Infections Due to Nontuberculous Mycobacteria in Children with Leukemia

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We reviewed the spectrum of infections due to nontuberculous mycobacteria (NTM) in children with leukemia. Three children acquired such infections. One patient developed pneumonia after the cessation of chemotherapy when Mycobacterium xenopi was identified in his lung biopsy specimen. He required 2 years of treatment with antituberculous agents and clarithromycin. Cultures of central and peripheral blood specimens from two patients yielded Mycobacterium fortuitum and Mycobacterium chelonae, respectively. Broviac catheters were likely the source of infection. Removal of the catheters and antibiotic treatment resulted in cure. Central venous catheters in leukemic children are potential sources of infection. For febrile neutropenic children with leukemia who do not respond to antibiotic therapy, cultures positive for diphtheroids or negative routine bacterial and fungal cultures should raise a suspicion for infections due to NTM. Systemic infections may require up to 2 years of therapy. Removal of the infected catheters during persistent or recurrent infections is necessary for control of the infection.

Strains of nontuberculous mycobacteria (NTM) were recognized as human pathogens in 1950 [1] and have been found to be associated with skin infections [2, 3], pulmonary disease [3–7], otitis media [8], osteomyelitis [2, 3], septic arthritis [2, 9], and lymphadenitis [2, 3, 10, 11] in children. NTM strains are also pathogens of opportunistic infections in patients with AIDS [2, 3, 7, 11] and in patients with malignant diseases [2, 3, 12–14].

Despite numerous reports about infections caused by Mycobacterium fortuitum [2, 3, 12, 14], Mycobacterium xenopi [4, 5, 7, 9], and Mycobacterium chelonae [2, 3, 13] in patients with various immunocompromised states, reports of infections due to NTM in children with cancer are exceedingly rare; only one case of M. chelonae infection has been described in a child with leukemia [13]. In our pediatric oncology population, we detected three infections due to NTM with M. fortuitum, M. xenopi, and M. chelonae in patients with acute leukemia, all of whom were treated successfully.

Case Reports

Case 1. A 7-year-old boy was admitted to the hospital for recurrent pneumonia. He had completed chemotherapy for acute lymphoblastic leukemia 6 months before admission. During his previous hospitalization, he was persistently lymphopenic (absolute lymphocyte count [ALC], <600/mm³), and bacterial, viral, and fungal cultures of blood specimens were negative. Since an extensive workup of a tracheal aspirate for identification of bacterial, viral, mycoplasmal, fungal, and protozoan pathogens had not yielded the etiology, he was empirically treated with broad-spectrum antibiotics.

At the time of the current admission, he had cough and fever, his temperature was 39°C, and he was in moderate respiratory distress (oxygen saturation by pulse oximetry, 90%). Diffuse crepitant rales were audible in both lung fields. The liver and spleen were both palpable 5 cm below the costal margins. A complete blood cell count revealed lymphopenia (ALC, 500–1000/mm³). A chest roentgenogram showed a diffuse parenchymal infiltrate with nodulations. Additional cavitary lesions were seen on a CT scan (figure 1). Anergy testing with candida, mumps virus, trichophyton, and PPD was nonreactive.

Analysis of bronchoalveolar lavage fluid did not show legionella, mycoplasma, fungi, Pneumocystis carinii, mycobacteria, or viruses. An open lung biopsy was performed. Pathological examination of the biopsy specimen demonstrated confluent granulomatous inflammation with necrosis. Viral, bacterial, and fungal cultures were sterile, and direct immunofluorescence of the tissue specimen was negative for P. carinii and legionella. An acid-fast bacillus (AFB) was identified. Culture of the biopsied lung tissue yielded M. xenopi.

In vitro antibiotic susceptibility testing of the isolate indicated that the organism was susceptible to rifampin, ethambutol, and clarithromycin. Hence, he was treated for 2 months with rifampin (20 mg/[kg·d] orally q12h) and for 2 years with ethambutol (15 mg/[kg·d] orally q.d.) and clarithromycin (15 mg/[kg·d] orally b.i.d.) until there was complete abatement of clinical symptoms and no radiological evidence of pneumonia. His leukemia remains in remission.

Case 2. A 2-year-old boy with Down syndrome and acute megakaryoblastic leukemia was hospitalized for treatment of persistent fever (temperature, 38°C–39°C). He had received a
M. fortuitum.

Fungal cultures of blood as well as viral cultures of specimens continued to do well.

Figure 1. CT scan of the lung bases of a boy with leukemia and Mycobacterium xenopi infection (case 1) that shows large coalescent cavitary lesions (black arrowheads).

10-day course of treatment with vancomycin (45 mg/[kg⋅d] intravenously q8h) earlier because of cellulitis at the exit site of a Broviac catheter and cultures of pus and a central blood specimen were positive for diphtheroids. During the course of his illness, he was persistently lymphopenic (ALC, 224/mm³). Fungal cultures of blood as well as viral cultures of specimens from the nasopharynx and rectum were negative for pathogenic organisms. Stool cultures did not yield enteropathogenic organisms. A chest roentgenogram showed bilateral interstitial infiltrates; however, his symptoms persisted. Blood culture yielded an AFB that was identified as M. fortuitum on the same day that the diphtheroids were isolated.

