Prevalence of Mycobacteremia Among HIV-infected Infants and Children in Northern Tanzania

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Abstract: *Mycobacterium tuberculosis* is a common cause of bloodstream infections among HIV-infected adults in sub-Saharan Africa, and is associated with high morbidity and mortality. We found no cases of mycobacteremia among 93 ill, HIV-infected children in northern Tanzania, despite optimization of laboratory methods and selection of patients thought to be at highest risk for disseminated infection.

Key Words: mycobacteremia, children, Africa, Tanzania, tuberculosis

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Mycobacterium tuberculosis (Mtb) is a major HIV coinfection. Multiple studies from Tanzania and other countries with generalized HIV epidemics and high tuberculosis (TB) incidence rates have demonstrated that Mtb is a leading cause of bloodstream infections among HIV-infected adults, responsible for up to 44% of all bloodstream infections in some series.^{1–4} Our study sought to characterize the prevalence of mycobacteremia in an ill, HIV-infected, pediatric population. Special attention was given to collecting adequate blood volume for mycobacterial culture, as well as inclusion criteria to select patients likely to be at highest risk for mycobacteremia.

MATERIALS AND METHODS

This study was conducted in Moshi, a city of >144,000 people in northern Tanzania. Study participants were enrolled from the inpatient wards and outpatient HIV clinics of 2 local hospitals over a period of 16 months. The first, Kilimanjaro Christian Medical Centre, is a consultant referral hospital with 458 beds serving a catchment area of >1.4 million. The second, Mawenzi Regional Hospital, is a 300-bed government hospital, also serving

the Kilimanjaro Region. All patients under the age of 16 years with documentation of HIV infection were screened for eligibility.

Enrollment criteria were chosen by the investigators to select patients thought to be at highest risk of disseminated TB; patients met inclusion criteria if at least 2 of the following were present: axillary temperature of $\geq 37.5^{\circ}\text{C}$ at the time of admission or screening, subjective fever ≥ 2 weeks, cough ≥ 2 weeks, moderate or severe (less than -2 or -3 standard deviations [SD], respectively, below the median weight/height for children >5 years old and less than -2 or -3 SD, respectively, below the median body mass index/age for children <5 years old) malnutrition,⁵ presence of any adult in the patient's home who was currently receiving treatment for TB or had been treated for TB in the previous 6 months, presence of oral candidiasis, lethargy or evidence of immunosuppression based on CD4-positive T-lymphocyte count or percentage. Children were excluded if they had received anti-TB therapy or isoniazid prophylaxis in the 3 months preceding screening. Re-enrollment was allowed if the child met inclusion criteria, >6 weeks had elapsed from the initial enrollment and the initial mycobacterial culture result showed no growth at 42 days.

Upon enrollment, demographic information was collected and a standardized medical history and physical examination were performed by a clinical officer with study-specific training. Twelve microliters of whole blood was collected via venipuncture using a sterile procedure, which was allocated as follows: 5 mL for mycobacterial blood culture, 3 mL for aerobic blood culture, and the remaining volume used for complete blood count, CD4 count/percentage, and confirmatory HIV testing via 2 different rapid antibody tests if >18 months and HIV-1 RNA polymerase chain reaction if <18 months. Six weeks after enrollment, all patients' health statuses were reassessed either in clinic or via telephone by the research team.

Samples were analyzed on site at the Kilimanjaro Clinical Research Institute Biotechnology Laboratory; this laboratory successfully participates in multiple external quality assurance programs in the United States and Europe. Blood culture bottles were assessed for volume adequacy by comparing the weight before and after inoculation with blood. Adequate volume was defined as $\pm 20\%$ of the recommended volume. BacT/Alert Pediatric FAN (PF) and Mycobacteria Blood (MB) culture tubes (BioMérieux Inc., Durham, NC) were loaded into the BacT/Alert 3D Microbial Detection system (BioMérieux), where they were incubated for 5 and 42 days, respectively. Standard methods were used for identifying bloodstream isolates. Complete blood count and CD4 cell counts were performed using standard laboratory methods. HIV-1 antibody testing was performed on whole blood pursuant to the Tanzania Ministry of Health HIV testing guidelines.⁶

Ethical approval was obtained for this study from the Kilimanjaro Christian Medical Centre Research Ethics Committee, the Tanzania National Institutes for Medical Research National Research Ethics Coordinating Committee and an Institutional Review Board of Duke University Medical Center. Written, informed consent was provided by a parent or guardian for all children enrolled.

