METHODS

Patients

Adult patients (≥18 years) referred to the Division of Pulmonology of Tygerberg Academic Hospital from April 2010 until June 2011 with radiological evidence of a pleural effusion were candidates for this study. Patients were actively recruited from two health community centres through a community outreach programme. Referral time was less than 24 hours in all cases compared to several weeks for regular appointments at a tertiary referral centre. Our institution is a 1,200-bed academic hospital in Cape Town, South Africa. It is one of two academic referral centres in the city and renders a tertiary service to a population of approximately 1.5 million. In 2010 the incidence of TB in South Africa was 981 cases per 100,000 [1]. Routine evaluation included history of previous antituberculosis treatment and HIV status at the time of referral. Patients were included in the study if they had at least 3 of the following indicators of possible TB: (1) persistent cough lasting >2 weeks, (2) shortness of breath, (3) haemoptysis, (4) weight loss >4 kg, (5) intermittent fever >3 weeks, (6) drenching night sweats >2 weeks, and (7) pleuritic chest pain [2]. Patients were excluded if transthoracic ultrasound (US) failed to confirm a pleural effusion of at least 10 mm width or if patients reported allergies to local anaesthetics. The Stellenbosch University Human Research Ethics Committee approved the study (reference number N10/02/055). Written informed consent was obtained from all subjects on enrolment and prior to any invasive procedures.

Sputum collection
Patients able to produce sputum spontaneously were asked to provide a spot sputum sample for smear microscopy and TB culture. Specimens were decanted into a 50ml Falcon tube (BD Biosciences, New Jersey, USA). An equal volume to the volume of the sputum specimen of a 5% NaOH/Trisodium-Citrate-NALC solution (Merck, New Jersey, USA) was added for decontamination. After 20 min phosphate buffer (Merck, New Jersey, USA) with a pH of 6.8 was added to stop the decontamination process. The mixture was centrifuged at 3000 rpm for 20 min, the supernatant decanted and 1ml of phosphate buffer was added. The solution was diluted if necessary to a McFarland standard of 0.5 and 0.5ml of this were inoculated into a liquid mycobacterial culture tube (Bactec Mycobacteria Growth Indicator Tube [MGIT], Becton Dickinson, Maryland, USA). One drop was put on a slide together with TB precipitating fluid and dried for 2 hours before auramine-O-staining [3] was performed.

**Transthoracic ultrasound**

A consultant respiratory physician or a senior registrar under supervision performed the sonography (Toshiba Just Vision 200 SSA-320A; Toshiba Medical Systems Corporation, Tochigi-ken, Japan). The preferred patient position for the procedure was sitting, with the arms folded across the chest and supported by a bedside table. We used a standard 3.75 MHz sector probe to document the cranio-caudal extent of the effusion: minimal (confined to the costophrenic angle); small (greater than the costophrenic angle but still within the range of one probe); moderate (greater than a one-probe range but within a two-probe range); and large (larger than a two-probe range) [4]. The site for aspiration and biopsy was chosen in midscapular line unless another location appeared safer. In sufficiently large effusions the puncture site was chosen to be as low as possible, but not within 25 mm of the diaphragm.

**Diagnostic thoracentesis and fluid analysis**
Thoracentesis was performed with a 20 G needle under sterile technique and local anaesthesia with lignocaine 1%. Samples were sent for chemical analysis, bacteriology, mycobacteriology, cell count and cytology. The chemical analysis included total protein, albumin, lactate dehydrogenase (LDH) and adenosine deaminase activity (ADA).

Bacteriological investigations included gram stains and aerobic and anaerobic cultures. For mycobacteriology we collected 5ml and 100ml of pleural fluid into separate containers. The 5ml specimen was collected in a sterile 5ml tube, the 100ml specimen was collected in two sterile 50ml Falcon tubes. Both specimens were processed the same day in the same fashion as the sputum samples. The two pellets of the large volume were pooled. Pellets of both specimens were separately resuspended in phosphate buffer to a McFarland standard of 0.5.

For liquid culture 0.5ml of the suspension were inoculated into a MGIT tube and one drop was used for auramine-O-staining [3]. Further processing included the addition of OADC growth supplement and PANTA to the MGIT tube as part of the MGIT culture kit. In positive MGIT tubes the presence of mycobacteria was confirmed by Ziehl-Neelsen staining and mycobacterial speciation by PCR [5 6]. Bacterial contamination was excluded with blood agar culture.

Closed pleural biopsies

Under sterile technique and local anaesthesia experienced clinicians performed closed pleural biopsies with an Abrams needle according to standardised guidelines [7 8]. Specimens were harvested until at least three macroscopically satisfactory specimens for histological evaluation (transported in 4% formalin) and at least one specimen for microbiological investigations (transported in 0.9% saline) had been collected. Pleural biopsy specimens were crushed in an automated grinder and processed in the same fashion as the sputum samples. All biopsy specimens were cultured for mycobacteria using the MGIT liquid culture system.
Post procedure care and follow up

All procedures were performed in a single session, whereafter all subjects were observed for one hour prior to discharge, and complications noted. Before discharge patients were re-examined with US to exclude post-biopsy pneumothorax, and a chest radiograph was obtained if the pre- and post-procedure US findings differed and at the discretion of the attending physician. Cases that remained undiagnosed were further investigated with medical thoracoscopy if they consented and followed up for a total of six months with the choice of further investigations guided by the attending physicians. Cases that remained undiagnosed after six months were deemed “undiagnosed pleural exudates”.

Definitions and statistical analysis.

All mycobacterial stains were scored according to the IUATLD/WHO scale as negative, scanty positive, 1+, 2+, 3+ [9]. We accepted a positive culture for *Mycobacterium tuberculosis* from any specimen and/or histology compatible with TB (epitheloid granulomata with or without central necrosis, with or without acid fast bacilli) as diagnostic for pleural TB. Time to positivity (TTP) of mycobacterial culture was recorded in hours. All data are presented as means +/- SD. Continuous data were compared with Student’s t-test and proportions with chi²-test, McNemar’s test and Fisher’s exact test. Although this was essentially an exploratory study, we estimated based on McNemar test a sample size of 60 patients to show a significant difference between the two culture volumes of 30%. We anticipated that 25% of patients would not have an effusion or tuberculosis and thus aimed to include 80 patients.
SUPPLEMENTARY RESULTS

We screened a total of 80 patients. 3 patients had no evidence of a pleural effusion on ultrasonography. As mentioned out of 77 patients pleural TB was diagnosed in 58 (75.3%) of whom only 5 (8.6%) had detectable cavitary changes on chest radiograph. In 11 of the 19 patients in whom a diagnosis of pleural TB could not be made an alternative diagnosis was established: empyema (n=2), mesothelioma (n=1), metastatic adenocarcinoma (n=1), liver abscess (n=1), pleural echinococcosis (n=1) and transudates secondary to systemic disease (n=5). Four patients remained undiagnosed following thoracoscopy and a further four were empirically treated for pleural TB based on an elevated ADA and a lymphocyte predominant effusion. If all 8 (10.4%) undiagnosed patients were carried forward as false negative for tuberculous pleurisy the total number of TB cases would increase to 66 (85.7% of all patients) and the yield of fluid culture, pleural biopsy histology and culture would drop to 53.0%, 74.2% and 57.6%, respectively.
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