The Diagnostic Value of Adenosine Deaminase Activity in the Ascitic Fluid of Patients with Tuberculous Peritonitis.

Submitted for the completion of part 3, of the Degree of Master of Medicine, University Of Cape Town.

Michael D. Voigt 1988.

The University of Cape Town has been given the right to reproduce this thesis in whole or in part. Copyright is held by the author.

The copyright of this thesis vests in the author. No quotation from it or information derived from it is to be published without full acknowledgement of the source. The thesis is to be used for private study or non-commercial research purposes only.

Published by the University of Cape Town (UCT) in terms of the non-exclusive license granted to UCT by the author.

University of Cape Town

Adenosine Deaminase in Tuberculous Ascites

Michael D. Voigt

Supervisor: Prof. R. E. Kirsch

To Paddi, Sean and Lara.

Table of Contents

Introduction and Review of the Literature. 1.

Prevalence and significance of tuberculous peritonitis. 1
Pathogenesis of tuberculous ascites. 4
The organism. 4
Mechanism of infection 4
Mechanism of ascites formation in tuberculous peritonitis. 6

The Diagnosis of Abdominal Tuberculosis 7

Characteristics of the ideal diagnostic test for tuberculous peritonitis. 7
- Specificity
- Sensitivity
- Ease of performance
- Rapidity
- Safety

Analysis of currently available diagnositic tests. 10
- Clinical
- Protein
- Lactate Dehydrogenase.(LDH)
- White Cell Count.
- Bacteriology.

Histology. 12
Blind peritoneal biopsy 12
Laparoscopy 12
- Visual appearance.
- Directed biopsy.

Mini-Laparotomy 14
Immunological tests for tuberculosis. 16
- Antibody detection.

Direct tests 16
Adenosine deaminase (ADA) 17

Materials and methods. 19

Aims 19
Study design. 19
Case finding
Criteria for inclusion into the tuberculous ascites group.
Criteria for inclusion into the control group.
Parameters studied.
Case finding
Criteria for inclusion into the tuberculosis group
Parameters studied.
Methods 20
Adenosine deaminase 21
Statistical analysis. 21

Results 23

Demograhic Data 23
Retrospective group.
Prospective group
Diagnosis 24
Retrospective limb
Prospective limb
Biochemical and ascites fluid results. 26
Retrospective group.
Prospective group
Multivariate Linear Discriminant Analysis 26
Retrospective limb
Prospective limb
Univariate Logistic Regression analysis. 27
Retrospective limb
Prospective limb

Unmatched Retrospective Controls 30
Analysis of False Positive Cases. 34
Analysis of False Negative Cases 35

Discussion 38

References 44

TUBERCULOUS ASCITES

Introduction and review of the literature

Prevalence and significance of tuberculous peritonitis.

Tuberculosis is a common problem in developing countries, including South Africa (1-4). Prevalence studies in the Transkei in 1972 and 1977 identified positive sputum cultures in 6% of citizens over the age of 15 years, and chest x-ray abnormalities consistent with active pulmonary tuberculosis in 7.5% (5-6). The incidence of extrapulmonary tuberculosis appears to be increasing (7), *(fig 1)*. When the incidence of extrapulmonary tuberculosis is expressed as a percentage of all forms of tuberculosis, the increase is even more striking *(fig.2)*. In the Republic of South Africa excluding Transkei, Bophutaswana, Venda, and Ciskei (TBVC countries), extrapulmonary tuberculosis accounted for 2.1% of cases in 1979 and 6.4% in 1986 (7). In the Western Cape, the incidence of extrapulmonary tuberculosis, is higher than in the rest of the country, excluding the TBVC countries, and also appears to be increasing (7). Thus in 1980 extra-pulmonary tuberculosis accounted for 4.9% of cases of tuberculosis. In 1986 the percentage had risen to 17.5%. From this it can be seen that extrapulmonary tuberculosis is a major problem throughout South Africa and particularly in the Western Cape *(fig 2)*. The increased incidence may be real, or may be due to better diagnosis, case reporting, or migration of tuberculous patients from the rural areas (7).

The incidence of abdominal tuberculosis in the Western Cape also appears to be higher than the rest of South Africa. *Fig.3* shows the incidence rate of abdominal tuberculosis in the Western Cape compared to the rest of the RSA (excluduing the TBVC countries), for the period 1973-1986.

At Groote Schuur Hospital, 125 patients with abdominal tuberculosis were identified, over the 10 year period 1971-1980. Peritoneal tuberculosis was the predominant form, occuring in 85% of cases (2). Approximately 80% of cases of abdominal tuberculosis present with ascites (8, 30). Indeed, in areas where TB is common, tuberculosis is one of the commonest causes of ascites. Thus tuberculous ascites accounted for 42% of all cases of ascites evaluated prospectively at a rural hospital in Lesotho (9). In a study from Nigeria (10), TB was the second most common cause of ascites, accounting for 23.2% of cases. TB is also an important cause of ascites in other developing countries. In Argentina, 8400 patients with ascites were evaluated by laparoscopy. TB was the third most common cause of ascites in this series (56). On the Indian sub-continent, the disease is also extremely prevalent (35, 44).

Western communities are not exempt from tuberculosis. In the USA abdominal tuberculosis accounted for 3.9% of the extrapulmonary forms of tuberculosis , between 1969 and 1973 (11). In England, the majority of cases of abdominal tuberculosis occur in immigrants from areas where tuberculosis is endemic (12). Two factors are important in

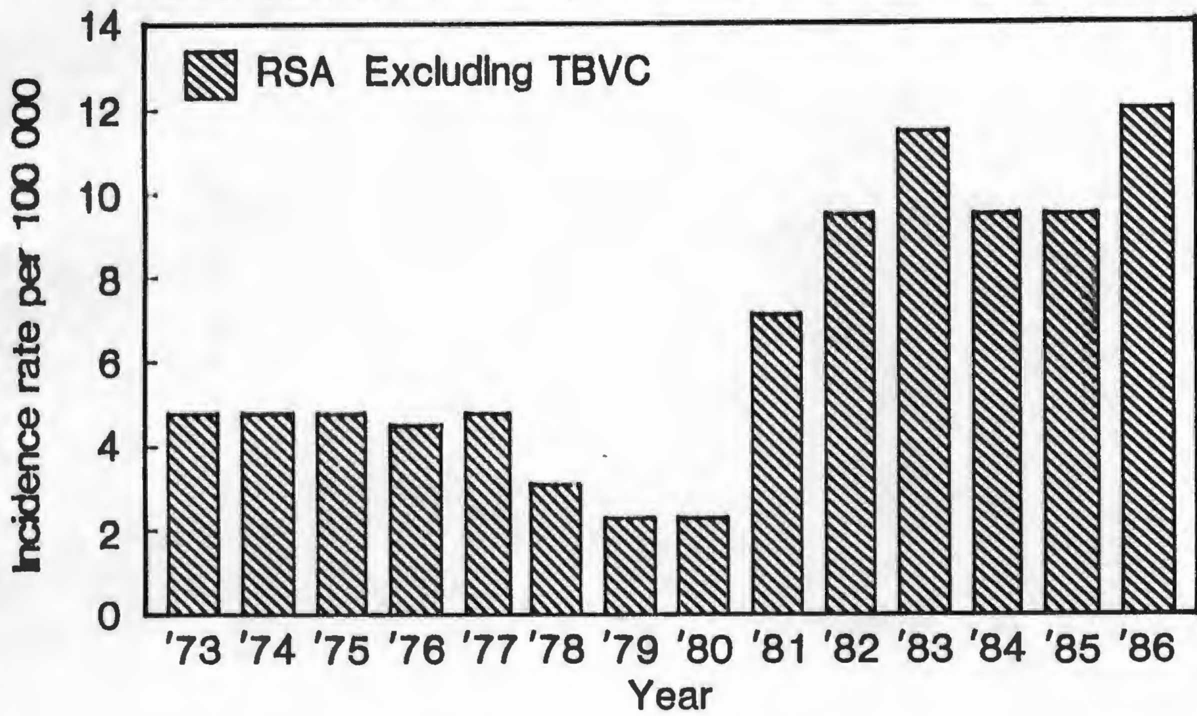


Fig 1.

