TUBERCULOUS PLEURAL EFFUSIONS : A prospective study of rapid diagnostic tests (adenosine deaminase, antigen capture enzyme-linked immunosorbent assay, and the polymerase chain reaction) and evaluation of a radiometric mycobacterial culture system.

FOR THE DEGREE MASTER OF MEDICINE PART 3, UNIVERSITY OF CAPE TOWN.

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ABSTRACT

A prospective study was undertaken to assess the diagnostic value of various rapid diagnostic tests for tuberculosis in pleural fluid, and to assess the sensitivity and speed of a radiometric mycobacterial culture system (BACTEC, Johnson Laboratories). Patients presenting to the Department of Medicine at Groote Schuur Hospital with pleural effusions for diagnostic pleural aspiration and biopsy over a 6 month period were entered into the study. Because the incidence of tuberculous effusions was observed to be high in this population (65% of 94 patients), patients from the Department of Radiotherapy with proven malignant disease and the development of new pleural effusions requiring diagnostic or therapeutic aspiration were included in the study in order to increase the number of control patients without tuberculosis.

The 111 patients (17 of whom were recruited from the Department of Radiotherapy) were divided into 4 diagnostic categories: tuberculosis - 62 patients, malignant - 28 patients, miscellaneous conditions - 10 patients, and undiagnosed - 11 patients (3 of whom probably had tuberculosis). There were 59 male patients. The racial distribution was 11 whites, 51 of mixed race, and 49 blacks.

Exudative pleural effusions were present in 109 patients. Closed pleural biopsies with the Abrams needle were performed on 100 patients using a modified version of the standard technique whereby larger specimens were obtained by stripping pleura off the chest wall. Seven pleural biopsies were reported as inadequate by the pathologist and the diagnostic yield of the procedure was 63%.

Tuberculosis was confirmed histologically or by culture in 62 patients. The age distribution of these patients was bimodal, with most cases occuring in the third decade. The presentation was usually acute, with 60% of patients being symptomatic for less than 4 weeks. Granulomata were found on initial pleural biopsy in 52 cases (84%). Pleural biopsy culture was positive in 44 cases (71%). The radiometric culture system tested (12B BACTEC) yielded the same number (14) of positive cultures as conventional mycobacterial culture media in pleural fluid, but was almost twice as fast. Bedside inoculation of pleural fluid into 13A BACTEC bottles more than doubled the yield in the 24 patients tested (11 positive cultures compared with 4 each for conventional and 12B BACTEC media, $p=0.046$).

The rapid diagnostic tests assessed on pleural fluid were adenosine deaminase (ADA), an antigen (BCG) capture enzyme-linked immunosorbent assay (ELISA), and a specific DNA probe after amplification with the polymerase chain reaction. ADA was found to have a sensitivity of 0.77 and a specificity of 0.83 in the 109 patients tested, and values were significantly higher in tuberculosis patients compared with the other three diagnostic categories ($p < 0.001$). The ELISA test was performed on 103 patients and showed a sensitivity of only 0.26 and a specificity of 0.72. The DNA probe was performed on 43 patients, and had a sensitivity of 0.93 with a specificity of 0.43. Contamination of samples or latent tuberculous infection may have been responsible for the poor specificity of the DNA probe.

CONTENTS

1. TUBERCULOUS PLEURAL EFFUSIONS

1.1 Pathogenesis

Mycobacterium tuberculosis, the organism causing tuberculosis in humans, usually gains access to the pleural space in the course of primary tuberculosis by contiguous spread from a subpleural pulmonary focus (1). The organism then invades the pleura, and an exudative effusion usually occurs. The effusion is considered to be a hypersensitivity response to mycobacterial antigens, but the organism may be cultured from the fluid in up to 52% of cases (2). The natural history of the primary form of pleural tuberculosis is spontaneous resolution within a few months, but the majority will develop reactivation tuberculosis within 5 years (2).

Pleural involvement occurs much less commonly with reactivation pulmonary tuberculosis. Bronchopleural fistulae and tuberculous empyemas are not infrequent in this stage of the disease. Pleural effusions are also found in up to 30% of patients with miliary tuberculosis (3).

1.2 Epidemiology

Pleural effusions complicate the course of primary tuberculosis more often in adults (4) than in children (5), and are are more frequent in older than in younger children (5). The reasons for the increasing incidence with age are unknown. A bimodal age distribution has been reported (6), with most cases occurring in the second or third decades.

Data on prevalence are not readily available. A South African study based on tuberculosis notifications unearthed only 4 cases from 1973 to 1986 in the age group 45 to 64 years (7). Clearly most cases are notified as pulmonary tuberculosis or are not notified at all. In a high prevalence area pleural effusions were found in less than 1% of cases of tuberculosis (8), but a study in a low prevalence area found effusions in 3.7% (9). In Nigeria tuberculosis accounted for 56% of pleural effusions (10), whilst a centre in the USA found an incidence of only 1.5% (11).

1.3 Clinical features

Tuberculous pleural effusions usually present acutely, which may cause diagnostic confusion with parapneumonic effusions (6). Older patients tend to have a longer symptom duration (6). Pleuritic chest pain generally precedes dry cough as a symptom (6), and dyspnoea is frequent. Fever is almost always present (6). Effusions are bilateral in 10% of cases, and may be of any size (12).

1.4 Diagnosis

Definitive diagnosis requires the culture of *Mycobacterium tuberculosis* from pleural fluid or tissue.

In low prevalence areas positive tuberculin skin tests are helpful, but are negative in a third of patients early in the disease (13). Skin tests are much less helpful in high

prevalence areas such as the Transkei, where the majority of chidren over the age of 10 have a positive Mantoux (14). Although the degree of positivity is generally held to be helpful, a study in Malaysia showed that Mantoux size in healthy tuberculin-positive controls was the same as in tuberculin-positive patients (15). The incidence of positive skin tests is likely to be high in the Western Cape, which has notification rates of 300 to 400 / 100 000 population (16), and this test is thus of little value in our area.

The characteristics of aspirated pleural fluid are generally unhelpful. Haemorrhagic effusions are more frequent in malignancy (17), but are also found in about 10% of tuberculous effusions (10,17). Lymphocytes predominate in all except the early cases (6), but this is often true in other effusions (18). The presence of >1% mesothelial cells or >10% eosinophils (except with pneumothoraces) is against the diagnosis of tuberculosis, but counts less than these values are non-specific (18).

Acid-fast bacilli are rarely seen in the fluid (10), but may be cultured in about a quarter of cases (13,19). Some workers have doubled this yield by performing both guinea pig inoculation and conventional cultures (2) or by culturing fluid aspirated on 2 or more occasions (20). Others have suggested that culturing large volumes increases the yield (21) - this is certainly the case in tuberculous ascites (22). Mycobacterial culture is a slow process, and may take up to 8 weeks using conventional media. New radiometric culture methods take about half as long (23).

