Diagnostic Accuracy of the Xpert MTB/Rif Ultra for tuberculosis adenitis

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Abstract

Background The WHO recently recommended the new Xpert MTB/RIF Ultra assay (Ultra) instead of the Xpert MTB/RIF assay because Ultra has improved sensitivity. We report the diagnostic accuracy of Ultra for tuberculous adenitis in a tuberculosis and HIV endemic setting.

Methods We obtained fine-needle aspirates (FNA) and lymph node tissue by core-needle biopsy in adult patients with peripheral lymphadenopathy of > 20 mm. Ultra and mycobacterial culture were performed on FNA and tissue specimens, with histological examination of tissue specimens. We assessed the diagnostic accuracy of Ultra against a composite reference standard (CRS) of 'definite tuberculosis' (presence of acid-fast bacilli (AFBs) or culture positive on FNA or lymph node tissue) or 'probable tuberculosis' (macroscopic caseation or granulomas on histology or tuberculosis proven from another site, and no other identified cause for lymphadenopathy).

Results We prospectively evaluated 99 participants of whom 50 were HIV positive: 21 had 'definite tuberculosis', 15 'probable tuberculosis' and 63 did not have tuberculosis (of whom 38% had lymphoma and 19% disseminated malignancy). Using the CRS, the sensitivity of Ultra on FNA was 70% (95% CI 51-85) and on tissue was 67% (95% CI 45-84); specificity on FNA was 100% (95% CI 92-100) and on tissue 96% (95% CI 88-99).

Conclusions Ultra performed on FNA or tissue of a lymph node had reasonable sensitivity and high specificity. Ultra on FNA would be an appropriate initial investigation for lymphadenopathy in tuberculosis endemic areas.

Introduction

Lymph nodes and pleura are the most common sites of involvement in extrapulmonary tuberculosis (EPTB), which is more common in people living with HIV (PLWH) [1]. EPTB is difficult to diagnose because it is usually paucibacillary and conventional methods for diagnosis, such as microscopy and culture, have low yields, culture results may take several weeks and histological findings (e.g. granulomas) are not specific for tuberculosis. Following a systematic review, the WHO has recommended that the rapid nucleic acid amplification test Xpert MTB/RIF assay (Xpert) should be used in the diagnosis of EPTB, including lymph node (LN) tissue[2]. In this meta-analysis (13 studies, 955 samples), the pooled sensitivity of Xpert on LN (tissue or fine needle aspirate (FNA)) was 83.1% (95% CI 72-91), and pooled specificity was 94% (95% CI 88-97%). Xpert has been recently superseded by the Xpert MTB/RIF Ultra assay (Ultra), which has greater sensitivity for the detection of *Mycobacterium tuberculosis* complex in sputum specimens but has lower specificity, especially in patients with previous tuberculosis [3].

The Ultra differs from the earlier Xpert assay in a number of ways: two different multicopy amplification targets (*IS*6110 and *IS*1081) have been added to improve detection of *M. tuberculosis* and melting temperature-based polymerase chain reaction (PCR) analysis (melting curve analysis) is used to improve

the detection of rifampicin resistance[4]. Because the limit of detection of Ultra is lower (15.6 bacterial colony-forming units (CFU) per ml compared with 114 CFU per ml with Xpert), the greatest improvement in detection is likely to be in paucibacillary samples. This has been demonstrated in cerebrospinal fluid, where Ultra has higher sensitivity (90%, 95% CI 55-100, versus Xpert at 60%, 95% CI 26-88); but lower specificity (90%, (95% CI 83-95) versus Xpert at 97% (95% CI 92-99). The performance on Ultra in lymph nodes has only been tested in one small retrospective study on 10 frozen samples where it showed an improvement of 50% compared with Xpert, (5/10 samples that were Xpert negative and culture positive were positive on Ultra) [5].

In this study, we determined the diagnostic accuracy of Ultra for the detection of *M. tuberculosis* in specimens of LN tissue obtained by FNA and core-needle biopsy.

