Screening for Tuberculosis in HIV-Infected Nigerian Children with a Urinary Antigen Assay

Holly E. Rawizza[1,2], Stephen Oguche[3], Godwin Imade[3], Oche Agbaji[3], David Olaleye[4], Georgina Odaibo[4], Kike Osinusi[4], Isaac Adewole[4], Prosper Okonkwo[5], and Phyllis J. Kanki[2]

[1] Brigham & Women's Hospital, Boston, USA; [2] Harvard School of Public Health, Boston, USA; [3] Jos University Teaching Hospital, Jos, Nigeria; [4]University College Hospital, Ibadan, Nigeria; [5]AIDS Prevention Initiative Nigeria (APIN), Ltd., Abuja, Nigeria

CROI 2014
Poster Abstract #810
Contact: Holly Rawizza, M.D.
Brigham & Women's Hospital
75 Francis Street, PBB-A4
Boston, MA 02115
hrawizza@hsph.harvard.edu

AIDS Prevention Initiative Nigeria

Abstract

Background:
Tuberculosis (TB) remains the leading cause of death among HIV-infected children in sub-Saharan Africa. Accurate and prompt identification of TB infection remains challenging, particularly among young children who are often unable to produce sputum. In this study, we evaluated the sensitivity and specificity of the lipoarabinomannan (LAM) TB ELISA for the detection of mycobacteria-specific antigen in urine samples of HIV-infected Nigerian children.

Methodology:
As part of routine care, all HIV-infected children enrolling in the APIN/Harvard PEPFAR program undergo routine screening for TB consisting of: clinical evaluation for symptoms or signs of TB infection, chest radiograph, and tuberculin skin testing (TST). In addition, for those children with a chronic productive cough, sputum samples are collected when possible. A composite of these standard TB diagnostic techniques was used as the "gold standard" and compared against results of the urine LAM assay.

Results:
184 HIV-infected pediatric patients were enrolled at two study sites; 40% were female with a median age of 5 years and median baseline CD4+ cell count of 434 cells/mm^3. Almost 36% of patients (66 children) were diagnosed with TB within 3 months after collecting the LAM sample. The sensitivity of the LAM assay to detect the composite diagnosis of TB infection is 10.6% (95% CI 4.7-21.2%), specificity is 97.4% (95% CI 91.9-99.3%), and the PPV is 70% (95% CI 35.3-91.9%). The median CD4 count was 103 cells/mm^3 (IQR, 25-331 cells/mm^3) among children with a positive LAM, compared to 393 cells/mm^3 (IQR, 177-717 cells/mm^3) among those diagnosed with TB but with a negative LAM result.

Conclusions:
Although the sensitivity of the LAM assay was low in this population, the relatively high PPV suggests that a positive result may add valuable information to the care of a sick child. However, more sensitive rapid diagnostic methods for the detection of TB in young children are urgently needed.

Background

- As of 2011, over 3.3 million children were estimated to be living with human immunodeficiency virus (HIV) infection in sub-Saharan Africa.
  - Nigeria is home to over 430,000 children with HIV, with approximately 60,000 children newly infected in 2012 alone, more than any other country in the world [1].
  - Compounding this burden, Nigeria also faces one of the highest rates of incident tuberculosis (TB) among HIV-positive patients [1].
- Despite improved access to antiretroviral therapy (ART), TB remains the leading killer of children with HIV [2-4].
  - An estimated 20-50% of HIV-infected children living in endemic areas are coinfected with TB, with nearly one-quarter of HIV-infected children diagnosed with TB every year.
  - For children, this combination is particularly devastating, with a mortality rate that is five times that for TB infection alone.
- Accurate and prompt identification of TB infection in HIV-infected children is challenging, as currently available diagnostic techniques lack sensitivity.
  - Additionally, children younger than 5 years of age cannot reliably produce sputum, making diagnosis with current techniques particularly challenging in this vulnerable population.
- Techniques that utilize rapid detection methods for lipoarabinomannan (LAM), a mycobacteria-specific antigen, in urine samples are particularly attractive due to ease of sample collection, low cost, and the ability to distinguish active from latent TB.
- In a recent review of the TB-LAM lateral flow urine antigen assay for HIV-associated TB, the sensitivity of the assay was widely variable and dependent upon the population being studied [5];
  - A sensitivity of 83% was reported among a subset of adult patients with CD4+ cell counts <100 cells/mm^3, most of whom were inpatients with suspected disseminated TB.
  - Low estimates were around 12% among mostly outpatient TB suspects in a South African cohort.
- The test appears to be particularly sensitive for the detection of TB in patients with advanced immune suppression.
- Thus, we hypothesized that this rapid urinary TB ELISA may have increased sensitivity in HIV-infected pediatric patients, who experience high rates of TB dissemination.