Unless the patient has associated reactivation pulmonary tuberculosis, the organism is rarely found in the sputum. Pleural biopsy, using a closed approach, is usually done to make the diagnosis. Granulomata are found on initial biopsy in 60 to 70% (6,13,19). Other causes of pleural granulomata are rare (24). The presence of caseation and particularly acid-fast bacilli is virtually diagnostic. A second biopsy will often be positive if the first was non-specific (25). However, a third biopsy usually is seldom helpful (25). Several biopsies should be taken with each procedure, and one sample should be cultured for mycobacteria. This gives a yield similar to histology (19,20). Culture not only confirms the tuberculous nature of the granulomata, but is often positive when histology is negative (20).

As the sensitivity of histology is not very high , a trial of antituberculous therapy should be given whilst awaiting culture. Therapeutic trial should only be considered after two adequate closed biopsies have been non-diagnostic. Open pleural biopsy, preferably under thoracoscopic guidance (26), should probably be reserved for cases not responding to therapy. Approximately 20% of patients with pleural effusions will remain undiagnosed after closed biopsy and aspiration for cytology and routine and mycobacterial cultures (17,26).

Experienced operators obtain adequate pleural biopsies with the Abrams needle in 90% of cases (27). The procedure has a low morbidity (27). In a study performed at Groote Schuur Hospital, where many biopsies are done by inexperienced operators, the adequacy rate was found to be only 63% (28).

1.5 Rapid diagnostic techniques

Histology of pleural biopsy is thus the usual way of making the diagnosis rapidly. As noted in **1.4**, a significant proportion of patients will be treated empirically pending results of mycobacterial culture. Admission, at least overnight, is usually required for the procedure which takes about an hour to perform. More time (and cost) is spent by the pathologist viewing the slide. The pathologist should also be experienced at reporting on pleural biopsies (27). Great savings of cost and hospital time could be achieved if a reliable rapid diagnostic test could be done on pleural fluid.

A variety of rapid diagnostic techniques in tuberculosis have been evaluated. The test should have a specificity approaching 1 if it is to replace histology or culture. Ideally the test should also have a sensitivity of 1, however specificity of diagnostic tests is usually lost with increasing sensitivity (36). Obviously the test should be more sensitive than Ziehl Neelsen staining and sensitivity should be similar to that of pleural biopsy.

In the diagnostic work up of pleural effusions a biopsy could be done if it and other tests, particularly cytology, were negative on aspirated pleural fluid. The other major cause of exudative effusions, malignancy, is diagnosed more often by cytologic analysis of pleural aspirate than by biopsy (11,17,29). Cytology has a specificity of 1 and reported sensitivity varies from 0.58 (11) to 0.73 (29).

Some authors feel, therefore, that pleural biopsy should be reserved for patients with negative cytology in areas having a low prevalence of tuberculosis (11,30). This would become universal practice if a test comparable to cytologic analysis existed for diagnosing tuberculosis.

Rapid diagnostic tests which have shown the most promise will now be reviewed:

1.5.1 ADENOSINE DEAMINASE

This enzyme is produced largely by lymphocytes. High levels have repeatedly been found in tuberculous effusions (31-35); but also frequently in lymphoma, rheumatoid arthritis, empyema, parapneumonic effusions, and mesotheliomas (32-35). Sensitivities have generally approached 1, but specificity is lower. Carcinomas can usually be separated from tuberculosis using this assay. The test is easy to perform, and is thus an attractive proposition for the underdeveloped world. It is widely used in South Africa, and in some centres treatment is given if the levels are high without doing a biopsy. A study in a low prevalence population, where high specificity is required before a test is clinically useful (36), found that adenosine deaminase (ADA) was not diagnostic of tuberculous pleurisy (37).

1.5.2 IMMUNODIAGNOSTIC ASSAYS

Immunological tests, for both antigen and antibody detection, have been extensively researched. Available techniques, especially enzyme-linked immunosorbent assays

(ELISA), are sensitive and could be done in underdeveloped countries. One reviewer found satisfactory sensitivity and specificity of serological antibody tests in sputum smear-positive pulmonary tuberculosis, but not in paucibacillary disease (38). As the need for the test only exists in smear-negative disease, currently available serological tests are not useful. Antigen assays have met with some success in body fluids, especially cerebrospinal fluid (39). Further development of assays based on antigen 5, which showed great promise (39), has been stopped as it is expensive to produce and unstable (Daniel,TM - personal communication). Research has been hampered by lack of commercially available reagents (39).

Several studies have been published on pleural fluid using immunoassays. The best results (sensitivity and specificity of 1) were obtained using combined antibody and antigen tests (40). That study used predominantly clinically diagnosed cases, and has not been repeated elsewhere. South African workers achieved good results in a small group of pleural effusions (most of their samples were cerebrospinal fluid) using a BCG based competition ELISA (41). BCG and antibodies directed against it are commercially available. More conventional antigen capture ELISA's using BCG and anti- BCG have given disappointing results (42), and one group found that the test was unable to distinguish between tuberculous and malignant effusions (43).

1.5.3 TUBERCULOSTEARIC ACID

This lipid is a cell wall product of mycobacteria as well as Actinomycetales (including nocardia). Pleural effusions caused by nocardia and mycobacteria other than tuberculosis are rare (24), and false positives with these infections could be diagnosed by culture. Published studies on sputum (44) and cerebrospinal fluid (45) have shown specificities approaching 1 and high sensitivities. Intrathecal amikacin, which is produced by an actinomycete, was responsible for a false positive result (45). Gas chromatography is technically demanding and the equipment is expensive - assaying tuberculostearic acid this way in underdeveloped countries is not feasible.

1.5.4 DNA PROBES

DNA probes specific for *Mycobacterium tuberculosis* have been successfully applied to clinical specimens, including pleural fluid (46). The test was shown to be specific, but was no more sensitive than conventional Ziehl Neelsen staining.

A DNA probe specific to *Mycobacterium tuberculosis* has been developed by Dr LM Steyn of the Department of Medical Microbiology. This 336 base pair DNA fragment is also represented several times in each organism (Steyn,LM - personal communication). Together with Dr D de Wit a system has been developed, using the polymerase chain reaction (47), to amplify the DNA in clinical samples before applying the DNA probe. Because of its exquisite sensitivity

great care must be exercised to avoid contamination which could cause false positives.

In summary, ADA and immunoassays are easy to perform, but they are not specific enough to replace existing diagnostic methods. Tuberculostearic acid fulfills most requirements for the ideal test for paucibacillary tuberculosis, but the currently available assay technique is too complex for underdeveloped areas. DNA probes have the required specificity, but are insensitive. Utilising DNA amplification techniques would improve sensitivity and hopefully retain specificity.

1.6 Study aims

There were two main aims of this study. The first was to prospectively evaluate the diagnostic value of some of the rapid diagnostic tests discussed in **1.5** in patients with pleural effusions requiring diagnostic aspiration. The tests that were assessed were the DNA probe developed by Dr LM Steyn (after amplification with the polymerase chain reaction), ADA, and an antigen capture immunoassay. The second aim was to compare the sensitivity and speed of a radiometric culture system (BACTEC - Johnson Laboratories) with conventional mycobacterial culture.