Methods

Study design and participants

We conducted a prospective diagnostic accuracy study of Ultra on both FNA and LN core-needle biopsy tissue in patients with suspected tuberculosis adenitis. The study was performed at Groote Schuur Hospital, a tertiary referral academic centre in Cape Town, South Africa. Eligible study participants were adults (≥18 years), both in- and outpatients, referred with enlarged lymph nodes of > 20 mm in the widest diameter located in either the cervical, axillary or inguinal region. Patients on tuberculosis therapy were enrolled provided this had been given for <1 month (sub-analyses were done in patients on tuberculosis therapy for <24hr). Patients with contraindications to core-needle biopsy (low platelets, other coagulopathy and bleeding risk, clinically unstable, site of biopsy unsafe) were excluded. Written informed consent was obtained from all participants. The Human Research Ethics Committee of the Faculty of Health Sciences, University of Cape Town, approved the study.

Patients came from within Groote Schuur and from secondary level hospitals and day clinics in the referral area. Results of prior tuberculosis investigations (sputum Xpert or tuberculosis culture from any site within 3 months of referral, or urine lipoarabinomannan (LAM)) were recorded. Details of HIV status, tuberculosis treatment and ART were obtained.

Data collection

Demographic information, symptoms, symptom duration, HIV test result, and other TB investigations performed were recorded at enrolment. Performance status was graded according to the Eastern European Cooperative Group (ECOG)[6]. The site of biopsy was recorded, along with other sites of lymphadenopathy. The presence and duration of constitutional symptoms (cough, loss of weight night sweats) were specifically enquired about as was the duration that the patient had noted the lymphadenopathy. Blood was taken for a full blood count with differential, lactate dehydrogenase and HIV status and, if positive, a CD4 count and a viral load for those on ART.

Study procedures and specimen collection

FNA was performed using a 22G needle and 5 mL syringe; further study procedures were determined by the volume of the aspirate sample obtained as shown in figure 1. A core-needle biopsy was only performed when <0.5mL of caseous material was obtained by the aspirate, due to the risk of causing a draining sinus. Initially, when both an FNA and a biopsy were performed on a participant the Ultra was performed only on the tissue, but there was a protocol change after 25 patients and Ultra was performed on both the FNA and tissue on the same patient. When a patient had both an FNA and a core-needle biopsy the TB culture was only performed on the tissue specimen. For the Ultra on the FNA, the needle and syringe were flushed into a sterile container containing 2 mL of saline. An air-dried smear for AFBs was made at the bedside from a second FNA. For culture from the FNA, the aspirate was flushed directed into mycobacterium culture medium (Middlebrook 7H9 broth medium). The core-needle biopsy was performed by an automated biopsy gun (BARD Magnum™, CR Bard Inc, Covington, GA, USA) with a 14G needle. If the lymph node was not obviously palpable the biopsy was performed under ultrasound guidance. Two or three cores were sent in formalin for histology (10-15 mm long), an additional core was cut in two with a sterile blade and sent for culture and Ultra, both in 2 ml of 0.9% saline. If all of the tests performed were inconclusive, the patient underwent a repeat core-needle biopsy or an excision biopsy at the discretion of the treating clinician.

Laboratory tests

FNA and tissue specimens were transported within 2 hours of collection to a centralized laboratory and processed individually using standardised protocols by trained laboratory staff. The smear slide made at the bedside was examined by Ziehl-Neelsen (ZN) for AFBs. The tissue obtained by core-needle biopsy was crushed using pestle and mortar. A small portion was smeared on a slide and examined with a ZN stain for AFBs. Mycobacterial culture was performed using an automated liquid mycobacterial culture system (BACTEC™ MGIT™ 960; Becton, Dickinson and Company, New Jersey, USA). Lymph nodes are considered a sterile site and decontamination was not performed prior to liquid biopsy. If the MGIT flagged positive, one droplet was inoculated on 2% blood agar and incubated for 24hr to check for bacterial growth. If there was no bacterial growth, the MGIT was reported as positive for mycobacterium, the specimen was decontaminated with sodium hydroxide (1%) and N-acety-L-cysteine and then repeat MGIT was performed. Positive isolates from culture media were identified by acid-fast staining followed by MRTBDR*plus* testing (Hain LifeScience, Hehren, Germany) to confirm the presence of *M. tuberculosis* and rifampicin and isoniazid sensitivity.

