ORIGINAL ARTICLE

Nasopharyngeal aspiration for diagnosis of pulmonary tuberculosis

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Background: Confirmation of pulmonary tuberculosis (PTB) in young children is difficult as they seldom expectorate sputum.
Aim: To compare sputa obtained by nasopharyngeal aspiration and by sputum induction for staining and culture of *Mycobacterium tuberculosis*.
Patients and methods: Patients from Mulago Hospital, Kampala with symptoms suggestive of PTB were considered for inclusion in the study. Those with a positive tuberculin test and/or a chest radiograph compatible with tuberculosis were recruited. Infection with human immunodeficiency virus (HIV) was confirmed by duplicate enzyme-labelled immunosorbent assay or in children <15 months by polymerase chain reaction (PCR). Direct PCR was undertaken on 82 nasopharyngeal aspirates.
Results: Of 438 patients referred, 94 were recruited over a period of 5 months. Median (range) age was 48 (4–144) months. Of 63 patients tested, 69.8% were infected with HIV. Paired and uncontaminated culture results were available for 88 patients and smear results for 94 patients. Nasopharyngeal aspirates were smear-positive in 8.5% and culture-positive in 23.9%. Induced sputa were smear-positive in 9.6% and culture positive in 21.6%. Overall, 10.6% were smear-positive, 25.5% were culture-positive and 26.6% had smear and/or culture confirmed tuberculosis. Direct PCR on nasopharyngeal aspirates had a sensitivity of 62% and specificity of 98% for confirmation of culture-positive tuberculosis.
Conclusions: Nasopharyngeal aspiration is a useful, safe and low-technology method for confirmation of PTB and, like sputum induction, can be undertaken in outpatient clinics.

The diagnosis of pulmonary tuberculosis (PTB) in young children is usually based on contact history, positive tuberculin test and compatible chest radiography. However, confirmation of tuberculosis by culture is important where, for example, there is difficulty in clinical diagnosis, the contact history is not known or drug sensitivities are not available. In developing countries the diagnosis of PTB is particularly difficult as the contact history is often not clear (especially where tuberculosis/human immunodeficiency virus (HIV) co-infection in the household is common) and the tuberculin test is often falsely negative. Chest radiography may be difficult to interpret, especially when there is HIV-related pulmonary disease such as lymphocytic interstitial pneumonitis (LIP).[1 2]

Obtaining sputum in young children who are unable to expectorate is problematic. Gastric aspiration usually requires a child to be admitted for up to 3 days and has to be undertaken in the early morning while the child is recumbent and fasted overnight.[3] Alternative methods include sputum induction (SI),[4 5] laryngeal swab (LS)[6 7] and nasopharyngeal aspiration (NPA).[8 9] This study aimed to compare the value of NPA with SI for confirmation of PTB.

METHODS

The study was undertaken at Mulago Hospital, Kampala during the period January to June 2004. A diagnostic room was set up for SI, NPA and tuberculin testing, organised by a dedicated paediatrician, a nurse (who undertook the SI and NPA) and an HIV counsellor. Outpatients from the tuberculosis and HIV clinics and inpatients from the general wards and malnutrition units were recruited. Patients with symptoms suggestive of PTB, for example cough for over 2 weeks, weight loss, severe malnutrition not responding to nutritional rehabilitation or an abnormal chest radiograph, were screened for tuberculosis. Consent was obtained from the guardian(s) and the child if old enough to give consent. Patients with a positive tuberculin test and/or a chest radiograph suggestive of tuberculosis were recruited. Where the tuberculin test was negative and the chest radiograph was non-specific, the child was given a 2 week course of antibiotics. If symptoms or chest radiograph did not improve, they were then recruited. If the patient was known to have HIV infection, was stable and had a chest radiograph with bilateral symmetrical hilar opacities and diffuse parenchymal infiltration suggestive of LIP they were usually not recruited as it was considered that the likelihood of tuberculosis would be relatively low.[10] NPA and SI were undertaken on each patient; NPA was done before SI and both were undertaken only once. Children with weights ⩽3 standard deviations below the mean for their heights or with oedema of both feet were classified as having severe malnutrition according to World Health Organization criteria.

Chest radiography

Probable PTB was suggested by hilar or mediastinal lymphadenopathy, local collapse/consolidation, severe bilateral but asymmetric disease, cavitation or miliary changes. Chest radiographs were reviewed by JBSC who was blinded to the clinical details.