Methods

- Since 2004, the Harvard School of Public Health has partnered with AIDS Prevention Initiative in Nigeria (APIN) utilizing PEPFAR funds to provide care and treatment for adults and children living with HIV-infection throughout Nigeria.
- As part of routine care, all HIV-infected children enrolled in the pediatric antiretroviral treatment program undergo screening for TB consisting of:
  - A clinical evaluation for symptoms or signs of TB infection, screening chest radiograph, and tuberculin skin testing (TST). In addition, for those children with a chronic productive cough, sputum samples are collected if possible and are evaluated by Ziehl-Neelsen staining.
- We carried out a prospective cohort study at two large tertiary care centers in Nigeria, namely Jos University Teaching Hospital (JUTH) and University College Hospital of Ibadan (UCH).
- HIV-infected children enrolled in the APIN pediatric program were recruited and provided consent for the collection of a single urine sample for testing with the Clearview® LAM TB ELISA.

Methods (cont.)

- For the purpose of analysis, patients were stratified into distinct TB risk groups, similar to categorization schemes used by other researchers [6,7]. Specifically, patients were retrospectively categorized into one of the following four groups: "confirmed TB infection", "highly probable TB infection", "clinically indeterminant TB", and "not TB" based on chart review.
- Patients were defined as not having TB (Group D) if they did not receive TB treatment and had a stable clinical course for at least 6 months after collection of the LAM sample.

Table 1. Criteria for TB Risk Categories

Group	TB Risk Category	Clinical or Radiographic Criteria
A	**Confirmed TB Infection**	AFB positive by sputum smear or clinical specimen MTB confirmed by culture
B	**Highly-probable TB infection**	CXR highly suggestive of active TB (cavitation in association with chronic pneumonia, miliary shadowing, non-pyogenic pleural effusion, OR hilar or mediastinal lymphadenopathy) Cervical LAN (with sinus tract formation or positive TST) Spinal gibbus formation Meningitis with CSF consistent with TB meningitis
C	**Clinically indeterminant TB**	Do not meet criteria for highly-probable TB, but active TB cannot be excluded
D	**Not TB**	Alternative diagnosis made Stable clinical course despite receiving no ATT for 6 months

Results

- From May 2010 to July 2012, 184 HIV-infected pediatric patients were enrolled at the two study sites; they had the following baseline characteristics:
  - 40% were female
  - median age of 5 years
  - median baseline CD4+ cell count of 434 cells/mm^3
- Almost 36% of patients (66 children) were diagnosed with TB within 3 months after collecting the LAM sample.
- Overall, the rate of LAM positivity was low, with only 10 patients testing positive.

Table 2. Characteristics of children with positive or negative tests for lipoarabinomannan in urine samples

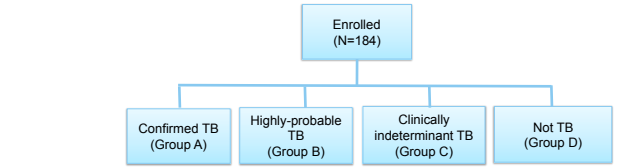
Characteristics	Median (IQR)^ LAM negative (N=174)	Median (IQR)^ LAM positive (N=10)	P-value
Age (years)	5.1 (2.5-9.4)^	7.2 (1.7-9.9)	0.78
Female Sex (%)	39%	40%	1.0
Anthropometrics			
Ages 0-5 years	n=82	n=4	
Weight for age z-score	-1.9 (<-3 to -0.5)	-1.2 (-2.0 to -0.7)	0.63
Weight for length z-score	-0.2 (-1.9 to 0.6)	0.3 (-1.2 to 0.4)	0.68
Ages ≥5 years	n=87	n=6	
Body Mass Index (BMI) z-score	-0.5 (-1.8 to 0.1)	-1.4 (-2.7 to -0.9)	0.22
WHO Clinical Stage (%)			
1 or 2	49%	50%	
3 or 4	51%	50%	1.0
CD4+ cell count (cells/mm³) at LAM	444 (225-927)	103 (25-331)	**0.018**
CD4 percentage at LAM	16 (10-23)	6 (2-23)	0.17
Receiving ART (%) at LAM	18%	20%	1.0
WHO 5 TB screening questions – positivity rate (%)	36%	70%	**0.045**
Cough ≥2 weeks (%)	30%	60%	0.07