For the Ultra, 1.4 mL of specimen reagent was added to 0.7 mL of aspirate or crushed tissue sample. Results were reported as: invalid (no internal assay control detected); not detected; or detected (with semi-quantitation: trace, very low, low, medium, or high) and rifampicin resistance (detected, not detected, or indeterminate). Staff performing Ultra were blinded to clinical and other microbiological results.

Histological review was performed by a qualified anatomical pathologist who was blinded to the results of the ULTRA and did not have access to the culture, but would have been able to see the results of AFBs on microscopy. If granulomas were identified, a separate ZN stain was performed by the pathologist and a Periodic acid–Schiff (PAS) stains were performed for fungi.

Case definitions and statistical analysis

We assigned participants to one of three diagnostic categories on the basis of clinical, histological and microbiological investigations: *definite tuberculosis* (culture-positive for *M. tuberculosis* or AFB identified on FNA or lymph node tissue*), probable tuberculosis* (no other diagnosis that would account for lymphadenopathy AND one or more of: macroscopic caseation, tuberculosis confirmed microbiologically [Ultra or culture] at another site, granulomas on histology) or *not tuberculosis*. The Ultra diagnostic accuracy was also reported separately using positive culture only as the reference standard.

Sample size estimation in diagnostic accuracy studies depends on the prevalence of disease[7]. We were expecting a high prevalence of tuberculosis based on a pilot study we conducted of core-needle biopsy in HIV-positive patients, 92% of whom had tuberculosis lymphadenitis[8], but the proportion of patients with tuberculosis in our study was lower than expected (40%) in a pilot phase of our study. We estimated the sensitivity and specificity of Ultra would both be around 90% based on the Cochrane meta-analysis[9]. With a 40% prevalence of tuberculosis a sample size of 87 is needed for 95% CI widths of 10% and with sensitivity and specificity of 90%[7]. We inflated the sample size to 100 because of uncertainties about the diagnostic accuracy of Ultra.

We calculated sensitivity, specificity, negative predictive value (NPV), positive predictive value (PPV) and likelihood rations by defining true or false positives and true or false negatives against the composite reference standard (CRS) of *probable* or *definite tuberculosis* for our primary analysis. As secondary analyses we also determined the accuracy of Ultra using mycobacterial culture alone as the reference standard. Data was entered into a REDCap® database and analysed using the STATAv14 software package (StataCorp, College Station, Texas, USA). Baseline clinical characteristics were compared using the chi-squared or Fisher's exact test for categorical variables and the Kruskal-Wallis test for continuous variables. We counted invalid tests (i.e. Ultra-error) as negative results. This study is reported in accordance with the Standards for Reporting of Diagnostic Accuracy Studies Guidelines [10].

Role of the funding source

The funders had no role in study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results

Demographic and clinical characteristics

Between November 2017 and October 2018, 154 consecutive patients were evaluated for suspected lymphadenopathy and we enrolled 99 participants for the study (figure 2). Ultra of both FNA and core-needle biopsy were performed in 56 participants, 25 participants had Ultra testing of a core-needle biopsy only (prior to protocol change), and 18 had Ultra testing of an FNA only. The majority of participants (84%) were seen as outpatients. Of the 51% (n=50) participants who were HIV positive, 62% (n=31) were on ART and had a median CD4 count of 216 cells/mm^3 (IQR 82-361) and in the participants on ART, 42% (13/31) were virally suppressed with a viral load lower than the detectable limit (<20 RNA copies/mL).

The yield of other tuberculosis investigations prior to referral was: sputum Xpert 3/22, urinary LAM 1/5, and tuberculosis culture by site: urine 0/1, blood 1/2, sputum 4/12, lymph node 0/1 (tissue). Chest x-ray had been performed in 36% and reported as 'suggestive of tuberculosis' by the referring clinician in 28% of these.