Skin testing

Chiron Vaccines Evans (Liverpool, UK) tuberculin (10 units) was used for the Mantoux test and ⩾10 mm induration, irrespective of the presence of a BCG scar, was regarded as

Abbreviations: AFB, acid-fast bacillus; HIV, human immunodeficiency virus; LIP, lymphocytic interstitial pneumonitis; LS, laryngeal swab; MTB, *Mycobacterium tuberculosis*; NPA, nasopharyngeal aspiration; PCR, polymerase chain reaction; PTB, pulmonary tuberculosis; SI, sputum induction

positive. In severely malnourished patients or patients known to have HIV infection, 6–9 mm was accepted.

HIV testing

All patients where the HIV status was not known were offered a rapid HIV test (Abbott, Abbott Park, IL, USA) with pre- and post-test counselling of the guardian(s). Confirmatory HIV tests were undertaken at the Uganda Virus Research Institute (UVRI), Entebbe using Vironostika Uniform II + O (bioMérieux, Marcy l'Etoile, France) and Murex HIV 1.2.0 (Abbott) enzyme immunosorbent assays (ELISA). For infants under 15 months with a positive ELISA, polymerase chain reaction (PCR) was undertaken in order to exclude false positive results arising from the transfer of maternal antibody.

Nasopharyngeal aspiration

Patients were in the sitting position. A graduated suction catheter (Caretip; Meddis, Chipping Campden, UK) was inserted through the nostril into the oropharynx which stimulated a cough reflex; secretions were aspirated mechanically.

Sputum induction

SI was undertaken with a DeVilbiss nebuliser (Sunrise Medical, Carlsbad, CA, USA). Salbutamol (500 µg) was nebulised initially for 5 min. This was followed by 15 ml of 3% hypertonic saline for 20 min. NPA was undertaken to obtain the secretions. Chest physiotherapy was not undertaken prior to suction.

Laboratory methods

NPA and SI specimens were kept in a cool-box until transported to the National Tuberculosis Diagnostic Unit, Wandegeya, Kampala each afternoon. Specimens were processed by routine methods. Briefly, sputum specimens were digested and decontaminated with NAOH-NALC-NA-citrate before undergoing centrifugation, fluorochrome staining and culture on Loewenstein Jensen media. The presence of 1 acid-fast bacillus (AFB) in 100 high-powered microscopy fields defined a positive smear.

Frozen NPA samples were transported from Kampala to Liverpool in order to carry out direct PCR using a commercially available real-time PCR assay (RealArt, M. tuberculosis RT-PCR kit, Artus, Hamburg, Germany). The real-time assay amplifies a region of the mycobacterial 16 Sr DNA using primer binding sites that are conserved in all members of the *Mycobacterium tuberculosis* (MTB) complex. The specific amplicon is detected by a dual-labelled flurogenic probe. In addition, the real-time assay contains a second heterologous PCR system which is used to control the DNA purification procedure as well as the existence of possible PCR inhibitors. This ensures high sensitivity. The analytical sensitivity of the real-time assay for MTB complex is 1.5 DNA copies/ml.

The Research Ethics Committee of The Liverpool School of Tropical Medicine and the Ethics and Research Committee of Mulago Hospital approved the study.

Data analysis

Confirmed tuberculosis was defined in the presence of a positive smear or culture for MTB. NPA was compared with SI for value in confirming tuberculosis. Diagnostic methods were compared between HIV-infected and non-infected subgroups. Agreement between SI and NPA results was calculated using κ statistics. Odd ratios, positive predictive values, sensitivities and specificities were calculated where appropriate.

RESULTS

Of 438 children referred with suspected tuberculosis, 96 were considered to have probable PTB based on study entry criteria. The results from two children were excluded, leaving 94 (21.2%) for analysis. The median (range) age of the study group was 48 (4–144) months and 57 (60.6%) were male. The median weight-for-height z score (interquartile range) was −1.30 (−2.79 to +0.04) and 22.9% were severely malnourished. Of 63 children who were tested, 44 (69.8%) were infected with HIV. Both SI and NPA were undertaken in all patients. A small number of children had coughing spasms and/or vomiting after SI and some had bloodstained aspirates following both procedures. A child with AIDS had dyspnoea and pneumonic changes on chest radiograph. He deteriorated during pre-induction with salbutamol and the procedure was abandoned. Unfortunately, he later died. Another child who became emotionally distressed during NPA developed a brisk epistaxis and the procedure was abandoned. Both children were excluded from analysis because their datasets were incomplete.