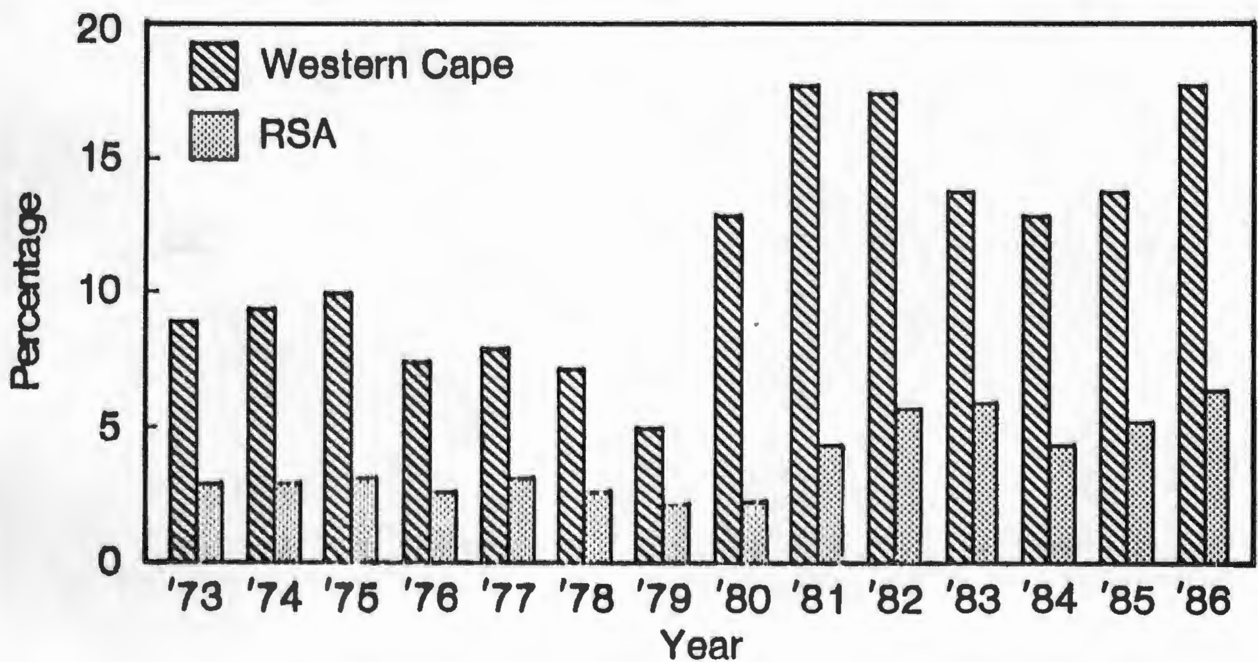
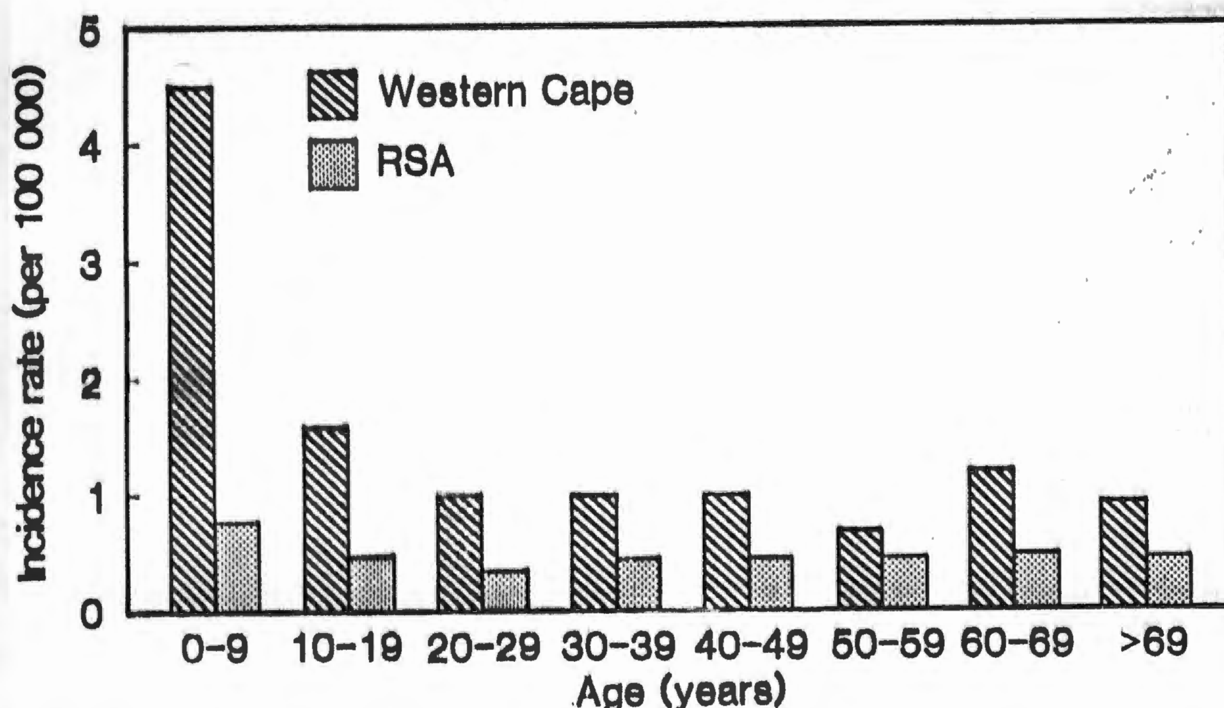


Fig 2.

Fig. 3.



the increasing prevelance of tuberculosis in western communities: the appearance of the human immunodeficiency virus (HIV) (13-16) and the aging population (17-18). There is also an increasing population of homeless people in many first world cities, many of whom are alcoholic, malnourished or stressed (19). Such persons are particularly at risk of developing tuberculosis.

Patients with aquired immunodeficiency syndrome are predisposed to infection with tuberculosis (16,20). Thus tuberculosis is common in areas, such as Haiti and Central Africa, where TB and HIV infection are endemic (16,20-21). The interaction of tuberculosis and HIV infection may become of great clinical significance as the HIV infection becomes more common in South Africa. Approximately three quarters of all tuberculous infections in patients with aquired immunodeficiency syndrome are extrapulmonary (13,16).

The elderly are at risk because of their living in closed communities such as old age homes and possibly because of carrying residual bacilli. Infection in the elderly tends to be extrapulmonary (22). Elderly alcoholics are particularly at risk (19,22). Health care workers also have a high risk of infection (23).

Tuberculosis is under-diagnosed, especially in low prevalence areas. Mortality is high when the diagnosis is missed. In Denmark, 20 patients out of 243 cases of tuberculosis died, without the diagnosis being considered (24).

Pathogenesis of tuberculous ascites.

The organism.

Tuberculous peritonitis in South Africa is, with rare exceptions, caused by *Mycobacterium tuberculosis*. In the past *M.bovis* acounted for many cases. A review of the University of Cape Town data confirms the fact that *M.bovis* is now rarely found. In 90 consecutive cases of culture positive abdominal tuberculosis, there was not a single case of *M.bovis*. In 2223 consecutive positive cultures, from all sites, only one case of *M.bovis* was found (Personal observation). This was in a patient who had been vaccinated in the standard way with BCG and had subsequently developed a mycobacterial granuloma on his finger. This was probably due to a secondary innoculation of the finger from the site of the original BCG injection.

Mechanism of infection

Tuberculosis is usually spread by uninfected persons inhaling infected droplets. The lungs are the most common portal of entry of the bacilli (25). With the eradication of *M.bovis* from cattle and the pasteurization of milk, ingestion of this bacillus, as a portal of entry of infection, has been almost completely eliminated. During the primary pulmonary infection, which is commonly asymptomatic, bacilli disseminate via lymphatics or haematogenously and establish metastatic foci of bacilli throughout the body. Following the primary infection, specific host immunity eradicates actively dividing bacilli, but dormant bacilli remain, especially in areas of high oxygen tension. These bacilli may reactivate weeks to decades after the initial infection when host control factors break down (25).

There is speculation whether secondary tuberculosis results from exogenous reinfection, or reactivation of endogenous latent bacilli aquired during a previous infection. In pulmonary tuberculosis, current evidence suggests that reinfection may be as important as reactivation in causing secondary disease, particularly in the elderly (26).

In tuberculous ascites the bacilli may gain entry to the peritoneum via abdominal lymphatics, through the bowel wall, from adjacent organs such as the genito-urinary tract, or via the blood. Nice (27) first postulated that tuberculous peritonitis was due to reactivation of latent mycobacteria in the peritoneum. The weight of current evidence supports this postulate (28,30,34-35). This will be discussed below.

Earlier workers suggested that gastrointestinal tuberculosis is caused by the invasion and local spread of ingested organisms through the abdominal wall. Several lines of evidence argue against this postulate. Firstly, *M.tuberculosis* is not commonly present in food or drink. Indeed, the only time bacilli are likely to be ingested, is in persons with open pulmonary tuberculosis, who swallow their sputum. Parenchymal pulmonary tuberculosis is found in the minority of patients with tuberculous ascites. (table 1) In several studies, patients have been classified as having pulmonary involvement on the basis of x-ray evidence of previous tuberculosis, pleural effusions, hilar adenopathy, miliary tuberculosis and pericardial effusions. In many studies, no distinction is made between parenchymal involvement and pleural or other involvement. Table 1 gives the prevalence of parenchymal involvement in cases of tuberculous peritonitis. Patients with miliary, pleural, and hilar lymph node involvement are not included in this analysis, because these are not potential sources of mycobacterial ingestion. Studies that did not distinguish parenchymal from other pulmonary involvement are not included.

In a prospective study, 50 cases with severe open cavitating pulmonary tuberculosis had in-

Table 1:
Parenchymal pulmonary involvement in tuberculous ascites

Study	Year	Cases	Active Parenchymal Disease	%
Singh(35)	1969	47	3	6
Bhansali(44)	1977	300	32*	10.6
Vyravanathan(48)	1980	18	3	16
Gilinsky(2)	1983	71	29* (16 pleural effusions)	40
Sochocky(62)	1966	100	44	44

Table 1: Studies reporting the incidence of parenchymal pulmonary tuberculosis are given.
The number of cases examined for pulmonary disease, and the the number and percentage of cases that had parenchymal involvement are given.
* Pleural involvement included.
Reference given in brackets

tensive investigation of their gastrointestinal tract. Colonoscopy with multiple colonoscopic biopsies, upper GI endoscopy and small bowel enemas and double contrast large bowel barium enemas were done. No case of symptomatic abdominal tuberculosis was found. One patient had macroscopic evidence of bowel disease, at colonoscopy, but neither AFB or caseating granulomas were found. In another patient, caseating granulomas were found in a mucosal biopsy, but AFB were negative. Acid fast bacilli were found in a further 12 patients, but none of them had clinical evidence of gastrointestinal involvement, macroscopic changes at colonoscopy, or caseating granulomas on histology. Thus, only insignificant bowel involvement was found, in 14 of the 50 cases despite a rigorous search (28). These findings suggest that ingestion of bacilli, even when the load of organisms is heavy, seldom leads to significant gastrointestinal disease by local spread.

A second line of evidence against the postulate that gastrointestinal and peritoneal tuberculosis is brought about by proliferation and invasion of ingested bacilli, comes from Britain. Patients presenting with tuberculous ascites are usually immigrants from areas with high prevalence rates for tuberculosis (12,29-33,112). The incidence of all forms of tuberculosis is maximal 2 to 5 years after entry into Britain and remains higher than the native population even at 20 years (120). This has also been shown for abdominal tubercu-

losis (12,112). If the tuberculosis was exogenously aquired, the rate of infection should be similar in the immigrant and indiginous British populations. However the prevalence of extra-pulmonary tuberculosis is 80 times greater in ethnic minorities in Britain, compared to the indigenous population (30). This evidence, although circumstantial, suggests that the high rate of tuberculosis in this group may be due to reactivation of dormant bacilli originally aquired in their home countries. Differences in socio-economic conditions may have contributed to this difference.

Phage and biochemical typing of *M.tuberculosis* has shown conclusively that tuberculous infections in Asian and Ugandan immigrants is caused by reactivation of bacilli aquired from their countries of origin (34).