The final diagnosis was *definite tuberculosis* in 21 (21%), *possible tuberculosis* in 15 (15%) and *not tuberculosis* in 63 (64%). Figure 2 shows the results of the different investigations on the lymph node for tuberculosis in each of the three groups. Table 1 shows baseline characteristics of the participants by diagnostic group. The groups had similar HIV prevalence and, in those who were HIV positive, similar median CD4 counts. The median age was significantly higher in the *not tuberculosis* group. Previous tuberculosis was common in all groups (29% of participants with *definite tuberculosis*, 40% with *probable tuberculosis* and 19% in participants classified as *not tuberculosis*). Participants in all groups frequently reported cough, night sweats and weight loss. There was also no significant difference in the clinical examination findings of the lymph node. Lymphocyte count, LDH and total white cell count (WCC) were similar in the groups, however, anaemia was more common in the participants with *definite tuberculosis* (present in 81%) compared with the other two groups (p=0.041).

In the *not tuberculosis* group the final diagnosis was: lymphoma in 24 (38%), other malignancy in 19 (30%), reactive lymphadenopathy in 8 (13%), and miscellaneous other causes (enlarged submandibular salivary gland (n=4), bacterial adenitis (n=4), sarcoidosis (n=2), sinus histiocytosis (n=1), branchial cleft cyst (n=1)). The diagnosis of 'bacterial adenitis' was based on >0.5 mL caseous material with lymphadenopathy resolving after drainage and antibiotics and culture of the caseous material not positive for tuberculosis. The Ultra was positive on 0/43 of the FNA samples in the *not tuberculosis* group, and in 2/57 (4%) of the tissue samples, in both of these cases the participants had a proven cancer diagnosis histologically and the Ultra was considered false positive (one had pulmonary tuberculosis 12 years previously; neither were on tuberculosis treatment). Granulomas were detected in 2 participants diagnosed with sarcoidosis; both had an excision biopsy which was negative on culture for tuberculosis and had features compatible with sarcoidosis on chest computed tomography.

The diagnostic accuracy of the reference tests against the CRS are shown in table 2.

The diagnostic accuracy of Ultra on both FNA and tissue is presented in table 3 using the CRS, CRS excluding participants on tuberculosis therapy, and by culture alone as the reference test. Rifampicin

resistance was identified in 2 of the 39 Ultra positive participants, which was confirmed on line probe assay.

The quantitative results of the Ultra with the number of false positives (FP) in each group are given in table 4. There were no *trace positive* results in the FNA group and 12 in the tissue group, 10 of which gave a *rifampicin indeterminate* result. When using *definite tuberculosis* as the reference standard, there were 10 FP in the FNA group: 5 were on tuberculosis treatment and 5 had previous tuberculosis (3 pulmonary and 2 tuberculous lymphadenitis) and 2 had neither previous tuberculosis nor current tuberculosis treatment. There were 6 FP in the tissue group: 2 had previous tuberculosis (both pulmonary), and 2 were currently on tuberculosis therapy (one was sputum positive on Xpert), and 2 had neither previous tuberculosis nor current tuberculosis treatment.

Discussion

Our prospective diagnostic accuracy study showed that Ultra on lymph node tissue obtained by core-needle biopsy or FNA had a specificity of 96-100% and a sensitivity of 67-70%, which was superior to detection of AFBs and culture. Ultra on FNA and core-needle biopsy had similar diagnostic accuracy. Our findings support the use of Ultra on FNA as an initial test when tuberculosis adenitis is suspected. Where the FNA does not yield a positive result core-needle biopsy would be an appropriate follow up investigation, with a repeat Ultra on tissue and histology allowing the diagnosis of other causes of lymphadenopathy, notably malignancies.

We had high precision for Ultra specificity, which is a key parameter given that specificity of Ultra was lower than Xpert in sputum[3]. The sensitivity of Ultra on FNA was 67% in our study, which is lower than the 87.6% reported in the Cochrane review[9], but they found sensitivity was lower in adults and with lower prevalence of tuberculosis.

The sensitivity of AFB detected on FNA and tissue was poor in our study (26%), consistent with other studies[11–16]. The higher sensitivity of Ultra indicates it should replace the air-dried smear for AFBs as a diagnostic test for lymphadenopathy. Although the sensitivity of culture on both FNA and tissue was low, culture may still play a confirmatory role in diagnosis particularly where drug resistance is suspected, but its utility is limited by both low yield and a long turn-around-time.