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RESULTS

During the study period, a total of 6579 inpatient and clinic screening visits were completed. Ultimately, 93 (2.3%) patients met inclusion criteria and were enrolled. Five patients were enrolled twice for a total of 98 enrollment visits. Mycobacterial cultures were not able to be drawn from 2 patients due to compromised clinical status.

Median (range) age of enrolled patients was 7.4 (0.2–15.7) years and 56% (57.1) were male. Detailed demographics and clinical presentation are shown in Table 1. Perceived weight loss in the past month (50/96, 52.1%), fever for >7 days (40/98, 40.8%),

TABLE 1. Characteristics of Enrollment Visits, Kilimanjaro Christian Medical Centre and Mawenzi Regional Hospital, 2010 to 2011

Demographic Characteristics	n/N (%)
Age, median (range) yr	7.4 (0.2–15.7)
Male sex	56/98 (57.1)
Recruited as inpatient	37/98 (37.8)
Bacille Calmette–Guérin scar	86/98 (87.7)
Household TB contact	10/96 (10.4)
Medications	
ART	50/98 (51.0)
Trimethoprim–Sulfamethoxazole prophylaxis	72/97 (74.2)
Antibacterial/antifungal in last 48 hr	35/98 (35.7)
Immunosuppression	
Immunologic ART eligibility in 3 mo before enrollment*	65/97 (67.0)
Severe immunosuppression at time of enrollment†	59/96 (61.5)
Oral candidiasis	16/98 (16.3)
Signs and symptoms at enrollment	
Days ill, median (range)	14 (0–90)
Febrile (>38.5°C)	24/98 (24.5)
Hypothermic (<35.5°C)	4/98 (4.1)
Tachycardic‡	23/95 (24.2)
Tachypneic§	52/97 (53.6)
Malnutrition (all)	52/97 (53.6)
Moderate malnutrition	28/97 (28.9)
Severe malnutrition	24/97 (24.7)
Wasting	
Recent perceived weight loss	50/96 (52.1)
Severe wasting on exam	31/98 (31.6)
Systemic	
Subjective fever >7 d	40/98 (40.8)
Night sweats within 4 wk	27/96 (28.1)
Poor feeding/appetite	38/98 (38.8)
Lymphadenopathy	14/93 (15.1)
Fatigue	43/98 (43.9)
Respiratory	
Cough >2 wk	54/98 (55.1)
Productive cough >4 wk	28/98 (28.6)
Difficulty breathing	22/98 (22.4)
Crepitations	44/97 (45.4)
Gastrointestinal	
Vomiting or diarrhea	27/98 (27.6)
Palpable liver	19/98 (19.4)
Palpable spleen	4/98 (4.1)
Central nervous system	
Comatose	2/98 (2.0)
Convulsions (in past 48 hr)	3/98 (3.1)

*Immunologic ART eligibility: <24 months, all patients; 2–5 years, CD4 ≤750 or CD4% ≤25%; ≥5 years, CD4 ≤350.

†Severe immunosuppression: ≤11 months, CD4 <25%; 12–35 months, CD4% <20%; 36–59 months, CD4% <15%; ≥5 years, CD4 <200 or CD4% <15%.

‡Tachycardia: <12 months, >160/minute; 1–5 years, >120/minute; >5 years, >100/minute.

§Tachypnea: <2 months, ≥60/minute; 2–11 months, ≥50/minute; 1–5 years, ≥40/minute.

||WHO guidelines define moderate malnutrition as less than -2 SD below the median weight/height for children >5 years old and less than -2 SD below the median body mass index/age for children <5 years old. The same applies to severe malnutrition, with the cutoff being less than -3 standard deviations below the median.