If the peritoneal infection is not caused by ingestion of the organism, it is possible that it arises in organs adjacent to the peritoneum. To study this, 47 patients with tuberculous peritonitis, were extensively investigated, looking for involvement of adjacent abdominal organs. Large and small bowel barium studies, intravenous pyelography, and salpingograms (where appropriate) were done on all patients. Not a single case of tuberculosis of an adjacent organ was found. The authors concluded that tuberculous ascites is not caused by spread from adjacent organs (35). Thus,although mycobacteria may spread to the peritoneum from adjacent organs (118), this is only a rare cause of tuberculous peritonitis.

Mechanism of ascites formation in tuberculous peritonitis.

The exact mechanism of ascites formation in tuberculosis is unknown. In general terms, ascites may form as a result of perturbations of the Starling forces in hepatic sinusoids, omental and peritoneal capillaries, or as a result of blocked abdominal lymphatic drainage as in other diseases associated with exudative ascites(36-39).

Ascites does not form in all cases of tuberculous peritonitis, approximately 15% being fibrinous or "plastic" (2). It is unknown why some patients develop ascites and others do not.

Tuberculous peritonitis usually causes raised ascites protein, (2-3,8-10,12,30-33,35,44-45,48,51,62), suggesting an exudative process. The peritoneum and omentum are inflamed, thickened, hyperemic and studded with tubercles (40-41). Numerous inflammatory mediators are produced during the cell mediated immune response to the bacilli. These mediators include products of the complement, clotting, fibrinolytic and kinin systems, lymphocyte, macrophage and neutrophil products, and phospholipid mediators such as acetyl glycerol ether phosphorylcholine, leukotrienes, and prostaglandins. These mediators cause vascular dilation, alter permeability and may lead to exudation of fluid (42-43).

Abdominal lymphatic obstruction may contribute to fluid exudation. Abdominal lymphadenopathy is a frequent finding in gastrointestinal tuberculosis (44).

Raised portal pressures due to liver disease may contribute to ascites formation in some patients with tuberculous peritonitis. Alcoholism is common in patients with tuberculous peritonitis (9,45,107,112). Portal hypertension is an early feature of alcoholic liver disease (46).

The Diagnosis of Abdominal Tuberculosis

The definitive diagnosis of tuberculosis requires the demonstration of *M.tuberculosis* in the patients tissue or secretions, either by microscopy or culture. Definitive diagnosis is extremely important, as it allows the exclusion of other, potentially treatable diseases, that may have similar clinical presentations. Culture of the organism is also important as the type of mycobacterium and its drug sensitivity may be tested. This is particularly important where atypical mycobacteria are prevalent, eg *M avium-intracellulare* in AIDS patients in the USA. The major disadvantages of bacteriological methods are that microscopy is insensitive, and cultures may take weeks to months, before a result is obtained. The general approach is to make a presumptive diagnosis on the basis of clinical biochemical or histological data, and to change therapy as culture results become available.

The ideal diagnostic test for tuberculous peritonitis will be defined below, and currently available diagnostic methods will be evaluated according to this framework.

Characteristics of the ideal diagnostic test for tuberculous peritonitis.

The ideal diagnostic test for tuberculous peritonitis should have the following characteristics:

Specificity

The test must first and foremost be highly specific. The relative importance of this will vary with the prevalence of the disease in the community. In communities with a low prevalence of tuberculosis, the specificity should approach 100%, otherwise the positive predictive value, in population studies, is extremely small. (If tuberculosis is present in 1 out of 100 000 cases of ascites, a 99% specific test will yield 1000 false positive tests for every true positive.) However, in areas where tuberculosis is common, eg. Lesotho, where up to 42% of all cases of ascites have been shown to be due to tuberculous peritonitis (9), a 99% specific test will yield approximately 84 true positive cases for every false positive result.

When the patient's socio-economic background, clinical history and examination are taken into account, the patient with tuberculosis will generally be stongly suspected of having TB, before any diagnostic tests are done. In other words, he will be assigned a high pretest probability of between 60%-90%, of having tuberculosis . A 99% specific test will yield only 1 false positive result for every 300-999 true positive tests. A test that is 90% specific, will yield 1 false positive in every 15 true positive tests, with a pre-test probability of 60% and 1 false positive in every 100 tests, with a pretest probability of 90%.

The relationship between the pre-test probability of the disease and the accuracy of positive and negative predictions is demonstrated in fig. 3. The influence of test specificity is shown. The X-axis represents the pre-test probability of disease. The Y-axis represents the accuracy of positive or negative predictions. Curves are shown for test specificities of 85%, 90%, and 99%. Although test specificity has a large influence on the accuracy of a positive prediction when the pre-test probability of the disease is low, it has only a small influence on the accuracy of a positive prediction when the pre-test probability is high. The specificity of a test has little influence on the accuracy of a negative prediction.

The danger of a false positive test is that the attending physician will stop his diagnostic workup and treat inappropriately.

Pos. and neg. predictive values
effects of specificity and prevalence

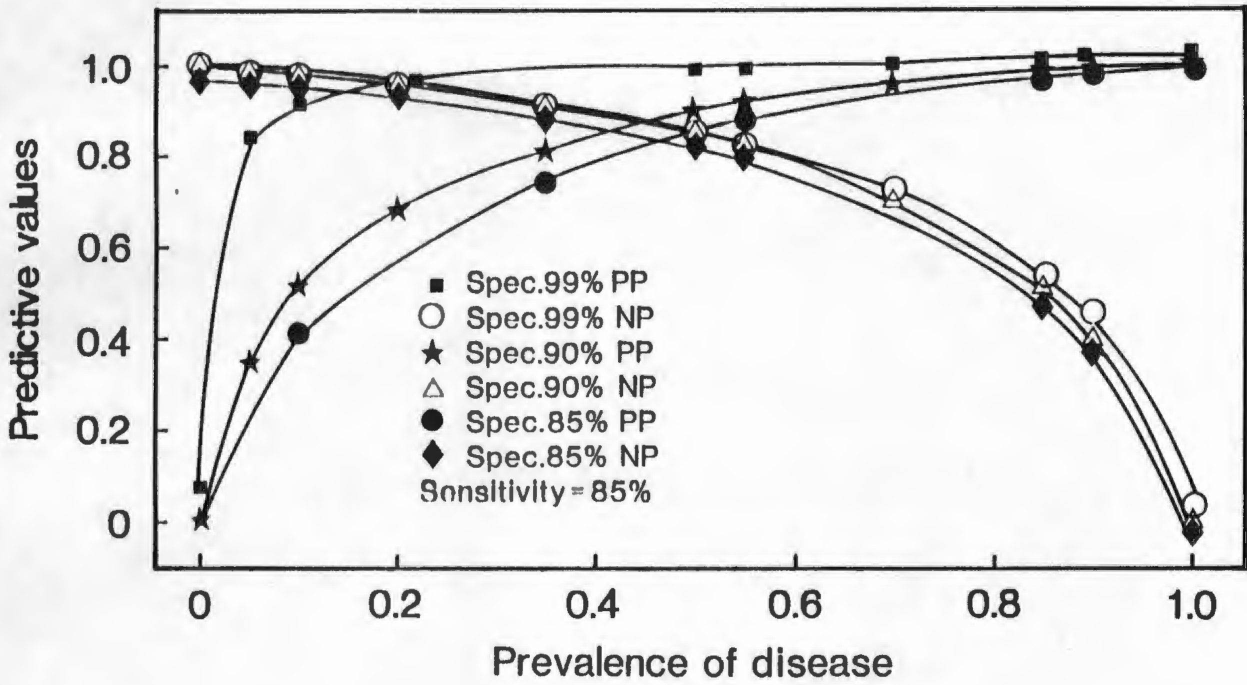


Fig 4.

Pos. and neg. predictive values
effects of sensitivity and prevalence

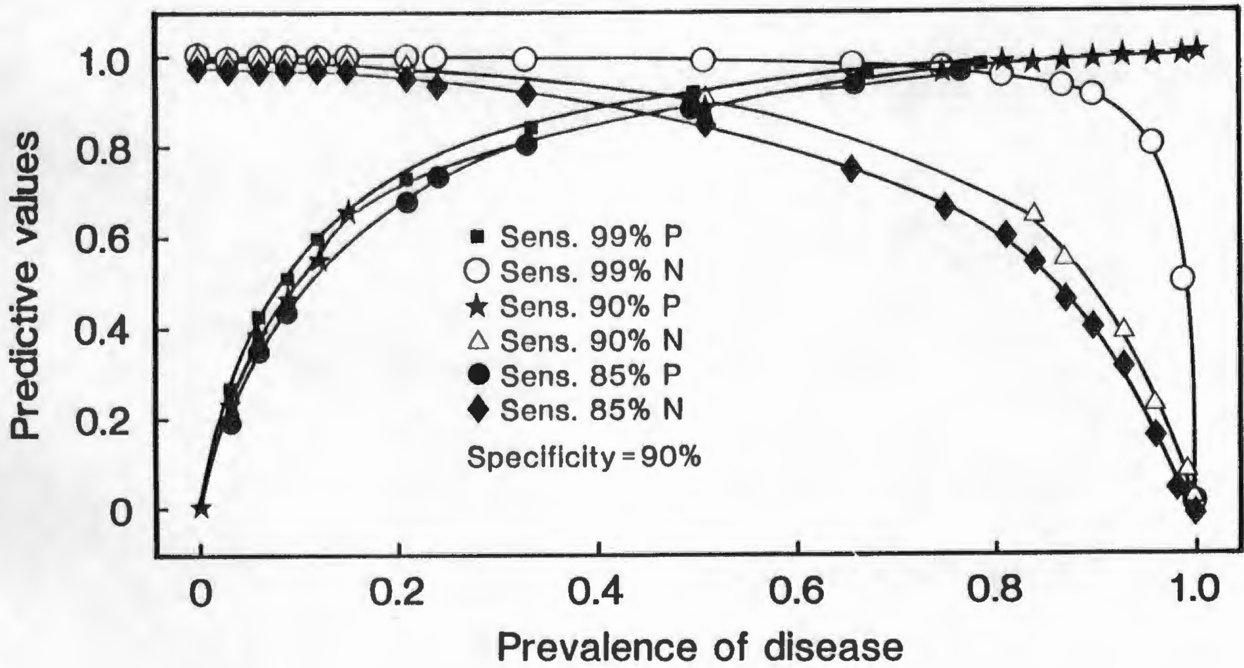


Fig 5.