2. PATIENTS AND METHODS

2.1 Patient selection

Patients presenting to the Department of Medicine at Groote Schuur Hospital for diagnostic pleural aspiration and biopsy in the 6 month period December 1988 to June 1989 were entered into the study. Those cases thought to have transudative pleural effusions on clinical grounds were excluded. A pilot study showed that the proportion of tuberculous effusions in medical patients was high (80% of the first 30 patients). With so few negative controls meaningful specificity of the various rapid diagnostic tests could only be calculated by recruiting large numbers of patients. Therefore patients were recruited from the Department of Radiotherapy. These patients all had malignant disease and had developed new pleural effusions requiring diagnostic or therapeutic aspiration. Only adults (13 years or older) were entered into the study. The study protocol was approved by the Ethics Committee of the Faculty of Medicine, University of Cape Town.

The type and duration of symptoms were noted. The results of Mantoux skin tests (performed and read by the ward staff) were recorded. This test was only performed on some patients as most were only admitted overnight. Effusions were graded according to radiographic size: small - costophrenic angle blunted, moderate - occupying less than half the hemithorax, large - more than half the hemithorax, and massive - the whole hemithorax. The presence of mediastinal shift was

recorded. Other radiographic features were also noted.

2.2 Tests performed on aspirated fluid

All pleural fluid was collected under sterile conditions. The aspirate was performed through the Abrams needle before the biopsy, to avoid traumatic blood-staining of pleural fluid. If the patient was short of breath then a therapeutic aspiration of not more than 1000 ml was carried out.

2.2.1 BIOCHEMICAL TESTS

5-10 ml of pleural fluid was collected in plain tubes and analysed for protein and lactate dehydrogenase concentration on an Hitachi 704 auto-analyser. ADA estimations were done using a standard method (48) by the staff at the Chemical Pathology laboratory. This test is offered as a routine service by the laboratory. Amylase levels were measured only when there was clinical suspicion of pancreatitis.

2.2.2 BACTERIOLOGICAL CULTURE

In almost all patients 5 ml of pleural fluid was sent for Gram staining and routine bacteriological culture, consisting of overnight incubation in cooked meat broth followed by plating onto MacConkey's medium, 4% blood agar, and boiled blood agar. These were then incubated for 48 hours (under anaerobic conditions with the 4% blood agar and under 5-10% carbon dioxide in air in the case of the boiled blood agar). This method would be adequate for culturing most aerobic and

anaerobic bacterial pathogens known to infect the pleural space (although nocardia occasionally require longer incubation periods).

2.2.3 ZIEHL NEELSEN STAIN

10 ml of pleural fluid was sent to the Microbiology Laboratory and centrifuged for 15 minutes at 1000 rpm. The spun deposit was fixed with heat onto a glass slide and stained by the Ziehl Neelsen method to search for acid-fast bacilli. These slides were carefully reviewed for 10 to 15 minutes by the author, but in some instances the material had been wiped off the slide by the technologist who viewed it initially.

2.2.4 CONVENTIONAL MYCOBACTERIAL CULTURE

The remainder of the spun deposit was used for conventional mycobacterial culture. This consists of Kirchner, Lowenstein-Jensen, and pyruvate enriched Stonebrink media. The cultures were aerated and read weekly for 8 weeks by the routine laboratory staff.

2.2.5 BACTEC MYCOBACTERIAL CULTURE

A further 10 ml sample of pleural fluid was centrifuged and the spun deposit injected into 12B BACTEC bottles. These contain liquid mycobacterial culture medium which contains radiolabelled carbon, ^{14}C. The organisms metabolise the ^{14}C-labelled nutrient, liberating radioactive carbon dioxide

into the closed atmosphere of the bottle. With a fully automated process (the BACTEC 460 analyser) a small amount of gas is removed from the 12B bottle and the amount of radioactivity measured. Positive readings are confirmed on Ziehl Neelsen staining. Organisms were identified as *Mycobacterium tuberculosis* by the niacin test.

The spun deposit of an exudative effusion frequently forms a clot which cannot then be injected through the sealed rubber stopper of the 12B bottle. A variety of anticoagulants (citrate, EDTA, and heparin) were added to separate samples of fluid at the time of aspiration to see whether clotting could be prevented. Despite this small clots continued to form, and eventually the clots were crushed before injection into the bottles.

The BACTEC bottles were incubated at $37^{O}C$ and read thrice weekly for the first 2 weeks, and then weekly for a total of 8 weeks by laboratory staff familiar with the BACTEC 460 analyser.

2.2.6 BEDSIDE INOCULATION

Dr George Strang of Frere Hospital, East London, has observed that the yield of mycobacterial culture of tuberculous effusions is higher with bedside inoculation directly into bottles containing liquid mycobacterial culture medium. We elected to assess the yield of bedside inoculation and to compare it with the yield of inoculating the spun deposit into culture media.

Bedside inoculation into BACTEC bottles would avoid the problem of trying to inoculate clotted pleural fluid through a rubber stopper. Using 12B BACTEC bottles for bedside inoculation would not lead to a valid comparison with conventional processing as they only accept 1 ml of fluid. Johnson Laboratories also manufacture a radiometric mycobacterial blood culture bottle, 13A BACTEC, largely for diagnosing disseminated mycobacterial infections in patients with the acquired immunodeficiency syndrome. The 13A bottle is evacuated and accepts 5 ml of fluid. It contains the same medium as the 12B, and is suitable for samples other than blood. Forty patients had 5 ml of fluid inoculated into 13A BACTEC bottles at the bedside. These 40 patients also had centrifuged fluid injected into 12B BACTEC bottles and conventional mycobacterial media. In this way the yield of bedside inoculation could be directly compared with laboratory inoculation of the centrifuged deposit. The 13A BACTEC bottles were then treated in the same way as the 12B bottles.

2.2.7 IMMUNODIAGNOSTIC ASSAY

2 ml of pleural fluid was stored at -20^{O}C and a BCG-based antigen capture ELISA was performed in a single batch by Dr Stan Ress of the Immunology Laboratory. The method involved preparing plastic plates containing wells to which commercial rabbit anti-BCG was fixed. The wells were then filled in duplicate with pleural fluid. After incubation the wells were washed with phosphate buffered saline and Tween 20 and peroxidase-labelled anti-BCG added. The wells were washed

again, and the substrate for the peroxidase (2,2'-azinobis 3' ethylbenzthiazoline sulphonic acid) added. This substrate undergoes a colour change, and the light absorbance is then measured with a spectrophotometer. The system was first calibrated with known concentrations of BCG antigen, and was capable of detecting 3 ng/ml of BCG (Dr. Stan Ress - personal communication). The operator performing the ELISA did not know the diagnosis in any patient.