Sputum smear and culture results are summarised in table 1. Of 94 children who had a sputum smear, eight (8.5%) were positive with NPA and nine (9.6%) with SI. Of 88 children who had paired and uncontaminated cultures for *M tuberculosis*, 21 (23.9%) were positive with NPA and 19 (21.6%) with SI. Combining the two methods, smear was positive in 10 (10.6%), culture in 24 (25.5%) and PTB confirmed on smear and/or culture in 25 (26.6%). The youngest child in whom tuberculosis was confirmed was 4 months old and the patient was positive by both methods.

Direct PCR was undertaken on 82 NPA specimens. PCR had 62% sensitivity and 98% specificity for culture-positive specimens. By comparison, direct PCR had 100% sensitivity and 91% specificity for smear-positive specimens.

Of the 44 children infected with HIV, 12 (27.3%) were confirmed with tuberculosis, which was the same proportion of confirmed cases in the total group. Of 19 children who were not infected with HIV, tuberculosis was confirmed in eight (42.1%). Consent for HIV testing was refused for the remaining 31 children.

Of the 53 patients with induration >5 mm on tuberculin testing, 46 (87.0%) had induration ⩾10 mm and seven (13.2%) had induration between 6 and 9 mm in diameter. The tuberculin test was interpreted as positive in 51 (54.3%) children, with 20/25 (80.0%) confirmed and 31/69 (44.9%) unconfirmed cases (positive predictive value 39.2%). Of the 44 children infected with HIV, the tuberculin test was positive in 21 (47.7%), with 9/12 (75.0%) confirmed and 12/32 (37.5%) unconfirmed cases (positive predictive value 42.3%). Of the 22 malnourished children, the tuberculin test was positive in 10 (45.5%) with 6/8 (75.0%) confirmed and 4/14 (28.6%) unconfirmed cases (positive predictive value 60.0%).

Chest radiographic changes compatible with tuberculosis were reported in 68 (72.3%) children, with 23/25 (92.0%)

Table 1 Smear and culture for nasopharyngeal aspiration and sputum induction

	Smear (n = 94)	Culture (n = 88)*
NPA+ SI+ (%)	7 (7.4)	16 (18.2)
NPA+ SI− (%)	1 (1.1)	5 (5.7)
NPA− SI+ (%)	2 (2.1)	3 (3.4)
NPA− SI− (%)	84 (89.4)	64 (72.7)
Total NPA or SI+ (%)	10 (10.6)	24 (27.3)
Cohen's κ	0.81†	0.74†

*Six cultures were contaminated and not included in this paired analysis.
†$p<0.001$.

confirmed and 45/69 (65.2%) unconfirmed cases (positive predictive value 33.8%).

Household contact with an active case of PTB was documented for 32 (34.4%) children, with 11/25 (44.0%) confirmed and 21/69 (30.4%) unconfirmed cases.

BCG status was recorded for 93 children and 47 (50.5%) had a BCG scar, with 7/24 (29.2%) confirmed and 40/69 (58.0%) unconfirmed cases (OR 0.3, 95% CI 0.1 to 0.9). There was no statistical difference between the confirmed and unconfirmed groups for severe malnutrition, disseminated lymphadenopathy or hepatosplenomegaly.

DISCUSSION

This study has shown NPA to be a simple and valuable method for confirmation of tuberculosis. The proportion of positive cultures (24%) for NPA is close to the 30%[8] and 23%[9] rates demonstrated previously in Peru. However, the smear positive proportion (8.0%) was lower than in the Peruvian study (17%).[8] Specimens obtained by NPA are probably similar to those obtained by LS but the latter (if done properly) might be expected to obtain sputum when directly coughed up as opposed to more general secretions in the pharynx as in the case of NPA. In a study using LS in Kampala, Lloyd[6] obtained a very high culture rate (63%), and Thakur *et al* in Lucknow, India[7] found a culture rate of 27% (table 2).

SI, NPA and LS can be undertaken during the daytime and in outpatients in contrast to GA, which is usually undertaken in the early morning (5–6 am) while the child is still recumbent and has not been fed. These restrictions make GA a more logistically difficult procedure to perform.

Studies of SI carried out to date have reported less than 30% culture-positivity compared with 30–45% in children and up to 75% in infants subjected to GA (table 2). Smears for AFB from SI and NPA appear to have higher positivity than most studies using GA (table 2). Smears are more likely to be positive in advanced PTB, cavitatory and miliary disease, especially if the child has a productive cough.[16]

A recent South African study compared SI with GA, both of which were generally undertaken on three occasions[5] (table 2). One SI had an 8.4% higher yield than one GA specimen. Undertaking SI over 3 days increased diagnostic yield by 13% and 8% for the second and third specimens, respectively, and for GA results were 24% and 8%, respectively. Although desirable, it is often impractical to undertake procedures over 3 days in low-income countries, especially in view of the cost of recurrent materials, especially for SI. In this study, chest physiotherapy was not undertaken before sputum collection. This may have improved mycobacterial yield.

