Urine lipoarabinomannan testing for diagnosis of pulmonary tuberculosis in children: a prospective study

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Summary

Background Urine tests for mycobacterial lipoarabinomannan might be useful for point-of-care diagnosis of tuberculosis in adults with advanced HIV infection, but have not been assessed in children. We assessed the accuracy of urine lipoarabinomannan testing for the diagnosis of pulmonary tuberculosis in HIV-positive and HIV-negative children.

Methods We prospectively recruited children (aged ≤15 years) who presented with suspected tuberculosis at a primary health-care clinic and paediatric referral hospital in South Africa, between March 1, 2009, and April 30, 2012. We assessed the diagnostic accuracy of urine lipoarabinomannan testing with lateral flow assay and ELISA, with mycobacterial culture of two induced sputum samples as the reference standard. Positive cultures were identified by acid-fast staining and tested to confirm *Mycobacterium tuberculosis* and establish susceptibility to rifampicin and isoniazid.

Findings 535 children (median age 42·5 months, IQR 19·1–66·3) had urine and two induced specimens available for testing. 89 (17%) had culture-confirmed tuberculosis and 106 (20%) had HIV. The lateral flow lipoarabinomannan test showed poor accuracy against the reference standard, with sensitivity of 48·3% (95% CI 37·6–59·2), specificity of 60·8% (56·1–65·3), and an area under the receiver operating characteristic curve of 0·53 (0·46–0·60) for children without HIV and 0·64 (0·51–0·76) for children with HIV. ELISA had poor sensitivity in children without HIV (sensitivity 3·0%, 95% CI 0·4–10·5) and children with HIV (0%, 0·0–14·3); overall specificity was 95·7% (93·4–97·4).

Interpretation Urine lipoarabinomannan tests have insufficient sensitivity and specificity to diagnose HIV-positive and HIV-negative children with tuberculosis and should not be used in this patient population.

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Introduction

Microbiological confirmation of pulmonary tuberculosis in children is difficult. Collection of a sample from the lower respiratory tract is challenging because young children rarely spontaneously produce sputum. Sputum induction or gastric lavage are useful methods for obtaining respiratory samples,[1] but require trained staff and basic equipment. Even when appropriate samples can be obtained, smear microscopy is rarely positive in children and mycobacterial culture is often required.[2] The main drawback of culture is that treatment decisions often need to be made before results are available because the clinical course of tuberculosis can be rapid in children younger than 5 years. Moreover, culture requires advanced infrastructure and trained staff and is therefore seldom available in countries with the greatest burden of disease. We recently reported on the accuracy of Xpert MTB/RIF testing of induced sputum[3] and nasopharyngeal aspirate[4] specimens, which holds promise as a rapid and feasible alternative to culture in low-resource settings.

A urine test would simplify specimen collection for children with suspected pulmonary tuberculosis. Studies of urine tests[5–9] for tuberculosis in adults with suspected tuberculosis have shown mixed results. Two approaches have been assessed. First, detection of small fragments of *Mycobacterium tuberculosis* DNA in urine[5] showed initial promise, but early findings have not been confirmed. Second, urine tests for mycobacterial lipoarabinomannan, in both ELISA and lateral flow assay format, have been assessed in adults with suspected tuberculosis.[6–8] The tests are sensitive in adults with advanced HIV disease but not in HIV-negative adults and HIV-positive adults with CD4 counts higher than 100 cells per L.[9] The lateral flow assay version of the lipoarabinomannan test might be the first point-of-care test for tuberculosis with diagnostic utility. In a recent Comment in *The Lancet Global Health*,[10] Van Rie outlined the need to assess the lipoarabinomannan test in children.

Young children with pulmonary tuberculosis develop disseminated disease more frequently than do adults; therefore, urine lipoarabinomannan testing might be useful for the diagnosis of pulmonary tuberculosis in children, including those without HIV. HIV-positive children are especially at risk for disseminated tuberculosis. Therefore, we prospectively assessed the

accuracy of urine lipoarabinomannan testing in ELISA and lateral flow assay formats in HIV-positive and HIV-negative children presenting with suspected pulmonary tuberculosis to a primary health-care clinic or to a referral hospital in Cape Town, South Africa.