2.2.8 DNA PROBE

Pleural fluid was stored at -20^{O}C and the DNA amplified using the polymerase chain reaction (PCR) by Dr Deo de Wit of the Department of Pathology under the guidance of Dr LM Steyn of the Department of Medical Microbiology.

The DNA was first extracted by mixing 2 ml of pleural fluid with 2 ml of 10% sodium dodecyl sulphide buffered phenol and shaken at 37^{0}C for 3 hours. This was then centrifuged and the supernatant extracted three times with an equal volume of phenol:chloroform:iso-amylalcohol (25:24:1). The DNA solutions were concentrated by precipitation with an equal volume of polyethylene glycol and centrifuged. The DNA pellet was washed twice with 70% ethanol, dried and dissolved in 40 ul of sterile distilled water.

20 ul of the extracted DNA solution was added to 80 ul of the PCR reaction mixture (10% dimethyl sulphoxide, 50 mM KCl, 10 mM Tris-CL pH8.3, 1.5 mM $MgCl_2$, 0.01% gelatin, 200 umoles dNTP, 1 to 2 umoles of the oligonucleotide primers

and 2.5 units of Taq polymerase). Thermal cycling at 95°C for 1 minute and 70°C for 2 minutes for 30 cycles. After amplification the DNA was concentrated by alcohol precipitation and agarose gel electrophoresis carried out. The DNA was then transferred to Hybond-N membrane and hybridised with the radiolabelled probe.Autoradiography of the hybridisation products was performed for 24-48 hours and the readings carried out by 2 independent observers. Further details can be obtained from Dr D de Wit's MD thesis (in preparation).

The first 10 samples assessed with the DNA probe were from known cases of tuberculosis so that the number of cycles required in the polymerase chain reaction to detect DNA could be determined. The remaining samples were from patients whose diagnosis was unknown to the operator.

2.2.9 CYTOLOGY

Fluid for cytologic analysis was collected in heparinised tubes and fixed with 50% alcohol. The sample was then centrifuged at 2500 rpm for 8 minutes. Unless there was an obvious cellular residue, 1.5 ml of the fluid was then subjected to additional high speed Cytospin centrifuge. The spun deposit was fixed onto 3 slides with Fencott fixative and stained with Haemotoxylin and Eosin. A further slide was fixed by air drying and stained by the Giemsa method. The slides were prepared and read by technologists in the Cytology Laboratory and reviewed by the pathologist.

2.3 Pleural biopsies

A closed pleural biopsy was performed on all medical patients using the Abrams needle. Because patients from the Radiotherapy Department had confirmed diagnoses of malignacy from other sites, it was felt that it would be unethical to subject them all to pleural biopsy. A biopsy was only performed on those Radiotherapy patients whose medical attendants requested it. Procedures were performed under local anaesthetic. All patients gave informed consent.

The technique used was a variation of the standard technique described by Cowie et al (27) which achieves larger samples by stripping pleura. In the standard method the Abrams needle is slowly withdrawn until it hooks onto the chest wall which is then biopsied by completely closing the needle. The stripping method differs in that the needle is only closed until snug, and is then pushed back into the patient thus stripping pleura off the chest wall.

Three to 4 biopsies were taken from each patient. One sample was placed in saline and then crushed for inoculation into conventional mycobacterial culture media (as described in 2.2.4). The other samples were fixed in formalin, sectioned, and stained with conventional tissue and Ziehl Neelsen stains for histopathologic analysis. The histology was reviewed by one pathologist, Dr Mark Hanly. Further sections were cut from those samples initially reported as inadequate (defined as the absence of pleura).

The author attempted to perform all the pleural biopsies as

the rate of adequate biopsies had been shown to be low at Groote Schuur Hospital (28).

Post-procedure chest radiographs were done on all patients to exclude a pneumothorax.

2.4 Statistical analysis

This was performed by a statistician from the Medical Research Council of South Africa. Standard SAS software (SAS Institute Inc., Box 8000, Cory, North Carolina, USA) was used in the analysis. Median values together with the 25th and 75th quartiles were given for non-parametric data. Continuous variables (such as age) were compared by the Wilcoxon 2-sample test. When many variables were identical (thus making ranking of data difficult) the median test was used. Discrete variables (such as presence of a certain symptom) were compared uing the Chi-square test. When the culture yield of bedside inoculation into 13A BACTEC bottles was compared with that of inoculating the centrifuged deposit, Chi-square could not be used as several patients were culture-positive with both methods. In this instance Mc Nemar's test was used.

The clinical differences between patients with malignant and tuberculous effusions were analysed. The other data subjected to statistical analysis were the values of ADA in the different diagnostic categories and the yield and time differences of the various mycobacterial culture methods tested.

3.RESULTS

3.1 Demographic data

One hundred and eleven patients were entered into the study, of whom 17 were recruited from the Department of Radiotherapy. The mean age was 49.9 years (range 14-85). Eleven patients were white, 51 of mixed race, and 49 black. Fifty nine were males.

3.2 Findings on pleural aspirate

Blood-stained effusions were found in 11 cases: 7 were malignant, 2 tuberculous, and 2 undiagnosed.

Exudative effusions, as defined by Light et al (49), were present in 109 patients. The diagnosis in the 2 patients with transudative effusions was cardiac failure (in a patient from the radiotherapy group who had a persistent effusion after diuresis), and malignancy with large mediastinal lymphadenopathy and negative cytology on pleural aspirate and negative histology on pleural biopsy (the effusion in this case was presumably caused by compression of mediastinal lymphatics).

Routine bacteriologic cultures identified the causative organism in 1 patient with empyema (whose aspirate was frank pus). Organisms which were considered contaminants were grown in 4 patients: *Staphylococcus epidermidis* in 3 and a yeast in 1.

Results of cytology and mycobacterial culture are reported in the malignant and tuberculous effusion groups below.

3.3 Biopsies - complications and results

One hundred pleural biopsies were performed, 90 of them by the author. The remaining 10 were done by experienced registrars or by interns who had been instructed by the author. Sixty eight percent of closed pleural biopsies on medical patients during the 6 month study period were entered into the study.

Pneumothoraces complicated the procedure in 8 cases, but required drainage in only 1. Three patients had minor vasovagal reactions. Massive extravasation of pleural fluid into subcutaneous tissue occurred in 1 patient with a malignant effusion (1 litre had been removed at the time of the biopsy).

The biopsy was diagnostic in 61 patients. Seven biopsies were found to be inadequate, even after extra sections were cut. Review of the biopsies by the pathologist did not change the diagnosis in any case, but additional features (such as acid-fast bacilli) were found in several samples.

3.4 Diagnostic categories

The patients were placed into one of 4 different diagnostic categories: tuberculosis, malignant, miscellaneous, and undiagnosed.

3.4.1 TUBERCULOSIS

a) Demographic data

Sixty two patients were shown to have tuberculous effusions. Sixty one were from the Medical group, which thus has an incidence of tuberculosis of 65%. The age distribution was bimodal and is indicated graphically in figure 1 overleaf.