Sensitivity

The relative importance of sensitivity also depends on the prevalence of the disease in the population being studied. The accuracy of a positive prediction is hardly affected over all ranges of prevalence, by changes in the sensitivity of a test. However, the negative predictive value differs greatly, as the prevalence (or pre-test probability) of the disease increases. This becomes important in the clinical setting, where a patient from a high prevalence area, with signs and symptoms suggestive of tuberculosis, may have a pre-test probability of the disease approaching 80%. An insensitive test then has low negative predictive value. Although a false negative test may lull the attending physician into a false sense of security and delay the institution of treatment, it is less of a problem than a false positive test, because the diagnostic process will go on. Fig. 4 shows the influence of test sensitivity on the accuracy of positive and negative predictions over a range of pre-test probabilities, with a constant test specificity of 90%.

Ease of performance

Tuberculosis is predominantly a disease of rural communities in third world countries. Sophisticated laboratory facilities are not available in these areas. Therefore for a test to have any impact on the disease, it must be simple, inexpensive and it should not require the use of intricate equipment, techniques, or radioactiveisotopes.

Rapidity

A rapid diagnostic test is clearly important, so that the time taken to institute treatment is reduced to a minimum. The long delay associated with mycobacterial culture diminishes the value of this test in the the initial diagnostic workup of tuberculosis.

Safety

The ideal test should have no morbidity and no mortality. Tuberculous peritonitis is a treatable disease with nearly 100% survival if appropriate treatment is given in time. Diagnostic procedures that may cause the patients death are clearly not acceptable. Patients with tuberculous peritonitis are usually debilitated and malnourished, hence are not good candidates for major invasive procedures such as laparotomy. Both surgery and laparoscopy in these patients are associated with significant morbidity and mortality. This will be in more detail below.

Analysis of currently available diagnositic tests.

Clinical

The symptoms and signs of tuberculous ascites are similar to the symptoms and signs of ascites caused by other diseases. Patients presenting with tuberculous ascites often have underlying alcoholic liver disease, malignancy or other causes of ascites, which further confounds the clinical diagnosis of tuberculosis. Fever is absent at presentation in up to 42% of cases (2). The classical finding of a "doughy" abdomen is rarely seen. The mantoux skin test is often negative and evidence of tuberculosis in the chest or elsewhere is absent in more than 50% of cases (2), (Table 1).

Tuberculin skin testing is of little value in abdominal tuberculosis, in areas of high prevalence, for 2 reasons:

a). The high exposure rate to tuberculosis in the general community, means that most people will have a positive skin test, irrespective of whether active tuberculosis is present or not. (However, the degree of reaction may vary, and very strongly positive skin tests suggest that active tuberculosis may be present.)

b). With severe infection, as in abdominal tuberculosis, patients are frequently anergic (47). This is associated with increased numbers of suppressor T-cells (42). Repeat skin testing following treatment, may become positive, after an initial test is negative (45). 23

patients with tuberculous ascites who had negative skin tests were retested after treatment. 8 of these non-reactors developed a positive skin test after treatment. One possible explanation for the skin test being negative initially, is that the patients had only recently come into contact with tuberculosis and had not had sufficient time to mount an immune response. This is unlikely because tuberculous peritonitis usually results from reactivation of latent foci(qv) which have been present for a long time. Secondly, the onset of disease is insidious in the majority of cases, and patients usually have symptoms for 1-3 months before presentation (2).

Skin testing was positive in all the cases reported by Singh and co-workers (35). The patients were from New Delhi, an area of high prevalence. The degree of positivity was not recorded. Most other authors report lower percentages of skin test positivity, in abdominal tuberculosis. Only 55% of the skin tests were positive in the series reported from Groote Schuur Hospital (2), while Sarin (68) reported 74% positivity in 50 cases, and Vyravanathan (48) 78.5% positives in 35 cases with abdominal tuberculosis. In Karney's series (45), only 8 (30%) of 27 patients tested had positive skin tests with intermediate strength purified protein derivative (PPD). Of the 19 patients (70%) with negative skin tests, 4 patients had positive skin tests prior to developing tuberculous peritonitis. After developing tuberculous peritonitis, their skin tests had become negative. This indicates that the tuberculous peritonitis may suppress the host immune response, or that tuberculosis had developed as a result of suppressed immunity. Palmer (30) and co-workers found skin testing to be negative in 11%, weakly to moderately positive in 52% and strongly positive in 37% of 90 patients. Thus skin testing is an extremely insensitive and non-specific test.

In summary, skin testing is both insensitive and nonspecific, and is of little value in the diagnostic workup of a patient suspected of having abdominal tuberculosis.

Ascitic fluid analysis.

Protein

Ascites protein concentrations greater than 25-30 g/l have been related to exudative processes such as tuberculous peritonitis, peritoneal carcinomatosis etc. However, this separation of transudate from exudate is unreliable in practice. Transudates form from increased hydrostatic pressure, or loss of oncotic pressure in the capillary bed. When the increased hydrostatic pressure acts across the hepatic sinusoids, which have large fenestrae and are extremely porous, high protein ascites may result. Thus high protein ascites usually occurs in heart failure, constrictive pericarditis (49-50), and conditions associated with hepatic venous outflow obstruction, eg. hepatic vein thrombosis and inferior vena cava obstruction. Ascitic protein concentration is high in 12%-17% of patients with uncomplicated cirrhosis and may be as high as 43 g/l (51-52). Clearly the protein concentration does not reliably distinguish a transudate from an exudate, under these circumstances.

On the other hand, ascitic protein concentration may be low in up to 75% of cases of tuberculous peritonitis (2,68). Of the 60 patients with tuberculous ascites, reported by Gilinsky, 40 had ascitic fluid protein concentrations below 30 G/l. The low protein concentration may relate to underlying liver disease, malnutrition, or a dilutional effect in patients with pre-existing ascites. In a series reported by Karney (45), 62% of alcoholics with tuberculous peritonitis had ascites fluid protein concentrations below 30g/l. The mean ascites fluid protein concentration was also lower in the group of alcoholics compared to non-alcoholic controls (45).

The ascitic fluid protein concentration may be high in many conditions other than tuberculous peritonitis. Conversely, the concentration of protein is also low in many cases of tuberculous peritonitis. Thus the test is both insensitive and non-specific in detecting tuberculous ascites.

Lactate Dehydrogenase.(LDH)

An ascitic fluid LDH below 400 Sigma Units (SU) and an ascitic fluid to plasma ratio of LDH 0.6 are the criteria suggested to help distinguish the ascites of uncomplicated cirrhosis from ascites due to inflammatory conditions (119). In a prospective study of of 62 patients with ascites due to uncomplicated cirrhosis, LDH was <400 SU in all cases, although the ascites to serum LDH ratio was greater than 0.6 in 8% (51). However, LDH is not consistently raised in tuberculous ascites (119). Thus a low LDH does not exclude tuberculosis. Similarly, high LDH values are common in malignant ascites (51). LDH is both insensitive and nonspecific.

White Cell Count.

The white cell count is usually 500/mm^3 in tuberculosis, with a predominance of lymphocytes or monocytes. No study has systematically looked at this parameter in large numbers of patients with tuberculosis, making assessment of its diagnostic value impossible.

Bacteriology.

Although the identification of the organism forms the gold standard for the diagnosis of tuberculosis, there are several problems associated with bacteriology in tuberculous ascites.

1. Direct examination
Direct examination of fluid is extremely insensitive. Acid fast bacilli (AFB) are seldom demonstrated in ascitic fluid even when large volumes are spun down. Singh and co-workers spun down 1 litre of ascitic fluid and searched for a minimum of 15 minutes per case, in 47 patients. Despite this diligent search, acid fast bacilli were found in only 1 case (35). At Groote Schuur, AFB were found in the spun deposit of only 3 out of 60 (5%) cases with tuberculous ascites (2).

2. Culture
Three major problems are associated with ascitic fluid culture:

i) The prolonged period before culture results become avaialable. Culture of fluid takes up to 6 weeks. Patients with active tuberculosis clearly cannot wait that long before they receive treatment. The long delay means that culture results are only useful for confirmatory purposes, identification of the type of mycobacterium, and for determining drug sensitivities, but not in the primary diagnosticprocess.

ii) Ascitic fluid culture is an insensitive test for tuberculous peritonitis. At Groote Schuur Hospital, samples from 60 patients with tuberculous ascites were cultured. Only 4 were positive (6.6%) (2). Singh and coworkers(35) cultured 1 litre of fluid, and had positive cultures in 39 of 47 (83%) cases. Other series have culture results which vary between no positives (68), 50% (<200 ml cultured) (45) and 69% (70). The rate of positive culture appears to correlate with the volume of fluid used.

iii) Expense: Culturing mycobacteria is both expensive and requires reasonable access to laboratory equipment, which is often a problem in the rural areas of developing countries (9).

Histology.

The host response to tuberculosis is characterised by the formation of epitheloid granulomas, with caseation necrosis. Although caseating granulomas are the hallmark, histologically, of tuberculosis, they are also found in other conditions. Apart from *M. tuberculosis*, atypical mycobacteria and some yeasts (eg coccidia) may cause caseating granulomas (54, 119). *M. avium-intracellulare* is a relatively common pathogen in patients with AIDS (121). Rarely disseminated infection caused by "cultivable but unidentified" mycobacteria (53) occurs in immune-suppressed individuals. In South Africa, however, the abdominal organs are rarely involved in infections caused by these organisms.

Sarcoid granulomas may occasionally contain small areas of central coagulative or even apparent caseation necrosis. However, reticulin remains intact, which distinguishes it from true caseation necrosis (54). Thus, the finding of epitheloid granulomas with caseation necrosis is not entirely specific for tuberculosis. In practice, however, other causes of caseating granulomas in abdominal organs are so rare that they are of no practical significance.

Acid fast bacilli are difficult to identify in granulomas. If present, they strongly suggest the diagnosis of tuberculosis, but rarely, atypical mycobacterial infection may give an identical histological picture.

The methods most commonly used to obtain biopsies of abdominal tissue in patients with suspected tuberculous peritonitis are:

blind percutaneous peritoneal biopsy
laparoscopy
mini-laparotomy.

Blind peritoneal biopsy.

This technique has been used since 1959 in the diagnosis of patients with undiagnosed ascites and suspected abdominal tuberculosis (65) A blunt needle, such as the Cope or Abrams needle is inserted into the peritoneal space under local anaesthetic. Multiple biopsies are taken (66-69).

