

RESEARCH LETTER

The yield of different pleural fluid volumes for *Mycobacterium tuberculosis* culture

We prospectively compared the culture yields of two pleural fluid volumes (5 and 100 ml) inoculated in liquid culture medium in 77 patients of whom 58 (75.3%) were diagnosed with pleural tuberculosis. The overall fluid culture yield was high (60.3% of cases with pleural tuberculosis). The larger volume had a faster time to positivity (329 vs 376 h, $p=0.055$) but its yield was not significantly higher (53.5% vs 50%; $p=0.75$). HIV-positive patients were more likely to have positive cultures (78.9% vs 51.5%; $p=0.002$).

Pleural tuberculosis is a common form of extra-pulmonary tuberculosis particularly among HIV-infected individuals.¹ Mycobacterial cultures from pleural fluid have a reported average yield of 24%–58%, which is the highest when liquid culture media are used.^{1–2} Recent evidence suggests that a higher volume of pleural fluid submitted for cytological analysis or for bacterial culture does not increase the rate of detection of malignant cells or bacteria, respectively.^{3–4} We

compared the yields of 100 ml or 5 ml of pleural fluid inoculated in liquid culture medium in 77 patients with a high suspicion of pleural tuberculosis. Pleural tuberculosis was diagnosed in 58 (75.3%) following extensive examination including pleural biopsy, of whom 35 (60.3%) had positive fluid cultures (table 1). Time to culture positivity (TTP) was considerably shorter with the larger volume (329 vs 376 h, $p=0.055$) but the absolute yield was not significantly greater (53.5% vs 50%; $p=0.75$). HIV-positive individuals had culture-positive fluid more often, and spontaneously expectorated sputum had a remarkably high yield. Detailed descriptions of the methods and results can be found in the online supplement.

The fact that larger volume cultures had a lower TTP confirms that a higher number of viable bacteria were recovered from the larger volume. In vitro titration curves confirm that the TTP decrease found corresponds to a 10- to 20-fold increase of colony forming units inoculated.⁵ But why would this not translate into a significant increase in culture positivity? Bacterial concentrations among tuberculosis effusions could be distributed in dichotomous rather than continuous fashion. After initial invasion of the pleural space mycobacteria are either cleared from pleural fluid rapidly, thus leaving cultures negative irrespective of

technique, or the clearing process is insufficient or incomplete leaving behind bacteria in numbers large enough for culture positivity. It is in this group that the shorter TTP can be demonstrated. This theory would also explain the fact that HIV-positive patients, in whom bacterial clearance might be impaired, have higher yields for pleural fluid culture.

In conclusion, we confirmed that liquid mycobacterial culture has a high yield in pleural fluid. The volume of fluid used for inoculation in liquid culture did not seem to influence the proportion of positive cultures. Further studies are required to investigate if more than one pleural fluid sample should be submitted for mycobacterial culture. This study confirmed that HIV infected patients have a higher proportion of positive fluid cultures and that a substantial proportion of patients with pleural tuberculosis can be diagnosed on sputum.

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Table 1 Patients and pleural fluid

Parameter	All	TB	Non-TB	p Value
Number of patients (N)	77	58	19	NA
Age (years) \pm SD	33.4 \pm 12.8	30.8 \pm 11.4	41.1 \pm 14.1	0.002
Male gender (%)	59.7	60.3	57.9	1
HIV-positive, n (%)	23 (29.9)	19 (32.8)	4 (21.1)	<0.001
Effusion size				
Small or moderate, n (%)	39 (50.6)	25 (43.1)	14 (73.7)	0.01
Large, n (%)	38 (49.4)	33 (56.9)	5 (26.3)	0.03
Pleural fluid				
pH \pm SD	7.34 \pm 0.17	7.34 \pm 0.12	7.34 \pm 0.26	0.92
Protein (g/l) \pm SD	60.3 \pm 20.3	64.5 \pm 17.3	47.9 \pm 25.5	0.015
LDH (IU/l) \pm SD	1178 \pm 2466	947 \pm 1425	1859 \pm 4259	0.37
ADA (IU/l) \pm SD	88.1 \pm 45.3	98.5 \pm 37.5	53.8 \pm 52.5	0.004
Lymphocytes (%)	62.5 \pm 28.5	66.8 \pm 25.9	48.6 \pm 32.9	0.056
Lymphocytic effusion* (%)	80.9	84.6	68.16	0.16
Pleural fluid culture yield for MTB				
100 ml volume, n (%)		31 (53.5)		0.75
5 ml volume, n (%)		29 (50)		
Time to culture positivity for MTB				
100 ml volume (h) \pm SD		329 \pm 102		0.055
5 ml volume (h) \pm SD		376 \pm 160		

Continuous data were compared using Student t test as data were normally distributed and equal variance was assumed. Proportions were compared using χ^2 test and Fisher's exact test for non-matched proportions and McNemar's test for matched proportions.

*Lymphocytes/neutrophils >1.

ADA, adenosine deaminase; LDH, lactate dehydrogenase; MTB, *Mycobacterium tuberculosis*; TB, tuberculosis.