The median age was 37.5 years (25th-75th quartiles of 26-59.5 years) with a range of 14 to 83 years. The racial distribution was 1 white, 28 of mixed race, and 33 black patients. There were 34 males.

b) Clinical features

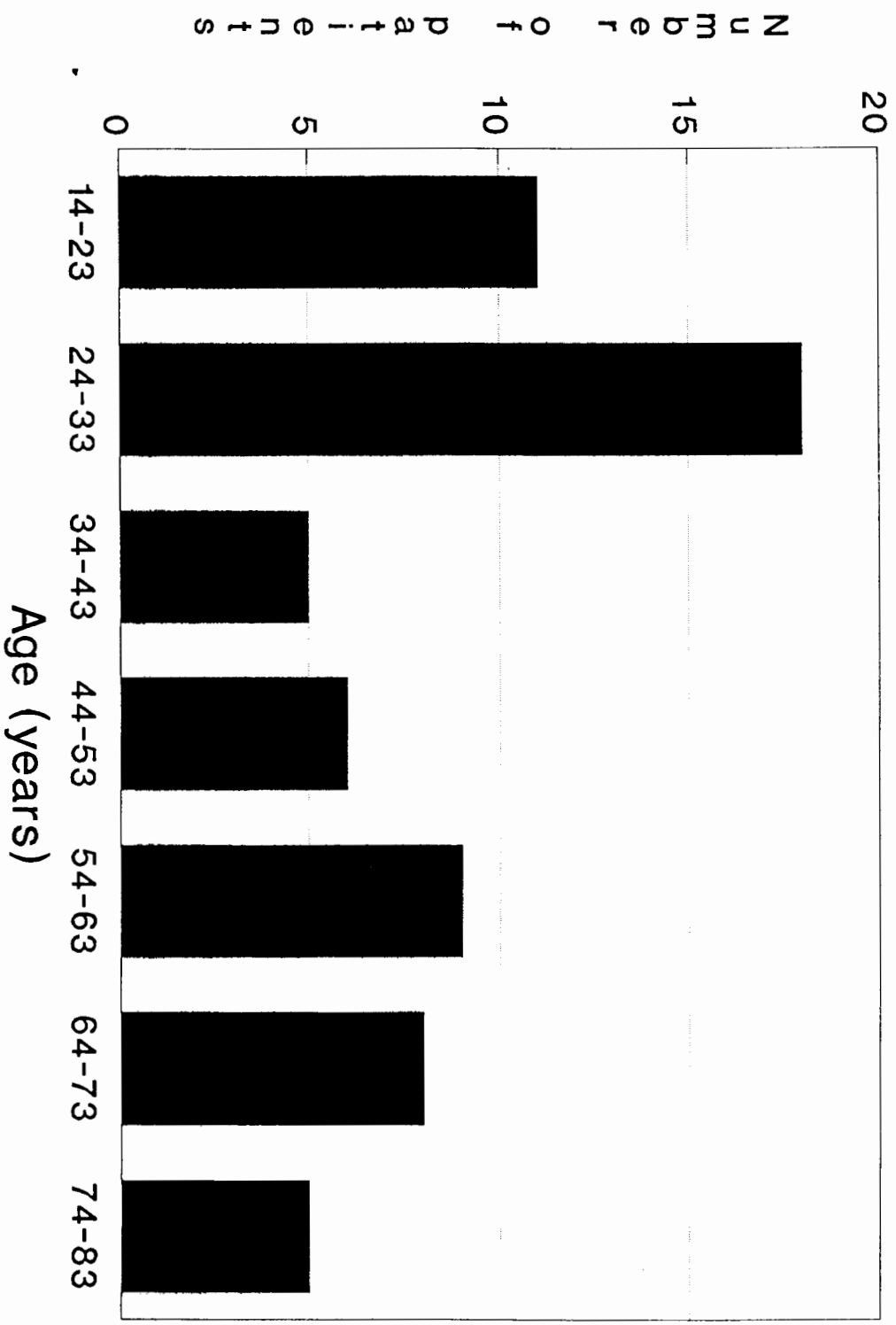
Initial symptoms were usually constitutional (fever, weight loss, or malaise) followed by pleuritic chest pain in most patients. All patients were symptomatic. Dry cough, when present, followed chest pain. Dyspnoea was regularly present with larger effusions. The frequency of symptom type is shown in table 1.

TABLE 1

Symptom type in tuberculous effusions.

Symptom	Number of patients (%)
Weight loss	37 (60%)
Malaise	5 (8%)
Fever	37 (60%)
Chest pain	49 (79%)
Cough	39 (63%)
Dyspnoea	23 (37%)

Figure 1 Age distribution in tuberculous pleural effusions



The median symptom duration in the 57 evaluable patients was 3 weeks (25th-75th quartiles of 2-7) with a range of 1 to 78 weeks. As shown in table 2, patients with a symptom duration of 4 weeks or more were older. The observed difference in age was significant (p=0.014 by Wilcoxon 2-sample test). Five patients were uncertain of their precise symptom duration, but most thought they had been ill for months. The mean age of this group was even older at 65.2 years.

TABLE 2

Comparison of age and symptom duration in tuberculosis

Symptom duration	<4 weeks	4 weeks or more
Sample size	32	25
Median age (years)	28	49
25th-75th quartiles	22-46.75	27-68

Five patients had tuberculosis involving other sites (2 ascites, 2 lymphadenitis, and 1 sternal abscess) and 4 patients had disseminated tuberculosis. Eight patients were aware of recent contact with tuberculosis.

Mantoux tests were positive (>9 mm) in 18 of 23 recorded cases (78%).

c) Radiographic features

Radiographic effusion size was small in 6 patients, moderate in 26, large in 16, and massive in 11 (6 of whom had contra-lateral mediastinal shift). The effusions were bilateral in 6 and loculated in 3. Radiographic features compatible with previous tuberculosis were present in 4 patients.

A miliary pattern was present in 4 cases. Pulmonary infiltrates compatible with tuberculosis were found in 5

patients (confirmed on sputum smear in 1).

d) Cytology and Ziehl Neelsen stain

One patient (with a very short history) had polymorphonuclear leukocytes predominating in the fluid, but lymphocytes predominated in all other samples. Cytologic features suspicious of lymphoma were reported in one patient (who was culture positive). Scanty acid-fast bacilli were found in only 1 pleural aspirate (only after review by the author).

e) Conventional compared with 12B BACTEC cultures

Conventional mycobacterial and 12B BACTEC cultures both yielded 14 positive cultures (23%). The BACTEC system was faster, however, with a median time to positive culture of 18 days (range 3-40). Mean culture time for conventional media was 33.5 days (range 21-48). Sixteen cases were culture-positive with only one method and 6 with both, giving a total of 22 (35%). The difference in time to positive culture between the 2 methods could only be compared in those 6 patients who were culture- positive with both methods. In this small group the 12B BACTEC method was significantly faster ($p=0.036$ by signed rank test).