The advantage of this procedure is that it is simple, readily available, and easy to perform. However, two main problems are associated with its use. Firstly, most studies have shown it to be very insensitive in the diagnosis of tuberculous peritonitis. Secondly, it has been associated with significant morbidity and mortality. Facilities for histological analysis are not always immediately available in the rural setting.

Levine (66), using a Cope needle, with 4 biopsies from the left lower quadant, claimed that blind biopsy to be 100% sensitive, in 20 patients. He neither specified his diagnostic criteria for tuberculous peritonitis, nor did he specify how patients with negative biopsies had tuberculosis excluded. Therefore, the sensitivity and specificity of blind biopsy in this study were not given. The study is uninterpretable without this data . Although there were no complications in the original series, he subsequently reported on a patient who died from shock, following massive haemoperitoneum, after a biopsy. This gives a mortality rate of 1.6% (67)

Singh (35) found positive histology in only 30 out of 47 (64%) blind biopsies, while Sarin and associates found blind biopsy to be 36% sensitive (68), and Jain and co-workers 23.8% sensitive (69).

Sherman performed Cope needle biopsy in 5 patients with tuberculous peritonitis. Perforations occured in 2 of these patients, one of whom died (70).

From the above it is clear that blind biopsy is both insensitive and dangerous. The slight gain from ease and convenience does not compensate for the insensitivity and excessive morbidity and mortality, and hence this procedure should not be used in patients with tuberculous peritonitis.

Laparoscopy

Laparoscopy or peritoneoscopy, to visualize abdominal organs, was first described in 1902 (55). Although seldom used until the late 1950's, it is currently extensively used in the diagnosis of intra-abdominal pathology. Several recent reports give data on the use of this technique in tuberculous ascites and in undiagnosed ascites.

The procedure is usually performed under local anaesthesia with sedation, after correcting coagulation defects. Some operators prefer general anaesthesia. The laparoscope is inserted through a small incision after insufflation of the abdomen with CO_2 or N_2O via a Verres needle inserted into the left iliac fossa. Biopsies of omentum, peritoneal structures and liver are taken. The procedure is used in many rural hospitals. Samples for histology and culture usually have to be sent to a central laboratory for processing, how-

ever, which may be associated with significant delay in obtaining results.

Laparoscopy may assist in the diagnosis of tuberculosis in 2 ways:

i). The visual appearance of abdominal structures at laparoscopy is characteristic (see below) and

ii). Biopsy may be performed through the laparoscope. The yield from the biopsy may be increased by selecting suspicious looking areas for biopsy, during laparoscopy. Both the visual appearance and directed biopsy contribute to establishing the diagnosis of tuberculosis in every case. The two aspects are considered separately below, for the sake of convenience.

Visual appearance.

The characteristic features of tuberculous peritonitis include the finding of scattered or confluent nodules of uniform size, over the visceral or parietal peritoneum, filmy adhesions between bowel loops, liver capsule, and abdominal wall (56), and hyperemic peritoneum and omentum (9). Turbid ascites , retraction of the omentum, and "fibrin" streaks on the bowel may or may not be present. Adhesions are sometimes absent, and nodules may vary in size, in tuberculous peritonitis (57). Occasionally carcinomatous seeding of the peritoneum, talc granulomas and nonspecific peritonitis may have a similar appearance to tuberculosis (56).

In a recent report from Lesotho, Menzies and co-workers (9) prospectively evaluated the diagnostic accuracy of visual appearance alone, at laparoscopy, in 92 patients with undiagnosed ascites. 37 of these patients had tuberculosis (42%). 35 were diagnosed on the basis of histology or positive culture and 2 patients were presumed to have tuberculosis on the basis of clinical response to therapy. The surgeon correctly diagnosed tuberculosis in 34 of the 37 patients, on the basis of the typical appearance of the abdominal organs at laparoscopy, giving a sensitivity of 92%. Visual appearance at laparoscopy was 94% specific in their hands. Clearly histology and culture of fluid obtained during this procedure, added to its diagnostic accuracy. The mean delay, before laparoscopy was performed, was 8.7 days (range 2-25 days)

6 patients (6.5%) developed prolonged ascitic fluid leak after laparoscopy. Of these, 3 developed bacterial peritonitis, and 2 died (2.2% mortality). Of note is that very ill patients were excluded from the study,viz. those with cardiac, renal disease, jaundice or coagulopathy. Thus there was a moderately high mortality and morbidity in a selected group of patients.

Directed biopsy.

The laparoscope facilitates targeted biopsy and increases diagnostic yield.

Geake and co-workers, from Durban, reported their experience with laparoscopy in 74 patients with tuberculous ascites (56). They do not give sufficient information to allow assessment of the sensitivity or specificity of visual appearance at laparoscopy in tuberculous peritonitis. However, of the 74 cases of TB peritonitis, 56 had a firm diagnosis made on histology of tissue obtained at laparoscopy. Thus targeted biopsy was only 76% sensitive. Of the 56 patients diagnosed on the basis of positive histology, the appearance of the abdominal structures was sufficiently characteristic of tuberculosis, to allow a confident diagnosis, in 42. (sensitivity of 75%) The analysis of false positives was not given, therefore the specificity of the procedure in diagnosing tuberculosis could not be assessed.

Although Jorge (57), in his report on 42 cases of peritoneal tuberculosis diagnosed at laparoscopy, claims that all tuberculous patients had a positive peritoneal biopsy, this data may be misleading. He does not specify what additional tests were done to exclude tuberculosis in patients who did not have a positive biopsy. It may be that only those that had the positive biopsy were identified, whereas those with tuberculosis but a negative biopsy would have been missed. Diagnostic laparoscopy with targeted peritoneal biopsy was 100% sensitive in 8 patients reported by Wolfe and co-workers (58). There was no

morbidity in these patients who had laparoscopy done under general anaesthesia.

Nafeh et al (59) performed laparoscopy in 59 patients with undiagnosed ascites. They did not specify their diagnostic criteria for tuberculosis, thus making it impossible to assess the diagnostic accuracy of laparoscopy in this series. However, the procedure modified the presumptive clinical diagnosis in 14 of the 59 cases. Laparoscopy yielded important additional information about unsuspected liver cirrhosis in 6 patients.

The important complications of laparoscopy include

i). persistent ascitic fluid leak (9, 57) which may go on to bacterial peritonitis,

ii). perforation of bowel or blood vessel,

iii). air embolism,

iv). haemorrhage from the liver biopsy site.

The indications for laparoscopy vary from institution to institution, hence the underlying degree of illness of the patients varies. Complication rates are therefore not comparable at all institutions and results from large European institutions performing hundreds of laparoscopies on relatively well patients should not be extrapolated to the local situation.

In Cape Town laparoscopy is generally reserved for patients with ascites that remains undiagnosed after initial clinical, biochemical and bacteriological workup. Patients with tuberculosis are at increased risk of death, haemorrhage, bowel perforation, ascitic fluid leak and sepsis from laparoscopy for 3 reasons:

i). They are often chronically ill and malnourished.

ii). Adhesions are common and increase the risk of perforation of the bowel and

iii). coagulopathy is not uncommon in this group of patients.

While the morbidity and mortality of laparoscopy in tuberculous patients was low in 2 small retrospective studies (58-59), the study by Menzies (9), with 6.5% morbidity and 2.2% mortality from the procedure, alerts one to the potential hazards of this procedure in ill patients. The complication rate from peritoneoscopy may have been under-rated in previous retrospective studies. A large prospective study of the complications of peritoneoscopy has shown that the complication rate is far higher than that which previous retrospective studies had shown (60).

Bowel perforation is one of the major complications of laparoscopy (60). Tuberculous patients are at increased risk because of the presence of adhesions. 1 of the 10 bowel perforations which occured in patients with tuberculosis of the bowel, at Groote Schuur Hospital over 13 years, was due to laparoscopy (61).

In summary, laparoscopy is an important procedure in the diagnostic workup of patients suspected of having tuberculous ascites. It allows rapid diagnosis, is 75%-90% sensitive and specific, and is available at both large and small hospitals. The initial information is obtained at the time of surgery from the visual appearance of the abdominal structures. This information can be obtained without the use of skilled laboratory workers and sophisticated laboratory equipment. These laboratory requirements are necessary, however, for the full work-up of the histology and culture.

Even when special care is taken, mortality and morbidity from laparoscopy is relatively high (particularly in rural areas).

Laparoscopic biopsies are sometimes inadequate. The sensitivity of laparoscopic biopsy is surprisingly low (qv).

An accurate screening test would thus be a very useful to help select the patients most likely to benefit from laparoscopy. This would reduce unneccessary laparoscopies in ill patients, and reduce the morbidity and mortality associated with the procedure.

Mini-laparotomy.

This is the most effective method of obtaining histologic proof of tuberculous involvement of the peritoneum. Singh (35) found blind peritoneal biopsy to be 64% sensitive. The addition of laparoscopy increased sensitivity to

85%, but the remaining 15% of patients were diagnosed only after laparotomy. In this series, however, the diagnosis of tuberculosis depended on the demonstration of caseating granulomas, thus laparotomy served as the "gold standard" for diagnosing tuberculosis. Similarly Sochocky resorted to laparotomy in 65% of his cases to make the diagnosis of tuberculous peritonitis (62). It is unclear whether other less invasive methods of diagnosis were first tried, before resorting to laparotomy.

Clearly, laparotomy is significantly more invasive than laparoscopy or blind peritoneal biopsy, and usually requires general anaesthesia. There is a resultant increase in morbidity and mortality. Diagnostic laparotomy resulted in 10% mortality and had a complication rate of 22% in a recent report of 86 patients with miscellaneous abdominal complaints (63). Similarly, in patients undergoing emergency surgery for tuberculous peritonitis, mortality was 24% (44) The high prevalence of liver disease (which is often unsuspected) in patients with tuberculous peritonitis has previously been mentioned. Surgery in these patients is extremely hazardous. In a recent report from Kings College (64), 36 patients with unsuspected liver disease underwent exploratory laparotomy. The morbidity and mortality were 61% and 31% respectively. Although these were not patients with tuberculosis, it is clear that exploratory laparotomy is not an insignificant procedure. It should be reserved for difficult diagnostic problems, where all other, less invasive diagnostic methods have failed.

Immunological tests for tuberculosis.

Because of the problems with bacteriological techniques mentioned above, much research effort has been directed at developing direct and indirect methods to identify mycobacterial infections. The direct methods look for mycobacterial products in the infected fluids, while the indirect methods look for specific host responses to mycobacterial infections.