Therapy with rifampin (20 mg/[kg⋅d] orally q12h) and amikacin (15 mg/[kg⋅d] intravenously q8h) was started. Even though his fever abated, repeated blood cultures continued to yield M. fortuitum, and the Broviac catheter was removed. Culture of the catheter tip yielded M. fortuitum. After 2 weeks of antibiotic treatment, blood cultures became negative, and diarrhea and skin induration resolved; however, his cough and the pulmonary infiltrate persisted. The Broviac catheter was reinserted into the right internal jugular vein in an area in close proximity to the previous insertion site. A few days later, fever recurred, and blood cultures were again positive for M. fortuitum. The central catheter was removed; culture of the catheter tip yielded M. fortuitum. Following 1 month of therapy with amikacin and rifampin, pneumonia resolved on the basis of clinical and radiographic findings, and blood cultures became sterile. The patient received 6 months of antibiotic therapy. His leukemia remains in remission, and he is asymptomatic for M. fortuitum infection.

Case 3. A 3-year-old boy had persistently high temperatures 3 months after acute lymphoblastic leukemia was diagnosed. He was asymptomatic for cough, diarrhea, vomiting, and headaches. Because of chemotherapy, he was persistently lymphopenic (ALC, 253/mm³). His temperature was 39°C. Physical examination was unremarkable. Cultures of throat and urine specimens were negative; blood culture yielded diphtheroids. A chest roentgenogram revealed normal findings. He was treated with vancomycin (45 mg/[kg⋅d] intravenously q8h) and ceftazidime (120 mg/[kg⋅d] intravenously q8h) for 10 days, and blood cultures became negative; however, fever persisted, and he developed a cough. Culture of a throat specimen and viral cultures of nasopharyngeal specimens were negative. A repeated chest roentgenogram showed a minimal bilateral interstitial infiltrate. He was then treated with co-trimoxazole (trimethoprim, 20 mg/[kg⋅d] orally q6h) for 2 weeks for presumed P. carinii pneumonia; however, fever persisted. Because of the new onset of hepatosplenic megaly and an enlarged fatty liver demonstrated by ultrasound examination, a presumptive diagnosis of hepatosplenic candidiasis was made, and empirical amphotericin B treatment was started. Cultures of central and peripheral blood specimens taken 1 week later yielded diphtheroids, and the next day, an AFB was isolated (which was eventually identified as M. chelonae). Amphotericin B therapy was discontinued, and treatment with amikacin sulfate (15 mg/[kg⋅d] intravenously q8h) was started.

The fever abated, and successive blood cultures yielded M. chelonae. The Broviac catheter was removed. Culture of the catheter tip yielded M. chelonae. He completed a 2-week course of therapy with amikacin sulfate while still receiving his chemotherapy. Hepatosplenic megaly and the pulmonary infiltrates resolved. Repeated blood cultures became sterile. It has been 5 years since he received chemotherapy, and he continues to do well.

Isolation and Identification of NTM

An AFB was isolated from all three patients; these organisms were identified by standard procedures after their growth in Löwenstein-Jensen slants and BACTEC 12B bottles (Becton Dickinson, Sparks, MD) containing 7H12 broth with radiolabeled carbon (14C) that was developed for the enhanced isolation of the organism. DNA probes for Mycobacterium tuberculosis were analyzed; when the results were negative, biochemical tests, including testing for the presence of arylsulfatase, semiquantitative testing for the presence of catalase, testing for hydrolysis of Tween (Remel, Lenexa, KS), and testing for reduction of tellurite and nitrate, were performed. In addition, strains were tested for iron uptake and utilization of inositol, mannitol, and sodium citrate as a sole source of carbon in the presence of ammoniacal nitrogen on the basis of previously reported procedures [15].

Findings. M. fortuitum and M. chelonae strains from patients 2 and 3, respectively, grew within 5–11 days at 31°C.
The nonpigmented colonies produced arylsulfatase for 3 days and grew on MacConkey agar without crystal violet. *M. fortuitum* reduced nitrate and absorbed iron at 31°C during 3 weeks of incubation; *M. chelonae* did not reduce nitrate or absorb iron and was identified on the basis of utilization of sodium citrate as a sole source of carbon for growth. *M. fortuitum* biovar *fortuitum* was identified as it failed to utilize mannitol as a sole source of carbon (*M. fortuitum* biovar *peregrinum* utilizes mannitol). Testing for the presence of catalase and urease and the reduction of tellurite was positive for strains isolated from patients 2 and 3 but not for the strain isolated from patient 1. On the other hand, slow growth of nonpigmented smooth-surfaced colonies was observed after 4 weeks of incubation at 35°C of the lung tissue specimen from patient 1 in culture media. Less than 45 mm of foam in the semiquantitative test for the presence of catalase, a 3-day production of arylsulfatase, and the presence of the “X” colony on 7H10 agar were consistent with the features of *M. xenopi* [15].