One sample was contaminated with yeasts, but a colony of *Mycobacterium tuberculosis* was picked off the slope of the Lowenstein-Jensen medium.

f) Bedside inoculation

Twenty four patients with tuberculous effusions had bedside

inoculation into 13A BACTEC bottles as well as having fluid centrifuged and placed into conventional and 12B BACTEC media. A higher yield was obtained with 13A bottles in these 24 cases - 11 (46%) positive cultures compared with 4 (17%) each for the other methods. This difference in yield was statistically significant (p=0.046 by Mc Nemar's test). The observed difference between the yields of 13A and the other methods was 29% (i.e. 46% minus 17%). The 95% confidence intervals of this difference is 3% to 55%.

The median time to positive culture in the 13A bottles was 25 days (range 17-56). Valid statistical conclusions concerning the difference in culture time between 13A and 12B bottles could not be made in view of the small sample size.

Twenty nine patients overall (47%) had positive fluid cultures. Six patients had positive fluid cultures with negative pleural biopsy cultures, and in 4 of these histology of the pleural biopsy (including 1 open biopsy) was also negative.

g) Pleural biopsy

Pleural biopsies were done in all cases. Granulomata were present in 52 (84%). Caseation was noted in 41 and acid-fast bacilli found in 27 (44%). The tuberculous nature of the granulomata was confirmed by culture of fluid or biopsy in 42 cases. Five biopsies were deemed inadequate by the pathologist. Two patients with non- specific histology had repeat biopsies (using an open approach in 1 case) - both

failed to show granulomata. Acid-fast bacilli were found on review in 10 cases which had been reported as negative.

Mycobacterial culture of pleural biopsy was positive in 44 patients (71%), 4 of whom had negative histology (including 3 samples reported as inadequate).

h) Other diagnostic methods

In 2 cases tests on pleural aspirate and biopsy were non-specific, but the diagnosis was established by histology of supraclavicular lymph node biopsy in one and by aspiration of a "cold" sternal abscess in the other.

3.4.2 MALIGNANT

a) Demographic data

Twenty eight patients had malignant effusions, 15 of whom were recruited from the department of Radiotherapy. The median age was 65 years (25th-75th quartiles of 48.75-74.5 years) with a range of 40 to 85 years. This was significantly older than the median age of 37.5 years in patients with tuberculosis ($p < 0.001$ by Wilcoxon 2-sample test).

Females predominated (17 patients) as many of the Radiotherapy patients were being followed with carcinomas of the breast. There were 8 white patients, 17 of mixed race, and 3 blacks.

b) Clinical features

The duration of symptoms could only be evaluated in 15

patients. Median symptom duration was 4 weeks (25th- 75th quartiles of 3-12) with a range of 2 to 104 weeks. This was not significantly different from the symptom duration in tuberculosis patients (p=0.269 by median test). Several patients from the Radiotherapy group were asymptomatic and had their effusions detected on routine follow up.

Symptom type was elicited from 18 patients. The difference in the frequency of symptom type between malignant and tuberculous patients is shown in table 3. Fever and chest pain were reported significantly more often by patients with tuberculosis, whilst dyspnoea was significantly more common in the malignant group. There was no significant difference in the frequency of weight loss or cough.

TABLE 3

Comparison of symptom type in malignant and tuberculous effusions.

Symptom	Number with malignancy	Number with tuberculosis	P value*
Weight loss	11 (61%)	37 (60%)	0.913
Fever	2 (11%)	37 (60%)	<0.001
Chest pain	7 (39%)	49 (79%)	0.001
Cough	7 (39%)	39 (63%)	0.070
Dyspnoea	13 (72%)	23 (37%)	0.008

*by Chi-square

c) Radiographic features

Radiographic effusion size was massive in 8 (with contralateral mediastinal shift in 6), large in 9, moderate in 8, and small in 3 patients. Three effusions were bilateral. Bony or pulmonary metastases were found in 5, and a large mass lesion was present in 4 cases. Ipsilateral

mediastinal shift occurred in 1 patient.

d) Diagnosis

Biopsies were done in 17 patients, and malignancy was found in 9 (53%). Cytologic analysis of the pleural aspirate was done in all cases, and 15 (54%) were reported as malignant. This includes 1 patient whose initial cytology was suspicious. The combined yield of cytology and pleural biopsy was 17 (61%). Malignancy was confirmed in a further 5 cases by histology of other tissue (lymph nodes in 3 and transbronchial lung biopsy in 2).

The remaining 6 patients were all from the Radiotherapy group, and had confirmed diagnoses of malignancy from various sites. Cytology was reported as suspicious of malignancy in 3 of these patients.

Type of malignancy:

breast	8
adenocarcinoma	7
squamous	5
mesothelioma	1
lymphoma	1
ovary	1
cervix	2
unspecified	3

3.4.3 MISCELLANEOUS

Ten patients had a variety of diagnoses, listed below:

systemic lupus erythematosis (SLE)

empyema (*Bacteroides* sp.)

parapneumonic (2 patients)

spontaneous pneumothorax (2 patients)

pulmonary infiltrate with eosinophilia (PIE)

cardiac failure

amoebic liver abscess with sympathetic effusion

abdominal surgery

The 2 patients with spontaneous pneumothoraces had non-specific findings on pleural biopsy, which was repeated in 1 patient. Eosinophilia was present in the blood and pleural fluid of the patient with the PIE syndrome. The patient with cardiac failure had a persistent effusion after diuresis and was from the Radiotherapy group. One patient had an otherwise unexplained effusion (with a low amylase) after abdominal surgery, which is a recognised cause of pleural effusions (50).

3.4.4 UNDIAGNOSED

Eleven patients remained undiagnosed after pleural aspiration and biopsy. Three of these patients had repeat closed biopsies, and 1 had an open biopsy. The biopsy was inadequate in 1 patient, whose effusion resolved without therapy.

Three patients had probable tuberculous effusions. All received antituberculous therapy with a good clinical

response. Only 1 had a Mantoux done, which was positive.

Two deaths occurred in the short follow-up period (maximum 8 months). No follow-up data were available for 2 patients.

3.5 Adenosine deaminase

ADA activity was measured in 109 patients. The sample from the patient with empyema was deemed unsuitable for analysis by the laboratory, and the result from 1 patient with a tuberculous effusion could not be traced.

The results are represented graphically overleaf in figure 2. Values obtained in the various diagnostic groups are shown in table 4. ADA was significantly higher in the tuberculosis group ($p < 0.001$ when compared with each of the other 3 groups by Wilcoxon 2- sample test).