^Values represent: Median (IQR) unless otherwise indicated

- The overall sensitivity of the LAM assay to detect the composite diagnosis of TB infection was 10.6% (95% CI 4.7-21.2%), specificity was 97.4% (95% CI 91.9-99.3%), and the positive predictive value (PPV) was 70% (95% CI 35.3-91.9%).
- The median CD4+ cell count among children with a positive LAM was lower, at 103 cells/mm^3 (IQR, 25-331 cells/mm^3) compared to 393 cells/mm^3 (IQR, 177-717 cells/mm^3) among those diagnosed with TB (n=66), but with a negative LAM result (p=0.045).
- The proportion of children who screened positive by the World Health Organization's (WHO) five TB screening questions was higher in the LAM positive group, but missed 30% of patients.

Results (cont.)

Figure 1. LAM positivity by TB Risk Categories



- When the sensitivity and specificity were calculated based on patients categorized only into Groups A and B, the sensitivity was 13.6%, similar to that determined using a "composite" diagnosis of TB based on overall physician assessment of TB infection.
- Similarly, the specificity calculated based on those patients categorized only to Group D was also high at 98.6%.

Follow-up of LAM positive patients:
- Of the 10 patients found to be LAM positive, 7 were diagnosed with TB and initiated on treatment based on clinical, radiographic, and/or sputum findings.
- For the 3 patients who were LAM positive, but not identified as TB suspects, the outcomes were:
  - A 16 month old severely malnourished infant with failure to thrive for 6 weeks and fever for 2 weeks who was lost to follow-up after a single visit, following a plan for hospital admission; CD4+ cell count was 83 cells/mm^3
  - A 14 year old who had been treated for TB 2 years earlier, who transferred to a new site, off ART with one week of cough, whose symptoms improved with successful ART
  - A 2.5 year old with a CD4+ cell count of 1670 cells/mm^3 (CD4% of 27%) who did not receive TB treatment and has subsequently been followed on ART for almost 2 years with virologic suppression; this may represent a false positive
- Although most LAM positive TB suspects were identified by clinical, radiographic, and/or AFB microscopy, use of this rapid detection test has the potential to provide critical information that may alter the care being delivered.

Conclusions

- Although the sensitivity of the LAM assay was low in this pediatric population, the relatively high PPV suggests that a positive result may add valuable information to the care of a sick child.
  - For infants and young children at highest-risk of mortality related to disseminated TB, this rapid diagnostic may add to therapeutic decisions.
  - Further performance characteristics of potential combined TB predictors are underway.
- Similar to other studies, we found that LAM positivity was associated with severe immunocompromise as indicated by the association with significantly lower CD4+ cell counts.
- Although malnutrition was not associated with an increased risk of LAM positivity, a generalized epidemic of undernutriton was noted in this population and evaluations of implementation projects to further reduce undernutrition are needed.
- Evaluation of the impact of the point-of-care LAM urine dipsticks on TB and HIV outcomes are needed in pediatric populations.
- Most urgently, more sensitive rapid diagnostic methods for the detection of TB in young children are desperately needed.

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Acknowledgements: This research has been supported by a Harvard University Center for AIDS Research grant (HU CFAR NIH/NIAID fund P30 AI-060354) and by the President's Emergency Plan for AIDS Relief (PEPFAR) through HRSA under the terms of Cooperative Agreement #U51HA02522. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funding institution. The authors would like to acknowledge the hard work and dedication of the clinical, data, and laboratory staff at all of the Harvard/APIN PEPFAR ART sites.