Methods

Study design and participants

We enrolled children aged 15 years or younger who presented with suspected pulmonary tuberculosis to Red Cross War Memorial Children's Hospital (a tertiary referral hospital) or Nolungile Clinic (a primary health-care facility), between March 1, 2009, and April 30, 2012. Suspected pulmonary tuberculosis was defined on the basis of cough of any duration and one of the following: household contact with an infectious tuberculosis source case within the preceding 3 months, loss of weight or failure to gain weight in the preceding 3 months (established by either documented weight loss on growth chart or parental report), a positive tuberculin skin test to purified protein derivative (2TU, PPD RT23, Staten Serum Institute, Denmark, Copenhagen), or a chest radiograph suggesting pulmonary tuberculosis (including airway compression or lymphadenopathy, diffuse miliary pattern, pleural effusion, or cavitary disease). A positive tuberculin skin test was defined as 5 mm or more of transverse induration in children with HIV or 10 mm or more in children without HIV. Children were excluded if they had received more than 72 h of treatment or prophylaxis for tuberculosis, if they were not resident in Cape Town and could not be followed up, if informed consent was not obtained, or if two induced sputum and one urine specimen could not be obtained.

Written, informed consent for enrolment in the study was obtained from a parent or legal guardian. The Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, and the Provincial Government of the Western Cape approved the study. Tuberculosis treatment was started at the discretion of the treating doctor on the basis of clinical, radiological, and microbiological information. Follow-up visits were done at 1, 3, and 6 months for children on tuberculosis treatment and at 1 and 3 months for those not treated. To assess response to treatment at follow-up, we recorded symptoms, signs, and weight, and repeated chest radiograph at completion of tuberculosis treatment.

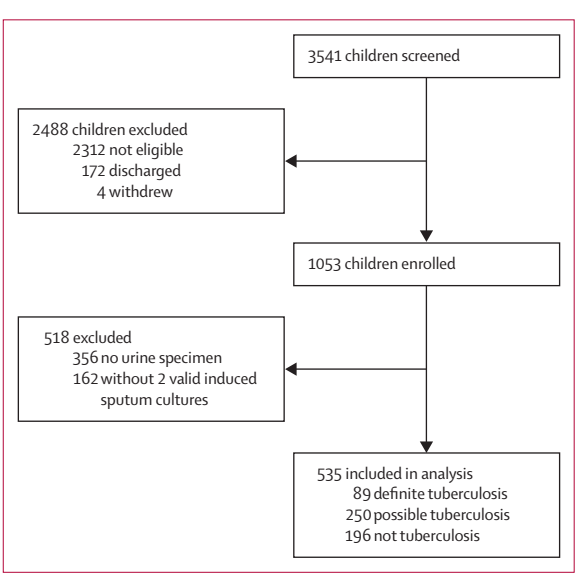

Figure 1: **Study profile**

Procedures

A history and physical examination were done in all children by a study doctor. Routine clinical investigations included chest radiography, tuberculin skin test, and HIV testing in children with unknown HIV status (HIV rapid test, followed by a confirmatory PCR for children aged <18 months or HIV ELISA in children aged >18 months). CD4 count and HIV viral load were tested, and HIV-positive children were categorised with WHO clinical and immunological classification.[11]

Two consecutive induced sputum specimens were obtained in children for microbiological confirmation of tuberculosis as previously described[3] and submitted for smear and liquid culture. Induced sputum specimens were transported within 2 h of collection to an accredited, centralised laboratory at Groote Schuur Hospital, Cape Town, and processed individually with standardised protocols by trained technologists. For both specimens, after decontamination with N-acetyl-L-cysteine and sodium hydroxide (1·0% final concentration), centrifuged deposits were resuspended in 1·5 mL phosphate buffer. A drop of induced sputum sediment was used for fluorescent acid-fast smear microscopy. Automated liquid culture testing (BACTEC MGIT, Becton Dickinson, Cockeysville, MD, USA) was done with 0·5 mL resuspended pellet. Cultures were incubated for up to 6 weeks. Positive cultures were identified by acid-fast staining followed by MTBDR*plus* testing (Hain Lifescience, Nehren, Germany) to confirm *M tuberculosis* and to establish susceptibility to rifampicin and isoniazid.

Urine was collected from children with specimen bags, unless children were old enough to voluntarily produce a specimen on demand. All urine specimens were frozen within 2 h of collection at –80°C, and were tested within 24 months of storage. For the lateral flow assay, 60 µL of thawed urine was applied to the test strip, incubated at room temperature for 25 min, visually inspected, and the intensity of any visualised test band was graded by comparison of band intensity with the manufacturer-supplied reference card by one research laboratory technician trained in this technique. Test band intensity was graded as zero if no band was visualised, and grade 1–5 for visualised bands, according to the band on the reference card that most closely matched the test band. Clearview tuberculosis ELISA (Alere, Waltham, MA, USA) was done according to the manufacturer's