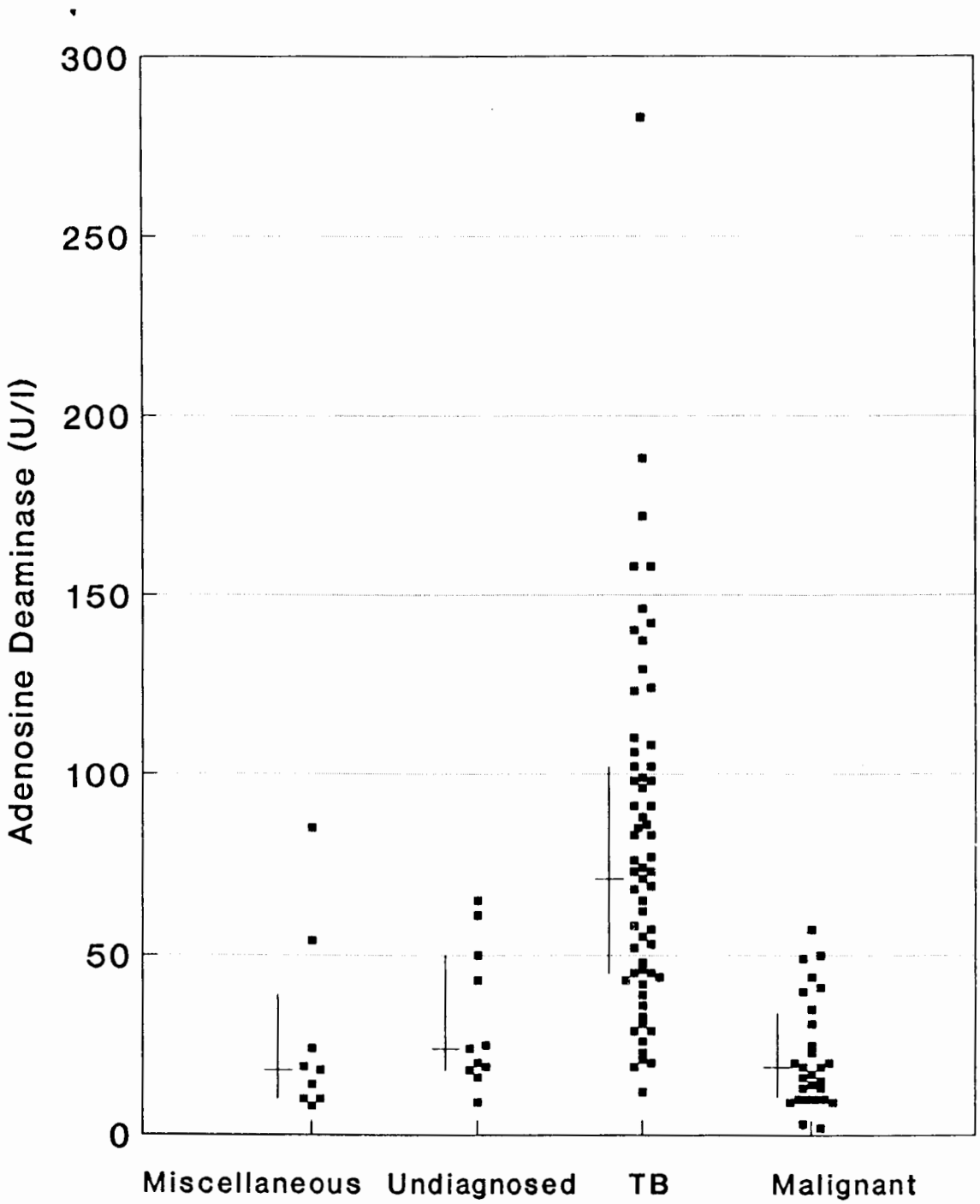
TABLE 4

ADA values (U/l) in the different diagnostic groups.

Diagnosis	Number	Median	25th-75th quartiles	Range
Malignant	28	19	10.75-34	2-57
Miscellaneous	9	18	10-39	8-85
Tuberculosis	61	71	45-102	12-283
Undiagnosed	11	24	18-50	9-65

A cut-off value of 45 U/l was used to identify tuberculous cases (33). Other authors have used 40 U/l (34) or 50 U/l (32), whilst a study of tuberculous ascites from this institution (51) showed excellent results with a cut-off of 35 U/l.

Figure 2 Adenosine Deaminase valaues in the various diagnostic group.



ADA levels <45 U/l were found in 14 tuberculous cases, giving a sensitivity of 0.77. Eight false positive cases were found (3 malignant, 3 undiagnosed, 1 parapneumonic, and 1 spontaneous pneumothorax), and the specificity was thus 0.83. Two of the patients with false positive ADA levels in the undiagnosed group had clinical diagnoses of tuberculosis. If these 2, together with the third probable case of tuberculosis, are included in the tuberculous group sensitivity remains 0.77 and specificity improves marginally to 0.87. The 3 false positive cases from the malignant group all had carcinomas.

Of note is that ADA correctly identified the single patient in the Radiotherapy group with tuberculosis, and the sensitivity and specificity of ADA in this small group was 1.

The positive and negative predictive values (with 45 U/l as a cut-off) were 0.85 and 0.74 respectively.

If 35 U/l is used as a cut-off, sensitivity improves to 0.84, but specificity drops to 0.73. As only 1 of the false positive cases had pleural ADA under 50 U/l, choosing this value as a cut-off would only further reduce sensitivity without significantly altering specificity.

3.6 Immunodiagnostic assay

The BCG antigen capture ELISA was performed on pleural fluid from 103 patients; 58 with tuberculosis, 26 with malignant

effusions, 9 with miscellaneous conditions, and 10 undiagnosed cases. The test was positive (BCG concentration of 3 ng/ml or more) in 28 cases. Thirteen tests were false positives: 5 malignant, 4 undiagnosed (2 of whom had probable tuberculosis), and 3 with miscellaneous conditions (SLE, empyema, and pulmonary infiltrate with eosinophilia). The sensitivity was 0.26 and specificity 0.71. Positive and negative predictive values were 0.54 and 0.43 respectively. If the 2 cases of probable tuberculosis are regarded as true positives then sensitivity and specificity remain virtually unchanged at 0.28 and 0.74 respectively.

3.7 DNA probe

The DNA probe after amplification with the polymerase chain reaction was only done on 45 samples of pleural fluid as there were technical problems with contamination of the primers. In 2 cases the results were recorded as equivocal with very faint bands present, and these 2 were excluded. Twenty nine tuberculous cases were done, and the test was positive in 27 (sensitivity of 0.93). The other 14 cases were made up as follows: 6 malignant, 3 miscellaneous, and 5 undiagnosed. There were 8 false positives in this group (specificity of 0.43); 5 from the malignant group and 3 undiagnosed (1 of whom had probable tuberculosis).

4. CONCLUSIONS AND DISCUSSION

4.1 Clinical features

Several of the patients with tuberculosis in the present study were incorrectly diagnosed as suffering from parapneumonic effusions due to their short histories. Acute presentation of tuberculous effusions has been well described (6). The bimodal age distribution and the longer symptom duration in older patients found in this study has also been previously noted (6).

Patients with malignant effusions were significantly older and experienced dyspnoea significantly more often, whilst patients with tuberculosis complained of chest pain and fever significantly more frequently. However none of these features are helpful in the individual case.