SI may be associated with transient hypoxaemia, bronchospasm and an increase in volume of pre-existing pleural effusion and some of these risks may be increased in debilitated patients such as those with AIDS.[17 18] In this study, both SI and NPA were generally well tolerated although suction was distressing for all patients and was associated with coughing spasms, retching and vomiting and minor epistaxis in some. One child who had AIDS and pneumonia did not tolerate pre-induction salbutamol nebulisation. Although we did not proceed to SI he later died on the ward. Caution is necessary in performing SI in ill, debilitated patients.

Sensitivity for direct PCR in culture-confirmed NPAs was 62%. In a Peruvian study, the direct PCR-positive rate for culture-positive GAs was 80% and for NPA 58%.[9] The reason for the lower sensitivity for NPA is not known. It is possible that GA simply yields more concentrated sputum.

Because we were testing new ways to confirm tuberculosis, it was important to recruit patients with the most likelihood of having PTB. We attempted to exclude relatively well patients with HIV-related pulmonary disease such as LIP. This was because the likelihood of these patients having tuberculosis might be lower than patients with similar symptoms who do not have HIV infection.[10] Approximately 70% of the children who were tested for HIV were infected. Tuberculosis was confirmed in 27.3% of children with HIV infection and in 42.1% of children without HIV infection.

There are limitations to the study. For completeness we could have included GA as a procedure and undertaken NPA and SI on 3 consecutive days. As many of the patients were outpatients this was not possible and for many guardians who lived far from the hospital it would not have been acceptable. Only 63/94 patients (67%) were tested for HIV. However, the 70% HIV infection rate for children suspected of tuberculosis is similar to other studies in sub-Saharan Africa.[10] It was only possible to test a proportion of specimens for PCR. Thus, we chose the relatively new method for diagnosis of tuberculosis, namely NPA.

Where facilities for smear and culture are available, NPA is a useful low-technology method for diagnosis of tuberculosis. Both NPA and SI are useful for outpatients as well as inpatients and are likely to have higher smear positive yields than GA.

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Table 2 Studies of smear and *M tuberculosis* culture using gastric aspirate, sputum induction, laryngeal swab and nasopharyngeal aspirate

Study/country	Date	Method	Sample	Smear +ve (%)	Culture +ve (%)	Comment
Starke,[11] USA	1984–7	GA	110	*	39	Included both pulmonary and extrapulmonary disease
Vallejo,[12] USA	1985–92	GA	47	*	75	Infants <1 year
Schaaf,[13] South Africa	1991	GA	235	*	43	Mediastinal lymphadenopathy and bronchial obstruction associated with TB
Garay,[14] Zimbabwe	1993–4	GA	115	<1	45	Lobar consolidation associated with HIV infection
Lloyd,[6] Uganda	1966–7	GA LS	60	Few	28 63	If 13 extrapulmonary cases were excluded culture rates would have been even higher
Thakur,[7] India	1997	LS	51	14	27	Smear/culture rate 33%
Shata,[4] Malawi	1994	SI	29	14	24	Smear/culture rate 28%
Zar,[5] South Africa	2000–2	GA SI	250 250	7 10	15 20	Smear/culture rate: GA 16%, SI 22%
Franchi,[8] Peru	1998	GA NPA	64 64	14 17	37 30	PCR positive in 27% of GA and 28% of NPA
Iriso,[15] Uganda	2000	SI	101	12	30	Included both pulmonary and extrapulmonary disease
Owens, Uganda	2004	SI NPA	94 94	10 8	22 24	Smear/culture rate for SI+NPA 27%. Sensitivity for PCR on NPA samples 62%

GA, gastric aspirate; LS, laryngeal swab; NPA, nasopharyngeal aspirate; SI, sputum induction.
*Smear negative or not mentioned.

What is already known on this topic

- Gastric aspiration and sputum induction (SI) are time consuming methods to obtain sputum for confirmation of tuberculosis and SI requires specialised equipment and dedicated staff
- Simpler methods appropriate for developing countries are required

What this study adds

- Nasopharyngeal aspiration (NPA) is a simple low-technology method to obtain sputum from children
- NPA has similar smear/culture positivity to SI

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