Indirect tests

Two main types have been used. Host antibody production to mycobacterial antigens is sought in the relevant fluid. The other consists of detecting adenosine deaminase as a marker of the cellular immune response.

Antibody detection.

The immune response to infection is specific, hence detecting antibody production to mycobacterial antigen should be specific for tuberculous infection. Numerous techniques have been used. Enzyme linked immunosorbent assays (ELISA) are the most commonly described tests because they can be used in third world settings, where they are clearly most needed. Numerous other tests have been devised, the earliest being haemagglutination in 1898 (71) and complement fixation in 1914 (72). Other methods reported to be of value in detecting antibodies to *M.tuberculosis* include: passive haemagglutination, gelprecipitation, immunoelectrophoresis, kaolin and latex agglutination, single cell plaque assay, immunofluorescence, precipitation of immune complexes by heterologous anti-immunoglobulin, and ammonium sulphate and radio-gel-electrophoresis(78).

The use of ELISA tests in tuberculosis has recently been reviewed (73). The major problem with ELISA has been the lack of specificity. Agglutination (74-75), radioimmunoassay (76), soluble antigen fluorescent antibody

assay (SAFA) and indeed all other tests detecting anti-mycobacterial antibody have their usefulness limited by nonspecificity .

One of the reasons for this lack of specificity is that antibodies to tubercle bacilli are universally found in the normal population (78) Sera from 53 patients with current or previous mycobacterial infection and 33 non-tuberculous controls were tested for the presence of mycobacterial antibodies. All the control sera reacted specifically with antigen from sonicated mycobacteria, as did all the sera from patients with tuberculosis.

Quantitative tests confirmed that specific antibodies to mycobacterial antigen were present in non-tuberculous control sera, although the levels in the control patients were lower than in patients with tuberculosis (13% binding in control patients vs 16% in tuberculous patients). There was marked overlap in the level of antibody binding in the various groups (78).

Exposure to saprophytic mycobacteria, PPD skin testing, prior asymptomatic tuberculosis and cross reactivity of the immune response with bacteria unrelated to mycobacteria (77), are all thought to play a role in bringing about this non-specific response (78).

Direct tests

Detection of mycobacterial products or constituents in body fluids is potentially a more specific method of diagnosing tuberculous infection, because it is unlikely that these products will be present non-specifically. Both biochemical and immunological methods of detection have been described. The biochemical methods are cumbersome and require sophisticated equipment (79-81), making them impractical for use where they are most required: in developing countries. Until cheaper and simpler batch methods are developed to detect these proteins, they will remain in the researchlaboratory.

Immunological detection of mycobacterial antigen was pioneered by Sada (82). A host of techniques have been developed to detect mycobacterial antigen in cerebrospinal fluid and blood. These include direct (82-83) and competitive inhibition (84) ELISAs, latex-agglutination immunoassay (85), passive haemagglutination (86) and biotin-avidin radioimmunoassays (87). These tests have generally been moderately (83,84,86) to highly (85,88) specific, although sensitivity has been low in all but the indirect ELISA of Bal and co-workers (84).

Application of these assays to intestinal disease has yielded similar results to those obtained in cerebrospinal fluid and blood. A small series reported from Durban using direct enzyme linked imunosorbant assay for mycobacterial antigen in the ascitic fluid of 10 tuberculous and 14 control patients gave false positive results in 3 of 14 controls and false negative results in 3 of 10 tuberculous patients (89) Chawla and co-workers (90) tested for mycobacterial antigen in the sera of patients with intestinal tuberculosis using an enzyme linked imunoabsorbant assay. They identified antigen in 22 cases out of 24 (92%) with intestinal tuberculosis, but also found it in 3 out of 24 non-tuberculous controls (87.5% specificity).

Adenosine deaminase (ADA)

Adenosine deaminase (EC 3.5.4.4) catalyses the conversion of adenosine to inosine. Adenosine is vital for the normal functioning of the cell. It has, for example, structural and functional roles in DNA, RNA, cyclic AMP and in nicotinamide and flavine nucleotides. It thus is important at numerous levels of cellular activity.

The distribution of ADA is greatest in intestine and lymphoid tissue. Kidney and liver show moderate levels of adenosine deaminase activity (91).

ADA has an important role in lymphocyte proliferation and differentiation. Genetic ab-

sence of ADA leads to severe combined immune deficiency, characterised by lack of T-lymphocytes, thymic hypoplasia, and functional failure of the T-cell compartment (92). ADA is required in the earliest stages of lymphocyte proliferation and differentiation (93) and is specifically released into the medium when certain subsets of T-lymphocytes are cultured in vitro. ADA activity was high in early mixed lymphocyte cultures (2000 U/mg) but low in selective cultures of T cytolytic lymphocytes (110 +/- 10 U/mg). Other T-lymphocyte subcultures expressed high levels of ADA in culture (94). High levels are also released during monocyte/macrophage maturation (95).

This enzyme is thus released into surrounding fluid during proliferation and differentiation of macrophages and certain sub-sets of T-lymphocytes. Cell mediated immune responses, such as that mounted against *M.tuberculosis*, are characterised by the selective proliferation of T-lymphocytes and macrophages. This explains why the immune response to tuberculosis gives rise to raised adenosine deaminase activity in surrounding fluid.

High ADA activity has indeed been found in many diseases. Raised serum activity is found in patients with tuberculosis of the lung, intestine, meninges (96), typhoid (97), and infectious mononucleosis (98). Activity is raised in the cerebrospinal fluid in tuberculous and bacterial meningitis, but not aseptic meningitis (96,99). Activity is also raised in the pleural fluid of patients with tuberculous pleural effusions, parapneumonic effusions (100), empyema (99,100) and effusions secondary to rheumatoid arthritis (100). High ADA has also been found in urine of patients with renal tuberculosis (101).

Summary

From the above, the following is clear:

Abdominal tuberculosis is common, particularly in the Western Cape.

Tuberculous infection often occurs in association with other diseases, particularly alcoholic cirrhosis and malignancy. Because clinical features of tuberculous peritonitis are non-specific, it is difficult to detect this complication.

Clinical examination, skin testing and currently used biochemical screening of both blood and ascitic fluid are of little use in detecting tuberculous infection.

Bacteriological studies take 4-6 weeks, and their main use thus is in confirming the diagnosis, identifying the type of mycobacterium and checking drug sensitivity. Direct microscopy on ascites fluid is extremely insensitive while culture has poor to moderate sensitivity.

Direct tests for mycobacterial products in body fluids are promising, but are still cumbersome and expensive and thus impractical for routine use.

Indirect immunological tests for tuberculous infection, which depend on the detection of anti-mycobacterial antibodies, are non-specific, due to antigenic cross-reactivity with non-pathogenic mycobacterial products and non-mycobacterialantigens.

Invasive procedures, such as laparoscopy and laparotomy are fairly sensitive and specific in detecting tuberculous ascites, but are associated with significant morbidity and mortality. This restricts their free use.

A sensitive and safe screening test to detect tuberculous infection in patients with ascites is clearly required. This test should be readily available, easy to perform, safe and quick. High sensitivity is important, because of the common association of tuberculosis with other diseases which cause ascites. The presence of another disease which could explain the presence of ascites lowers clinical suspicion of tuberculosis. A sensitive test will alert the clinician to the possibility of superadded tuberculous infection.

If doubt still exists as to whether tuberculosis was present, after the test result was available, diagnostic laparotomy (or laparoscopy) may be performed. This approach would reduce the number of invasive procedures, and hence the morbidity and mortality associated with these procedures.

Because raised adenosine deaminase activity has been found in various body fluids of patients with tuberculous infection of meninges, pleura, lung, bowel, and renal tract, and because the test for ADA is readily available, easy, safe and quick to perform, this study was performed to assess the value of ADA activity in ascites fluid as a screening test, and define its limitations, by studying a large population of tuberculous patients and controls.

Materials and methods.

Aims

This study was designed to:

1. Examine the diagnostic value of ADA levels in ascitic fluid,
2. Establish the sensitivity and specificity of this test in the diagnosis of tuberculous peritonitis, in a large number of patients,
3. Establish levels of adenosine deaminase activity which give the best discriminatory information in patients with ascites.
4. Determine what conditions may give rise to false positive or false negative results.
5. Finally, the study was designed to assess the relative diagnostic accuracy of previously used biochemical and haematological data, such as ascites total protein and white cell count. The diagnostic value of these tests alone, and combined with ADA activity in a descriminant analysis, was compared with the diagnostic accuracy of adenosine deaminase activity alone.

Study design.

This study was conducted in two phases. The first phase comprised a retrospective study of 41 patients with tuberculous peritonitis and 41 race and sex matched control patients with ascites due to other causes. The retrospective study period extended from January 1982 to January 1986.

In the second phase, from February 1986 to August 1987, all patients with ascites, of any cause, admitted to Groote Schuur Hospital, were studied prospectively. A total of 66 patients were included. 11 of these had tuberculous peritonitis and the remaining 55 served as random controls.

The detailed protocols of the retrospective and prospective studies are as follows:

Prospective limb

Case finding

Tuberculous ascites group.

Between January 1982 and January 1986, all patients admitted to Groote Schuur Hospital with a diagnosis of tuberculous peritonitis were studied. Their case records were obtained with the aid of a computer search of inpatient records. In addition, all ascitic fluids from which *M.tuberculosis* had been cultured, or in which AFB had been found, were identified by searching through the bacteriological records of the same period. Several additional patients were found in this way.

Control group

The control group consisted of patients with ascites of various causes, admitted between January 1982 and January 1986. Tuberculosis had been excluded in all of these patients. Their case records were obtained by means of a computer search of the Groote Schuur Hospital inpatient records. Consecutive, unselected case records were studied until 41 race and sex matched patients were found. A total of 54 unselected patients had to be studied before the required 41 race and sex matched control patients were found. The group of 41 patients served as the controls. The remaining 13 patients of the original 54 were unmatched for race and sex, thus were dealt with as a separate group.

Criteria for inclusion into the tuberculous ascites group.

Patients were included in the study group only if a definite diagnosis of tuberculosis had been made. No equivocal cases were studied. Patients were included if

1. Acid fast bacilli were present in the ascites.
2. *M.tuberculosis* was cultured from the ascitic fluid.

3. Biopsy of an abdominal structure showed caseating granulomas in which AFB were present.
4. Caseating granulomas were present without AFB, but *M.tuberculosis* was cultured from elsewhere, eg. from sputum.
5. Ascitic fluid adenosine deaminase activity had been measured.