4.2 Malignant effusions

It is particularly in older patients, with their higher incidence of malignancy, that diagnostic difficulties arise. Some would argue that there is little point in diagnosing malignant pleural effusions as they are incurable. Sahn (52) points out that effusions in malignancy are often not due to infiltration of the pleura, and uses the term "paramalignant" for these effusions (including treatable entities such as pulmonary emboli). Good palliative and even curative treatment exists for many malignancies (e.g. lymphoma, breast and ovarian carcinoma). Even if the

neoplasm itself cannot be treated, symptomatic effusions can be drained and sclerosants instilled intrapleurally to prevent recurrences (52). Apart from the above considerations, accurate diagnosis provides important prognostic information for the patient and family, and compensation (especially with mesotheliomas) is occasionally relevant.

4.3 Conventional compared with 12B BACTEC culture

The radiometric mycobacterial culture system (BACTEC) tested in this study had the same sensitivity as conventional media, but was almost twice as fast. This is consistent with published experience (23). The apparatus to detect growth in the culture bottles is expensive, but running costs are low and the system produces significant savings in technologist time (although the need to crush clots before injection into the bottles is a disadvantage). The 23% culture positive rate obtained with either method was similar to that found in previous studies (12,13).

4.4 The value of bedside inoculation

Bedside inoculation into BACTEC 13A bottles significantly increased the yield of pleural fluid culture, and produced culture rates similar to those reported with culture of 2 or more separate aspirations (20) or with combined conventional and guinea pig cultures (2). Decontamination procedures, which could reduce the number of viable mycobacteria, were not done on the pleural aspirates in this study. Delay in

getting the sample into culture media presumably leads to loss of a few viable organisms.

Recently published work (53) on tuberculous pericarditis with bedside inoculation into Kirchner culture bottles (which should give the same yield as 13A BACTEC bottles) achieved culture rates much higher than previously reported. The author of that study has obtained good results with direct inoculation in tuberculous ascites and pleurisy (Strang JIG - personal communication).

Bedside inoculation not only gives a better yield, but also saves technologists' time. Staining for acid-fast bacilli, which has a very low yield, could be done on the sample sent for routine bacteriologic culture.

4.5 Routine bacteriologic culture

In this study bacteriologic culture was only diagnostic in the patient with empyema who had a frankly purulent aspirate. However empyemas do not always yield macroscopically purulent fluid on aspiration. Fungal or nocardial infections of the pleural space (which are rare) are usually diagnosed by culture of pleural fluid (24). Routine culture should therefore be done on all exudates, but the yield will be very low in the patient population at Groote Schuur Hospital.

4.6 Pleural biopsy

Granulomata were found in a higher percentage of initial pleural biopsies in the tuberculous group than usually reported. This is probably attributable to the stripping technique of biopsy used, which produces larger specimens. However, since this method was not directly compared with the usual method, it is possible that other factors might have played a role. For example, most of the biopsies were performed by a single experienced operator.

Pleural histology was diagnostic in all 3 patients with tuberculous ascites, 1 of whom had miliary tuberculosis. This is contrary to the experience of other workers (13,54) who found that pleural biopsy was not useful in patients with tuberculous peritonitis and pleural effusions. Pleural biopsy is certainly safer and easier to perform than laparoscopy, which is the current method of choice for diagnosing tuberculous peritonitis (22).

The yield of pleural biopsy mycobacterial culture was similar to that found in previously reported series (19,20), and was positive in 3 cases whose histology showed inadequate tissue as well as 1 case with non-specific histology.

4.7 Adenosine deaminase

ADA activity was significantly higher in tuberculous effusions than in the other diagnostic categories evaluated

in this study. The specificity of 0.83 found was similar to most studies (32,34,35,37), although Ocana et al (33) found a specificity of 0.97 with a mixed group of ascites and pleural effusions. As a recent study from this institution has shown that ADA has high specificity for tuberculous ascites (51), the inclusion of patients with ascites might have improved their specificity. The original report of the value of ADA in pleural fluid (31) included an even larger proportion of patients with ascites and reported sensitivity and specificity of 1.

Ocana et al (33) also included significant numbers of transudates in their study, which would definitely increase specificity as ADA activity is invariably low in transudates (32-34). In a separate paper the same workers reported ADA specificity of 0.97 in a subgroup with lymphocytic pleural effusions (55), but excluded undiagnosed patients and those from the malignant group with bloody effusions or positive cytology.

The reported sensitivity of ADA approaches 1 (31-5). Despite the lower specificity reported, ADA could still be useful diagnostically in that tuberculosis could be excluded if the activity were low. The disappointing finding in the current study was the relatively low sensitivity of ADA. Similar sensitivity was reported from a low prevalence area (37), but that study included only 5 tuberculous effusions. Maritz et al (34) found a sensitivity of 0.93, which included many clinically diagnosed cases of tuberculosis. As shown, lowering the cut-off value only served to decrease

specificity with marginal gains in sensitivity.

4.8 Antigen capture ELISA

The BCG-based antigen capture ELISA tested had very low sensitivity and poor specificity. Dhand et al (43) found that this assay could not distinguish tuberculous from malignant effusions, and suggested that tumor antigens may cross-react with BCG. The present study shows that this ELISA has poor specificity with other conditions as well, and thus has no diagnostic value in pleural effusions. The same ELISA has given specificities of 0.95 (56) and 0.96 (57) in cerebrospinal fluid. False positives were found with bacterial and fungal meningitis, which can be distinguished from tuberculosis by culture. Sensitivity was only 0.39 in the larger study (57), which was improved with combined antibody and antigen ELISA's.

4.9 DNA probe after amplification

DNA amplification with the polymerase chain reaction is capable of detecting minute quantities of DNA and has found extensive application in virology (47). Because of its exquisite sensitivity false positves can easily arise from contamination. In this study pleural fluid was separated into 2 ml aliquots under a hooded extractor fan in the mycobacterial culture section of the bacteriology laboratory prior to storage at $-20^{O}C$. It is possible that aerosolised molecules of DNA could have contaminated the fluid under this hood as many samples from patients infected with

Mycobaterium tuberculosis are processed there daily. If this were the case one would expect to find false positive fluid cultures, which were not found in any of the patients whose DNA probe was a false positive. Another possible source of contamination is aerosolisation into the rubber teat which is inserted onto the ends of sterile disposable pipettes in order to create suction and re-used many times. These 2 potential sources of contamination need to be eliminated before this diagnostic test can be adequately evaluated. Another potential limitation of this method lies in the nature of tuberculosis; after primary infection a few organisms remain dormant in the host and may reactivate later to cause overt infection. The polymerase chain reaction would amplify the DNA of these dormant organisms if they were present in the sample and cause a false positive. In a high prevalence area for tuberculosis such as ours one would expect many patients to harbour organisms without having overt disease.

A very recent report has appeared of the value of DNA amplification in the diagnosis of tuberculosis (58). The samples tested were of various types and came from a pre-selected group with clinical diagnoses of tuberculosis. The test was shown to have similar sensitivity to mycobacterial culture, but the sample selected was such that specificity could not be assessed.

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