There is uncertainty surrounding the interpretation of trace positivity of the Ultra from respiratory samples[3] . In our study, two Ultra results were deemed false positive in our primary analysis – both were from lymph node tissue and were trace positive. However, 10 other trace positive Ultra results were deemed to be true positives. We suggest that trace positive Ultra results from lymph node tissue or FNA be regarded as positive, but that the patient is followed up to ensure response to anti-tuberculosis therapy.

Our study has several limitations. First, our CRS included participants with *probable tuberculosis* and our definition of *definite tuberculosis* included culture or AFBs. We felt it was justifiable to include detection

of AFB in the case definition for *definite tuberculosis* because adenitis caused by nontuberculous mycobacteria is rare, and typically only seen in non-BCG immunized children[17]. The most stringent reference standard would be culture, but this has relatively low sensitivity on lymph nodes, in particular on FNA [17,18]. The Cochrane systematic review of Xpert for extrapulmonary tuberculosis found the specificity of Xpert on FNA was 86% when culture was the reference standard, but in their latent class meta-analysis model specificity improved to 99%, which is likely to be a much more accurate reflection given that specificity was 98% or more with other extrapulmonary specimens[9]. Therefore, we feel that our CRS provided a more precise reflection of the diagnostic accuracy of Ultra for tuberculous adenitis. Second, we included participants on tuberculosis therapy, which had the potential to lower the sensitivity of culture and affect our reference standard. Excluding patients on tuberculosis treatment only minimally reduced specificity when culture was used as the reference standard in the Cochrane review of Xpert, and had no effect on specificity of Ultra in our study using the CRS (table 3). Third, in some of our participants the final diagnosis of cervical swelling was not lymphadenopathy. We decided to include these participants to best represent real life investigations that will be carried out for a clinical diagnosis of lymphadenopathy. Strengths of our study include the use of a standard protocol, strict inclusion criteria, and a single operator performed the FNA and core-needle biopsy.

Conclusion

The Ultra on FNA and on tissue had good sensitivity, and high specificity in our primary analysis. Ultra on FNA is a simple bedside procedure with a fast turnover time is the most appropriate the initial diagnostic test where tuberculosis adenitis is suspected. AFBs on air-dried smear on FNA has low sensitivity and should be replaced by the Ultra. Core-needle biopsy can be done as the next investigation if the Ultra is negative on FNA as it allows for histological examination and early diagnosis of other disorders, in particular malignancies, without the need for excision biopsy.

List Of Abbreviations

AFB: Acid-fast bacilli; ART: Antiretroviral therapy; CI: Confidence Interval; CRS: Composite Reference Score; ECOG; Eastern European Cooperative Group; EPTB: Extrapulmonary tuberculosis; FNA: Fine-Needle Aspiration; LAM; lipoarabinomannan; LN: Lymph node; OR: Odds Ratio; OS: Overall Survival; PLWH: People Living with HIV; Ultra: Xpert MTB/RIF Ultra assay; Xpert: Xpert MTB/Rif Assay.

Declarations

Ethics approval and consent to participate

Ethics was approved by the University of Cape Town Human Research Ethics Committee (HREC), approval number 674/2017. Written informed consent was obtained from each participant.

Consent for publication

Not applicable

Availability of data and materials

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Competing interests

The authors declare that they have no competing interests.

Authors' contributions

KA, EV and GM designed the research study. KA and JO ran the clinic under the supervision of VJL, FM and EV. KA and FM performed the biopsies. KA, EV and GM wrote the paper EV. The Xpert Ultra was performed in the lab under the supervision of MN. All authors read, critically appraised and approved the final manuscript.

Authors' information

MN is currently working at the School of Biomedical Sciences, University of Western Australia, Perth, Australia but was based at the University of Cape Town during the study period.