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1 **Online supplementary material to Research Letter**

2

3 **METHODS**

4 **Patients**

5 Adult patients (≥ 18 years) referred to the Division of Pulmonology of Tygerberg Academic
6 Hospital from April 2010 until June 2011 with radiological evidence of a pleural effusion
7 were candidates for this study. Patients were actively recruited from two health community
8 centres through a community outreach programme. Referral time was less than 24 hours in all
9 cases compared to several weeks for regular appointments at a tertiary referral centre. Our
10 institution is a 1,200-bed academic hospital in Cape Town, South Africa. It is one of two
11 academic referral centres in the city and renders a tertiary service to a population of
12 approximately 1.5 million. In 2010 the incidence of TB in South Africa was 981 cases per
13 100,000 [1]. Routine evaluation included history of previous antituberculosis treatment and
14 HIV status at the time of referral. Patients were included in the study if they had at least 3 of
15 the following indicators of possible TB: (1) persistent cough lasting >2 weeks, (2) shortness
16 of breath, (3) haemoptysis, (4) weight loss >4 kg, (5) intermittent fever >3 weeks, (6)
17 drenching night sweats >2 weeks, and (7) pleuritic chest pain [2]. Patients were excluded if
18 transthoracic ultrasound (US) failed to confirm a pleural effusion of at least 10 mm width or
19 if patients reported allergies to local anaesthetics. The Stellenbosch University Human
20 Research Ethics Committee approved the study (reference number N10/02/055). Written
21 informed consent was obtained from all subjects on enrolment and prior to any invasive
22 procedures.

23

24 **Sputum collection**

25 Patients able to produce sputum spontaneously were asked to provide a spot sputum sample
26 for smear microscopy and TB culture. Specimens were decanted into a 50ml Falcon tube (BD
27 Biosciences, New Jersey, USA). An equal volume to the volume of the sputum specimen of a
28 5% NaOH/Trisodium-Citrate-NALC solution (Merck, New Jersey, USA) was added for
29 decontamination. After 20 min phosphate buffer (Merck, New Jersey, USA) with a pH of 6.8
30 was added to stop the decontamination process. The mixture was centrifuged at 3000 rpm for
31 20 min, the supernatant decanted and 1ml of phosphate buffer was added. The solution was
32 diluted if necessary to a McFarland standard of 0.5 and 0.5ml of this were inoculated into a
33 liquid mycobacterial culture tube (Bactec Mycobacteria Growth Indicator Tube [MGIT],
34 Becton Dickinson, Maryland, USA). One drop was put on a slide together with TB
35 precipitating fluid and dried for 2 hours before auramine-O-staining [3] was performed.

36

37 **Transthoracic ultrasound**

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

48

49 **Diagnostic thoracentesis and fluid analysis**

50 Thoracentesis was performed with a 20 G needle under sterile technique and local
51 anaesthesia with lignocaine 1%. Samples were sent for chemical analysis, bacteriology,
52 mycobacteriology, cell count and cytology. The chemical analysis included total protein,
53 albumin, lactate dehydrogenase (LDH) and adenosine deaminase activity (ADA).
54 Bacteriological investigations included gram stains and aerobic and anaerobic cultures. For
55 mycobacteriology we collected 5ml and 100ml of pleural fluid into separate containers. The
56 5ml specimen was collected in a sterile 5ml tube, the 100ml specimen was collected in two
57 sterile 50ml Falcon tubes. Both specimens were processed the same day in the same fashion
58 as the sputum samples. The two pellets of the large volume were pooled. Pellets of both
59 specimens were separately resuspended in phosphate buffer to a McFarland standard of 0.5.
60 For liquid culture 0.5ml of the suspension were inoculated into a MGIT tube and one drop
61 was used for auramine-O-staining [3]. Further processing included the addition of OADC
62 growth supplement and PANTA to the MGIT tube as part of the MGIT culture kit. In positive
63 MGIT tubes the presence of mycobacteria was confirmed by Ziehl-Neelsen staining and
64 mycobacterial speciation by PCR [5 6]. Bacterial contamination was excluded with blood
65 agar culture.

66

67 **Closed pleural biopsies**

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

75

76 **Post procedure care and follow up**

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

85

86 **Definitions and statistical analysis.**

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

98

98 **SUPPLEMENTARY RESULTS**

99 We screened a total of 80 patients. 3 patients had no evidence of a pleural effusion on
100 ultrasonography. As mentioned out of 77 patients pleural TB was diagnosed in 58 (75.3%) of
101 whom only 5 (8.6%) had detectable cavitary changes on chest radiograph. In 11 of the 19
102 patients in whom a diagnosis of pleural TB could not be made an alternative diagnosis was
103 established: empyema (n=2), mesothelioma (n=1), metastatic adenocarcinoma (n=1), liver
104 abscess (n=1), pleural echinococcosis (n=1) and transudates secondary to systemic disease
105 (n=5). Four patients remained undiagnosed following thoracoscopy and a further four were
106 empirically treated for pleural TB based on an elevated ADA and a lymphocyte predominant
107 effusion. If all 8 (10.4%) undiagnosed patients were carried forward as false negative for
108 tuberculous pleurisy the total number of TB cases would increase to 66 (85.7% of all
109 patients) and the yield of fluid culture, pleural biopsy histology and culture would drop to
110 53.0%, 74.2% and 57.6%, respectively.

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