Criteria for inclusion into the control group.

Patients in the control group were matched with the patients of the tuberculosis group, for race and sex. Patients were included in the control group if there had been no clinical or bacteriological suspicion of tuberculosis and an alternative cause had been found for their ascites. Acid fast bacilli were absent from all body fluids examined, and cultures for *M.tuberculosis* were negative in all cases.

Parameters studied.

The following were recorded:
1. Age, sex and race.
2. Laboratory: Haemoglobin, white cell count, total serum protein and serum albumin, sedimentation rate in the first hour. Ascitic fluid total protein, ADA activity, and cytological examination results were recorded.
3. Bacteriology: microscopy and culture results.

Prospective limb.

Case finding

Consecutive patients with ascites, who presented to Groote Schuur Hospital, between February 1986 and August 1987 were included. All of these patients underwent a diagnostic paracentesis before receiving any treatment. Data on 66 patients was collected over this period. 11 of these patients were found to have tuberculous peritonitis while in the rest, a non-tuberculous cause for their ascites was found. The 55 patients without tuberculosis served as random controls for the tuberculosis group.

Criteria for inclusion into the tuberculosis group

The 11 patients in the group with tuberculosis were subdivided into groups with (1) definite tuberculous ascites and (2) probable tuberculous ascites

Definite tuberculous ascites was diagnosed if *M.tuberculosis* was cultured from ascitic fluid (6 patients), or if histology of a peritoneal or omental biopsy showed caseating granulomas which contained acid fast bacilli (3 patients, one of whom also had positive tuberculosis cultures).

Patients were classified as having probable tuberculous ascites if a peritoneal or omental biopsy contained caseating granulomas but no AFB and cultures for *M.tuberculosis* were negative (2 patients). 1 patient had negative TB cultures and peritoneal biopsy was not performed. His symptoms and signs were strongly suggestive of tuberculosis and he responded to anti-tuberculous treatment only. He was classified as having probable tuberculous peritonitis.

The ADA results were not available to the clinician caring for the patient and thus were not used in making the diagnosis

Parameters studied.

The prospective data was used to test the accuracy of the mathematical models for descriminant analyses, which had been developed in the first limb on the retrospective data. Therefore, the same data was recorded in the prospective group as had been collected in the retrospective group.

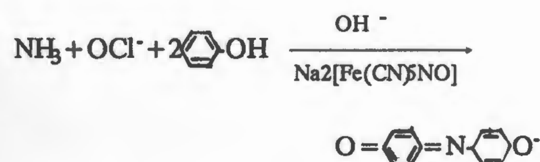
Methods

The haemoglobin and white cell count were measured by coulter counter, the sedimentation rate was done according to the method of Westegren. Serum protein and albumin were measured by automated SMAC multichannel analyzer.

Adenosine deaminase

Adenosine deaminase activity was measured by the method of Giusti and Galanti (103). Adenosine deaminase catalyses the release of ammonia from adenosine.

The equilibrium of this reaction is far to the right. Ammonia levels were determined using the Chaney and Marbach (104) modification of the Berthelot (105) reaction. The ammonia released forms an intensely blue indophenol with sodium hypochlorite and phenol in alkaline solution. Sodium nitroprusside is the catalyst. The ammonia concentration is directly proportional to the extinction of the indophenol. The reaction catalysed by ADA is stopped at the end of incubation period by the addition of the phenol-nitroprusside solution.



Solutions.
All solutions are prepared with distilled water.
Phosphate buffer:50 mM pH 6.5
Buffered Adenosine solution: 21 mM adenosine, 50mM Phosphate, pH 6.5
Ammonium Sulphate: 75uM; 0.15 umol NH_3/ml
Phenol/Nitroprusside solution:106 mM phenol, 0.17 mM sodium nitroprusside.

Alkaline hypochlorite: 11 mM NaOCl; 125 mM NaOH

Samples:

All samples were collected in standard plastic 10ml tubes ands stored at 4°C. They were assayed within 96 hours of collection. ADA has been shown to be stable at 4°C for at least 1 week (106) The samples were not spun down or deproteinated.

Assay System.

Adenosine, reagent and sample blanks were prepared.
0.05 ml ascites fluid was mixed with 1.0 ml buffered adenosine and incubated at 37°C for 60 minutes. 3.0 ml Phenol nitroprusside solution and 3.0 ml alkaline hypochlorite solution were then added and after incubation at 37°C for 30 minutes, extinction was measured at 650 nm on a Pye Unicam PU8610 spectrophotometer (Phillips)
Calculations

$E_{sample} - E_{sampleblank} = A$

$E_{Adenosineblank} - E_{reagentblank} = B$

$E_{Standard} - E_{reagentblank} = C$

Volume activity $= {}^{a-b}/_{c} \times 50$ [U/l]

Statistical analysis.

Non parametric tests (Wilcoxon) were done on both data sets to determine differences between tuberculous cases and controls.

Linear discriminant analysis was done between the cases and controls, of the retrospective data set, using the variables serum albumin, ascites adenosine deaminase, and ascites total protein. The prospective data set was then used to test the accuracy of the linear descriminant model.

A discriminant analysis was done between the cases and controls, of the retrospective data set, using logistic regression on the three variables. Multivariate as well as univariate analyses were done to improve the discriminatory power of the variables.

To confirm that the mathematical models generated on the retrospective data, in the logistic regression procedures, were accurate, the prospective data was then used to test the models in the multivariate and univariate discriminant analyses.

The univariate logistic regression analysis on the three variables was also used as an indication of the relative importance of each of these variables in the discrimination of cases from controls.

To confirm the accuracy of the model the prospective data was again used as test data. The same index was used and the above procedure followed.

Having established the optimum cut-off point from the logistic regression analysis, the results were expressed in terms of ADA activity (IU/l)

Results

There was a retrospective and a prospective limb to the study. The retrospective group comprised 41 patients with definite tuberculous peritonitis and 41 race and sex matched controls, who had a disease other than tuberculosis causing ascites, and in whom tuberculosis had been specifically excluded. An additional 13 retrospective control patients were analysed separately because they were not race and sex matched.

The prospective group consisted of 66 consecutive unselected patients admitted to Groote Schuur Hospital with ascites. Of these, 11 patients were found to have tuberculosis and in the other 55, tuberculosis was excluded. The 11 tuberculous patients served as the study group, and the 55 non-tuberculous patients served as random unselected controls.

Demographic Characteristics

Retrospective group.

There were 41 patients with tuberculous ascites and 41 race and sex matched control patients. 20 patients in each group were male, 5 (12.2% of the total), of whom were of mixed race, while 15 (36.6%), were black. Of the 21 females in the tuberculosis and control groups, 4 (9.8%) were of mixed race, and 17 (41.5%) were black.

The median age of the tuberculous patients, studied retrospectively, was 43 years, range 16 to 75 years (mean = 44.12, standard deviation (SD) = 17.2,) and that of the control group 48 years, range 12-80 years (mean 48.56 SD = 19.0,). The ages of the two groups were similar.

Prospective group

In the prospective limb, there were 11 patients with definite or probable tuberculous ascites and 55 control patients who did not have tuberculosis.

6 of the tuberculosis patients were male, and 5 were female. 9 were black (81.8%) and 2 were of mixed race (18.2%)

Of the 55 controls, 31 (61.8%) were male and 21 (38.2%) were female. 12 patients were caucasian (21.8%), 26 (47.3%) were of mixed race, and 17 (30.9%) were black. Overall, when all the tuberculosis patients in both retrospective and prospective groups are considered together, 79% were black, 21% were of mixed race and there no caucasians

The median age of the tuberculous patients in the prospective limb was 57 years with range 24-68 years, (mean 48.54, SD = 14.41). The control group had a median age of 61 years range 13-85 (mean 57.12 SD = 14.57). The age difference between the tuberculosis and control groups was not significant.

Demographic data of the retrospective and prospective groups are given in table 2.

Table 2.
Demographic Data of Retrospective and Prospective Groups.

Group	Tuberculosis		Control	
Retro-spective	20MALE	5Coloured 15 Black	20MALE	5 Coloured 15 Black
	21 FE MALE	4Coloured 7 Black	21 FE MALE	4 Coloured 17 Black
Pros-pective	6 MALE	6 Black	33 MALE	16 Coloured 13 Black 4 White
	5 FE MALE	3 Black 2Coloured	22 FE MALE	4 Black 8 White 10Coloured

Diagnosis

Retrospective limb

In the retrospective limb, tuberculosis was diagnosed as follows:

1. Acid fast bacilli were present in the ascites of 4 patients (9.8%). 3 of these simultaneously had positive *M.tuberculosis* cultures, while the fourth had a peritoneal biopsy which contained caseating granulomas which were negative for AFB.

2. *M.tuberculosis* was cultured from the ascitic fluid in 35 patients (85.4%). 5 of these patients also had AFB positive caseating granulomas, and 12 AFB negative caseating granulomas, on histology.

3. Biopsy of an abdominal structure showed caseating granulomas in which AFB were present, in 9 patients (21.95%). Cultures were simultaneously positive in 5 patients, whereas the diagnosis was made solely on the basis of histology in the other 4 patients.

4. Caseating granulomas, in which AFB could not be demonstrated, were present in 13 patients (31.7%). *M.tuberculosis* was cultured from sputum in 1 of these patients (2.4%), ascites culture was positive in 8, and AFB were found in the ascites of 1 patient, although his culture was negative. (Table 3)

Table 3.
Diagnosis of Tuberculosis in Retrospective Group

PRIMARY DIAGNOSIS	SECONDARY FINDINGS			
	positive culture	afb +ve caseating granuloma	afb -ve caseating granuloma	ADA
AFB in Ascites 4(9.8%)		3	1	114.5 (SD=8.7) (Range:102-117)
Positive Culture 27(65.8%)		5	11	89.6 (SD=45.6) (Range:12-160)
AFB Pos Granulomas 9 (22%)		5		109 (SD=61.7) (Range:43-250)
AFB Neg granulomas 1 (2.4%)	(sputum positive)			123

Primary diagnosis: The way in which the diagnosis of tuberculous peritonitis was first made.
Secondary findings: Associated findings which were confirmatory, after the diagnosis of tuberculous peritonitis had been established.
ADA: This was not a criterion for diagnosis.
Mean ascitic fluid activity, standard deviation and range shown in U/L. Only the individual value is given for the last category.