Disclaimer

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Tables

Table 1. Baseline characteristics by diagnostic group

	Total (n=99)	Definite TB (n=21)	Probable TB (n=15)	Not TB (n=63)	P-value
Sex					
Males	45 (45%)	9 (43%)	4 (27%)	32 (51%)	0.233
Females	54 (55%)	12 (57%)	11 (73%)	31 (49%)	
Age (median [IQR])	37 (30-49)	32 (28-35)	36 (31-41)	44 (32-57)	**0.003**
HIV status					
Positive	50 (51%)	13 (62%)	8 (53%)	29 (46%)	0.442
Negative	49 (49%)	8 (38%)	7 (47%)	34 (54%)	
On ARVS	31 (62%)	8 (62%)	7 (88%)	16 (55%)	0.263
CD4 count* (median [IQR])	216 (82-361)	100 (55-254)	351 (88-497)	252 (92-361)	0.106
<100	16 (32%)	6 (46%)	2 (25%)	8 (28%)	0.675
100-249	10 (20%)	3 (23%)	1 (13%)	6 (21%)	
≥250	24 (48%)	4 (31%)	5 (63%)	15 (52%)	
Performance Score					
ECOG 0	49 (50%)	10 (48%)	9 (60%)	30 (48%)	0.979
ECOG 1	28 (28%)	6 (29%)	5 (33%)	17 (27%)	
ECOG 2	4 (4%)	1 (5%)	0	3 (5%)	
ECOG 3	16 (16%)	4 (19%)	1 (7%)	11 (17%)	
ECOG 4	2 (2%)	0	0	2 (3%)	
Patient Type					
Inpatient	16 (16%)	4 (19%)	2 (13%)	10 (16%)	0.924
Outpatient	83 (84%)	17 (81%)	13 (87%)	53 (84%)	
Previous tuberculosis	24 (24%)	6 (29%)	6 (40%)	12 (19%)	0.205
On tuberculosis treatment	21 (21%)	6 (29%)	6 (40%)	9 (15%)	0.059
Consistency of LN					
Firm	81 (87%)	16 (80%)	12 (86%)	53 (90%)	0.491
Fluctuant	12 (13%)	4 (20%)	2 (14%)	6 (10%)	
Symptoms					
Cough	27 (27%)	6 (29%)	3 (20%)	18 (29%)	0.79
Weight loss	47 (47%)	13 (62%)	5 (33%)	29 (46%)	0.222
Night sweats	29 (29%)	8 (38%)	3 (20%)	18 (29%)	0.49
Location of lymph nodes					
Unilateral	61 (62%)	13 (62%)	12 (80%)	36 (57%)	0.262
Bilateral	38 (38%)	8 (38%)	3 (20%)	27 (43%)	
Lymph node FNA/biopsy site					
Neck	85 (86%)	19 (90%)	14 (93%)	52 (83%)	0.881
Axilla	10 (10%)	2 (10%)	1 (7%)	7 (11%)	
Inguinal	4 (4%)	0	0	4 (6%)	
Blood results (median [IQR])					
LDH (units/L)	267 (218-341)	292 (251-341)	218 (194-239)	273 (230-366)	**0.006**
Hb (g/dL)	12.1 (9.4-13.4)	10.5 (8.7-12.1)	11.8 (9.6-12.7)	12.4 (9.7-13.5)	0.041
WCC (cells x 10^9)	6.9 (4.9-9.1)	5.3 (4.0-8.0)	5.7 (4.8-7.3)	7.4 (5.2-9.9)	0.142
Lymphocytes (cells x 10^9)	1.7 (1.0-2.4)	1.2 (0.7-1.9)	1.7 (1.4-2.0)	1.7 (1.0-2.7)	0.197

P values <0.05 are shown in bold. Data are n/N; % (95% CI). ECOG = Eastern Cooperative Oncology Group.

LN=Lymph Node. LDH=Lactate Dehydrogenase. Hb=Haemoglobin. WCC=White Cell Count

Table 2. Reference test accuracy measured against the CRS

Tuberculosis test	Sensitivity	Specificity	PPV	NPV
AFBs on FNA (n=61)	7/27; 26% (11-46)	34/34; 100% (90-100)	100%	63%
FNA culture (n=17)	4/12; 33% (10-65)	5/5; 100% (48-100)	100%	38%
AFBs on tissue (n=81)	8/24; 33% (16-55)	57/57; 100% (94-100)	100%	78%
Tissue culture (n=75)	9/23; 39% (20-61)	52/52; 100% (93-100)	100%	79%
Necrotising granulomas on histology (n=81)	20/24; 83% (63-95)	55/57; 96% (88-100)	91%	93%

Data are n/N; % (95% CI). FNA = fine-needle aspirate. AFBs=acid fast bacilli. PPV=positive predictive value. NPV=negative predictive value. CRS=Composite reference standard.

