antibodies cannot distinguish between latent and reactivated infections. In the past decade, PCR assays have allowed rapid and sensitive detection of *Toxoplasma gondii* DNA in clinical specimens such as blood, spinal fluid, bronchoalveolar lavage, or amniotic fluid. Quantitative RT-PCR has been developed in the last decade, and can possibly be used to distinguish latency from acute infection. However, no commercial kits are available and in-house assays vary widely. This tool has been used prospectively in clinical settings: Foudrinier et al have described a patient with positive blood toxoplasmosis PCR 3 months prior the onset of toxoplasmosis encephalitis.⁵ Another group detected *Toxoplasma* PCR in 3 febrile patients less than 6 months after BMT without clinical or radiologic evidence for disease. Initiation of TMP-SMX therapy resulted in clinical and biologic recovery.⁶

Atovaquone is a naphthoquinone with ubiquinone analogy that inhibits protozoan ubiquinone attachment to cytochrome b⁷ and thus parasitic mitochondrial electron chain. It has a broad-

spectrum antiprotozoal activity, and is effective for the treatment and prevention of PCP. There is limited experience with other protozoans such as *T. gondii*. In vitro models have shown efficacy against *Toxoplasma* tachyzoites and, at high concentrations, against bradyzoites. In a phase I trial, atovaquone showed efficacy in the treatment of *Toxoplasma* retinochoroiditis in 17 immunocompetent patients.⁸ Most clinical experiences with atovaquone have been with HIV-infected, or AIDS patients. El Sadr et al found no difference in cases of toxoplasmosis in HIV patients receiving atovaquone for PCP prevention compared with those receiving dapsone.⁹ In case of TMP-SMX intolerance, dapsone and atovaquone are recommended as acceptable alternatives for *Toxoplasma* prophylaxis in immunocompromised patients.² Cytochrome b mutations have been associated with atovaquone resistance in *P. jirovecii*, *P. falciparum*, and *T. gondii*. In vitro models have shown variability in the susceptibilities of *T. gondii* strains to atovaquone. Because cytochrome b gene is located on the mitochondrial genome, mutations may occur at higher rates than on genes from the nuclear genome. Recently, breakthrough cerebral toxoplasmosis in a patient receiving atovaquone prophylaxis after BMT has been reported.¹⁰ Randomized controlled trials are needed to evaluate the efficacy on atovaquone in toxoplasmosis prophylaxis.

In conclusion, HSCT patients with atovaquone toxoplasmosis prophylaxis should nevertheless be considered at risk for *T. gondii* disease and should be monitored for protozoal replication by RT-PCR, when possible. Although the best strategy to treat reactivation or infection with *Toxoplasma* is not standardized, switching to another agent, such as pyrimethamine and sulfadiazine, or adding a second drug to atovaquone should be considered.

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