BCG indicates Bacille Calmette–Guérin vaccine; ART, antiretroviral therapy.

fatigue (43/98, 43.9%) and cough for >2 weeks (54/98, 55.1%) were the most common presenting complaints. The most common clinical diagnoses on admission were pneumonia (45/98, 45.9%), TB (25/98, 25.5%) and malnutrition (24/98, 24.5%). Most patients with a hospital diagnosis of TB were thought to have pulmonary TB by their clinical team (21/25, 84%), with the remaining patients clinically diagnosed with TB adenitis, miliary TB and TB meningitis.

Mycobacterial blood cultures were drawn from 91 patients over 96 enrollment visits. Bacterial blood cultures were collected from 87 patients over 91 enrollment visits. The median (range) volume of blood collected was 4.1 (1.9–6.7) mL for mycobacterial cultures and 2.8 mL (1.0–6.1) mL for bacterial cultures. Of mycobacterial cultures, 47 (49.5%) of 95 samples were classified as adequately filled. No mycobacteria were isolated from blood cultures after 6 weeks of incubation. A total of 4 aerobic blood cultures were positive with pathogenic organisms: *S. pneumoniae* (2), nontyphoidal *Salmonella* and *E. coli*. Overall, 4 (2.1%) of 187 bacterial and mycobacterial blood cultures were positive for an organism considered most likely to be a contaminant.

No patients were lost to follow-up. Deaths were reported for 8 (8.1%) of 98 patients either during inpatient admission or by family members during follow-up. Three of these patients had a clinical diagnosis of TB. At time of follow-up, anti-TB therapy had been initiated in 13 (13.3%) of 98 patients.

DISCUSSION

In this prospective cohort study, we found no cases of Mtb mycobacteremia among an ill, HIV-infected pediatric patient population in an area with a high TB burden. Previous pediatric studies showing a low prevalence of mycobacteremia were limited by low blood volume and broad inclusion criteria.^{7,8} Ours is the first study to focus enrollment criteria for identifying children at high risk of disseminated TB, as well as maximizing blood sample volume and for mycobacteremia detection. Our results suggest that either mycobacteremia was not present in our patient population or cannot be reliably detected even with the higher blood volumes that we sought.

The absence of blood cultures positive for Mtb in this study is in contrast to multiple adult studies showing that Mtb is a major bloodstream pathogen in HIV-infected adults in sub-Saharan Africa.^{1–4} Although the pathogenesis of disseminated TB is different in adults, with overwhelming lung destruction and subsequent hematogenous dissemination most often leading to mycobacteremia, this result is still surprising given the known tendency of TB to disseminate in children.⁹ We present here several potential explanations for this observation.

It is possible that the volume of blood collected was insufficient to recover mycobacteria. It is known that disseminated Mtb infection often occurs with a low magnitude of bacteremia, limiting culture sensitivity with conventional blood volumes.¹⁰ However, the amount of blood that can be drawn from children is limited both by physiologic concerns and cultural beliefs. Our study was able to increase the volume of blood inoculated in previous pediatric studies to a median (range) of 4.1 (1.9–6.7) mL.^{7,8} In adult studies, mycobacteremia has readily been demonstrated using 5 mL samples.^{1,4} Furthermore, the BacT/Alert MB blood culture system (BioMérieux) used in our study has been shown to produce similar yields with 5 mL blood samples as other commonly used mycobacterial blood culture systems.¹⁰ Nevertheless, it remains possible that insufficient blood volume could be a contributing factor to the absence of blood culture-positive TB in this study.