Table 3. Diagnostic accuracy of Ultra on FNA and tissue measured against the CRS and culture

	Sensitivity	Specificity	PPV	NPV	LR+	LR-
CRS (n=99 participants)						
Ultra on FNA (n=73)	21/30; 70% (51-85)	43/43; 100% (92-100)	100%	83%	inv	0.3
Ultra on tissue (n=81)	16/24; 67% (45-84)	55/57; 96% (88-99)	89%	87%	16.8	0.3
Excluding the 21 participants on tuberculosis therapy>24hr						
Ultra on FNA (n=57)	12/20; 60% (36-81)	37/37; 100% (91-100)	100%	82%	inv	0.4
Ultra on tissue (n=66)	10/18; 56% (31-78)	46/48; 96% (86-99)	83%	85%	14	0.5
Culture as the reference standard (n=99 participants)						
Ultra on FNA (n=73)	9/11; 82% (48-98)	50/62; 81% (69-90)	43%	96%	4.3	0.2
Ultra on tissue (n=81)	9/10; 90% (55-100)	62/71; 87% (77-94)	50%	98%	6.9	0.1

Data are n/N; % (95% CI). FNA = fine-needle aspirate. AFBs=acid fast bacilli. PPV=positive predictive value. NPV=negative predictive value. LR=likelihood ratio

Table 4. Quantitative results of the Ultra showing the number of false positive (FP) in each group using the CRS as the reference

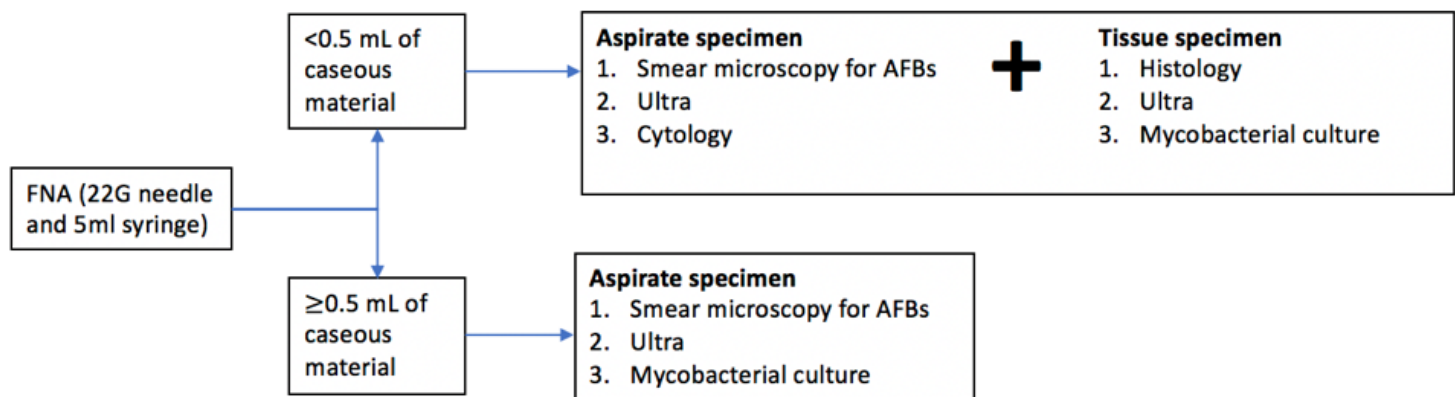|	Total no of FP	Trace n(FP)	Very low n(FP)	Low n(FP)	Medium n(FP)	Failed test
Ultra on FNA	0	0	4(0)	8(0)	9(0)	0
Ultra on biopsy	2	12(2)	3(0)	1(0)	2(0)	4

FNA = fine-needle aspirate.

Figures

Figure 1

Study procedures

Figure 2

Trial profile