### Discussion

*M. xenopi* pneumonia was diagnosed for patient 1. The clinical picture of *M. xenopi* pneumonia resembles that of pulmonary tuberculosis [3–7]. Chest roentgenograms often reveal bilateral thin-walled cavities, solitary or multifocal nodulations, and rarely abscess formation [5, 7]. To our knowledge, only one case of *M. xenopi* infection associated with leukemia has been reported [5]. This patient was a bone marrow recipient who had acute myelogenous leukemia; the patient was severely immunosuppressed when *M. xenopi* pneumonia developed.

The CD4+ cell count is known to be an important factor in the control of infections due to NTM. Patients with low CD4+ cell counts are more vulnerable to disease control [11, 16]. CD4+ cell counts for the pediatric age group range from 900 to 3,600/mm³ [17]. None of our patients had an ALC of >900/mm³, thus indicating that they were also lymphopenic with respect to CD4+ cells when they developed infections due to NTM.

Because of nonreactive anergy testing for patient 1 and chemotherapy for patients 2 and 3, all three patients were immunocompromised. We were unable to isolate mycobacteria from bronchoalveolar lavage fluid in case 1. This fact probably reflected the low number of organisms in the biopsy sample. Nevertheless, we established the diagnosis with the identification of an AFB in the lung tissue specimen and the growth of *M. xenopi* in culture [18]. The duration of treatment with efficacious antibiotics was based on the patient’s clinical and radiographically evident response; complete resolution was achieved within 2 years of therapy (figure 2).

Figure 2. CT scan of the lung bases of a boy with leukemia and *Mycobacterium xenopi* infection (case 1) that shows resolution of the largest cavitary lesion (white arrowhead).

Diphtheroids were isolated from pus and blood specimens from patient 2 and from blood specimens from patient 3. The growth of NTM in both cases in which cultures were initially positive for diphtheroids is rather interesting. The growth of an organism on 5% sheep blood and chocolate agars after 3 days of incubation, the development of radial striations during prolonged incubation, and irregular gram staining of slightly curved, beaded, gram-positive rods that revealed singly arranged filaments in small bundles were suggestive of *Corynebacterium* species. On the basis of prior experience with the colonial and microscopic morphology of rapidly growing NTM and because of the presence of diphtheroids in culture and the persistence of symptoms despite antibiotic treatment, there was a strong suspicion of infection due to NTM [19]. Despite treatment of both patients with efficacious antibiotics, cultures remained positive for NTM until the removal of catheters; these findings suggested colonization of the catheters with these organisms. We also assume that the local infection in the skin or the vein served as a focus for recurrent infection in patient 2, since the cultures were negative before the insertion of the second central venous catheter.

We did not perform cultures of sputum or lung biopsy specimens from either patient 2 or 3, which would have helped to elucidate the etiology of pneumonia. However, it is conceivable that in the presence of negative viral cultures of throat and nasopharyngeal specimens and with the abatement of clinical symptoms and no radiographic evidence of pneumonia after specific therapy, NTM were the causative agents. Patients with a less immunocompromised state may respond well to a conservative therapeutic approach (e.g., removal of infected catheters, surgical drainage of an abscess, and excision of the infected area) [8, 12–14]. However, for immunocompromised patients with active localized disease, 3 to 10 weeks of monotherapy with an efficacious antibiotic is recommended [14, 20]; 6 to 7
months of combination therapy with efficacious antibiotics is recommended for those with extensive disease [20]. Antibiotic therapy was completed in 3 weeks in case 3 because of the patient’s prompt clinical and bacteriologic response. However, despite removal of the Broviac catheter in patient 2, antibiotic treatment continued for six more months because of the persistence of cellulitis and pneumonia as well as repeated positive blood cultures. Although infections due to NTM in some patients may run a fulminant course leading to death [2, 3, 11], all three of our patients responded well to therapy, and their infections due to NTM were cured.

During the time when these 3 patients were treated, 43 other leukemic children were under our care. In addition, we have identified two other children with M. xenopi infection: one with severe malnutrition for whom a blood culture yielded M. xenopi and one with sickle cell anemia with a history of pulmonary tuberculosis whose sputum cultures yielded M. xenopi. Conceivably, both of these patients were immunocompromised.

When the etiology for febrile immunocompromised children is unclear, suspicion of infections due to NTM should be raised. Special attention should be paid to cultures positive for diphtheroids, since the growth characteristics and microbiological features of diphtheroids are similar to those of NTM. If diphtheroids grow in cultures or if gram-positive bacilli are identified, acid-fast staining should be done for AFB strains, and blood cultures with appropriate media should be repeated for NTM. We add NTM to the growing list of organisms causing catheter-related infections and emphasize the emergence of NTM as important nosocomial pathogens in children with leukemia.

References