It is also possible that the inclusion criteria for this study were not able to identify children with disseminated TB. Clinical guidelines are limited on this subject. Pediatric patients with disseminated disease tend to present with constitutional symptoms rather than respiratory symptoms, with hepatosplenomegaly, fever

and wasting being common.¹¹ Immunosuppression and young age are the most important risk factors for disseminated disease.⁹ Lack of fever was not an exclusion criterion in our study due to the physiologic response in severely malnourished children in which hypothermia may appear with sepsis rather than fever. However, because criteria for mycobacteremia are not well established in children, it is possible that our enrollment criteria were not sufficient to identify children at risk for disseminated TB infection. Our study also was unable to investigate gastric aspirates among research participants which, even with a low sensitivity, may have helped with a confirmatory diagnosis of TB in some cases.

A final consideration in the evaluation of mycobacteremia in children is the role of the bacille Calmette–Guérin vaccine. Almost 90% of participants had evidence of bacille Calmette–Guérin vaccination with visible scarring. The vaccine, although not consistent in prevention of pulmonary TB in adults, has been shown effective at preventing or attenuating severe and disseminated TB, especially in pediatric populations.¹² Protection from prior vaccination may help explain the lack of blood culture-positive Mtb in pediatric patients.

In conclusion, no blood cultures were positive for mycobacteria in this focused prospective cohort study. This result suggests that either mycobacteremia was not present in our patient population or cannot be demonstrated with current culture volumes. Given the high morbidity and mortality associated with TB, there is a need for the development of additional diagnostic modalities, as well as a high level of clinical suspicion for TB when evaluating at-risk children.

REFERENCES

- Lewis DK, Peters RP, Schijffelen MJ, et al. Clinical indicators of mycobacteremia in adults admitted to hospital in Blantyre, Malawi. *Int J Tuberc Lung Dis.* 2002;6:1067–1074.
- Arthur G, Nduba VN, Kariuki SM, et al. Trends in bloodstream infections among human immunodeficiency virus-infected adults admitted to a hospital in Nairobi, Kenya, during the last decade. *Clin Infect Dis.* 2001;33:248–256.
- Archibald LK, den Dulk MO, Pallangyo KJ, et al. Fatal *Mycobacterium tuberculosis* bloodstream infections in febrile hospitalized adults in Dar es Salaam, Tanzania. *Clin Infect Dis.* 1998;26:290–296.
- Crump JA, Ramadhani HO, Morrissey AB, et al. Invasive bacterial and fungal infections among hospitalized HIV-infected and HIV-uninfected adults and adolescents in northern Tanzania. *Clin Infect Dis.* 2011;52:341–348.
- World Health Organization. *Child Growth Standards and the Identification of Severe Acute Malnutrition in Infants and Children: A Joint Statement by the World Health Organization and the United Nations Children's Fund.* Geneva, Switzerland: World Health Organization; 2009.
- Welfare TURoTMOHaS. *Standard Operating Procedures for HIV Testing and Counselling (HTC) Service.* Dar es Salaam, Tanzania; 2009.
- Waddell RD, Lishimpi K, von Reyn CF, et al.; Dartmouth/UCLMS/UNZA Collaborative Study Group. Bacteremia due to *Mycobacterium tuberculosis* or *M. bovis*, Bacille Calmette–Guérin (BCG) among HIV- positive children and adults in Zambia. *AIDS.* 2001;15:55–60.
- Archibald LK, Kazembe PN, Nwanyanwu O, et al. Epidemiology of bloodstream infections in a bacille Calmette–Guérin-vaccinated pediatric population in Malawi. *J Infect Dis.* 2003;188:202–208.
- Swaminathan S, Rekha B. Pediatric tuberculosis: global overview and challenges. *Clin Infect Dis.* 2010;50(suppl 3):S184–S194.
- Crump JA, Morrissey AB, Ramadhani HO, et al. Controlled comparison of BacT/Alert MB system, manual Myco/F lytic procedure, and isolator 10 system for diagnosis of *Mycobacterium tuberculosis* Bacteremia. *J Clin Microbiol.* 2011;49:3054–3057.
- World Health Organization. *TB/HIV: A Clinical Manual.* Geneva, Switzerland: World Health Organization; 2004.
- Colditz GA, Brewer TF, Berkey CS, et al. Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA.* 1994;271:698–702.