Prospective limb

In the prospective group, there were 8 patients with definite tuberculosis and 3 with probable tuberculosis. The patients with definite tuberculous peritonitis were diagnosed as follows:

M.tuberculosis was cultured from the ascitic fluid of 6 patients, peritoneal or omental histology showed caseating granulomas which contained acid fast bacilli in 3 patients, one of whom also had positive tuberculosis cultures.

The 3 patients classified as having probable tuberculous ascites, were diagnosed as follows: 2 had peritoneal biopsies containing caseating granulomas but no AFB were detected and cultures for *M.tuberculosis* were negative . 1 patient had negative TB cultures and peritoneal biopsy was not performed. His symptoms and signs were strongly suggestive of tuberculosis and he responded to anti-tuberculous therapy only.

Table 4.
Diagnosis of tuberculosis in prospective Group.

PRIMARY DIAGNOSIS	SECONDARY FINDINGS			
	positive culture	afb +ve caseating granuloma	afb -ve caseating granuloma	ADA
Positive Culture 5 (45.4%)			1	94 SD=40.2) (Range=58-153)
AFB Pos Granulomas 3 (27.3%)	1			106 (SD=22.3) (Range=86-102)
AFB Neg Granulomas 2 (18.3%)				(84, 187)
Clinical Response 1 (9%)				(110)

Primary diagnosis: How tuberculous peritonitis was first diagnosed.
Secondary findings: Associated findings which were confirmatory, after the diagnosis of tuberculous peritonitis had been established.
ADA: This was not a criterion for diagnosis.
Mean ascitic fluid activity, standard deviation and range shown in U/L.
Individual values only are given for the last 2 categories, because of the small sample size.

Biochemical and ascites fluid results.

Logarithmic transformation on ascites adenosine deaminase was done to compensate for differences in the variances.

Retrospective group.

The variables in the retrospective data, that were found to be significantly different between the tuberculous and control groups, were ascites adenosine deaminase, ascites total protein, and serum albumin.

In the retrospective group the tuberculosis patients had a mean ascites adenosine deaminase of 99.83 IU (SD=49.15 SD),compared to 14.83 IU (SD=8.37), $p < 0.0001$.

Mean ascites total protein was 47.78g/l(SD=14.08) in the tuberculosis group, vs 26.35g/l(SD=16.16)g/dlinthecontrols,$p < 0.0001$ and mean serum albumin 25.54g/l(SD=5.26) vs 28.17g/l(SD=5.44) $p < 0.03$

Prospective group

The mean ascites ADA was 112.64 IU (SD=44.97) in the tuberculosis patients, compared to 16.27 IU (SD=26.72) in the control group ($p < 0.0001$). Mean ascites total protein was 44.36 (SD=12.08) vs 26.25 (SD=14.97)g/l, $p < 0.016$ and serum albumin 24.72 g/l (SD=5.51) vs 26.69g/l(SD=6.39), $p < 0.14$ in the tuberculosis and control groups respectively.

Multivariate linear discriminant analysis

Retrospective limb

Using the 3 variables found to be significantly different in the retrospective study, namely ascites adenosine deaminase, ascites total protein, and serum albumin, discriminant analysis was done. 36 tuberculous and 40 control patients were included in this analysis, because of missing values in 5 tuberculous and 1 control patient, . The linear descriminant analysis model correctly classified 39 of the 40 control patients and 34 of the 36 tuberculous patients. There were thus only 2 false negative results and 1 false positive result. (Table 5).

Table 5
Classification of Retrospective Data by Linear Discriminant Analysis.

	Predicted Negative	Predicted Positive	Total
True Negative	39	1	40
True Positive	2	34	36
Total	41	35	76

Sensitivity: 94.4%, Specificity: 97.5% Correct: 95.9%
False Positive Rate: 2.5% False Negative Rate: 5.6%

Prospective limb

The multivariate linear discriminent analysis model derived from the retrospective data was then applied to the prospective data to test its accuracy. Similar results were obtained. (Table 6).

Table 6
Classification of Prospective Data by Linear Discriminant Analysis.

	Predicted Negative	Predicted Positive	Total
True Negative	47	8	55
True Positive	0	11	11
Total	47	19	66

Sensitivity: 100% Specificity: 85.4% Correct: 88.2%
False Positive Rate: 14.5% False Negative Rate: 0%

Fig 6.

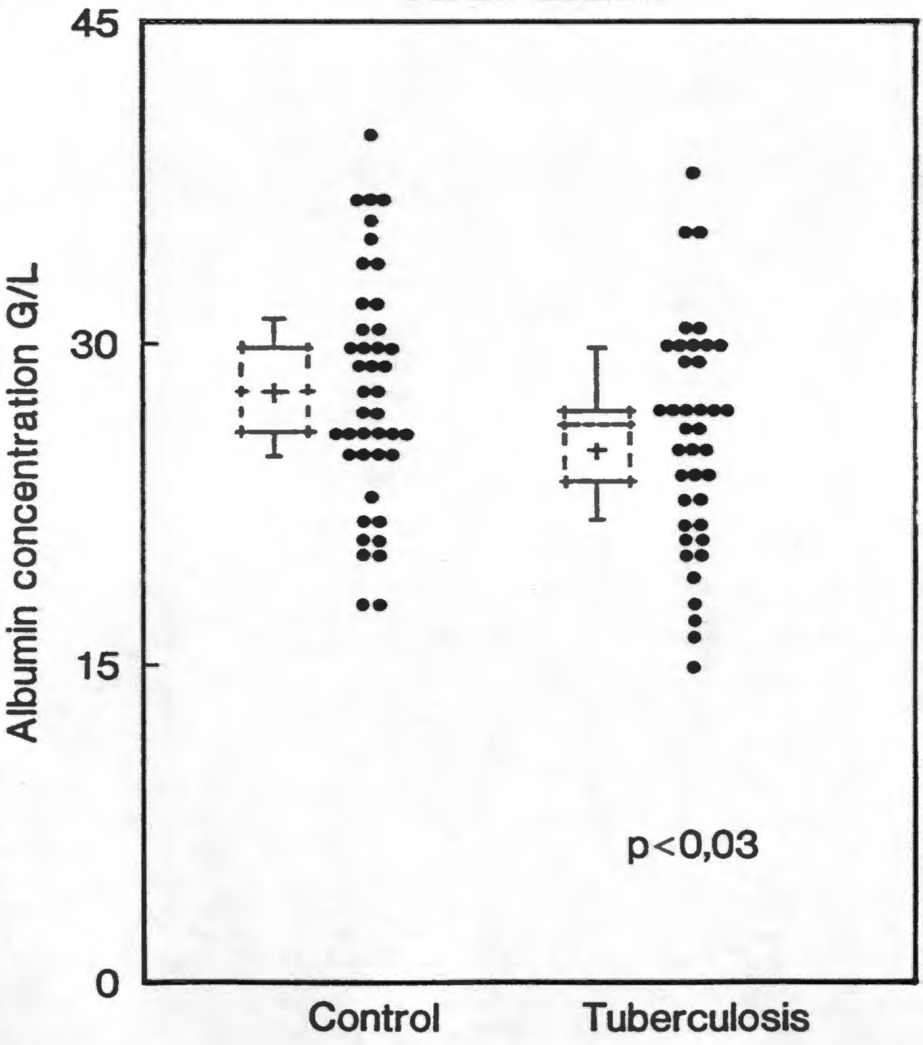


Fig 7.

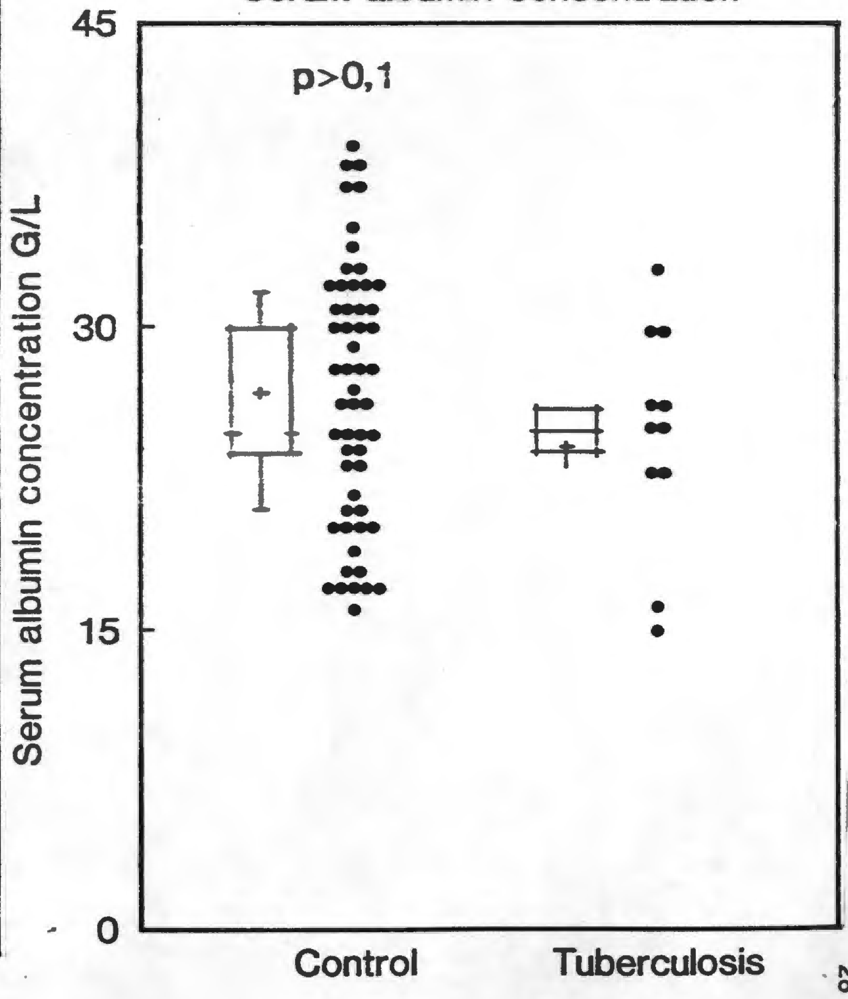


Fig 8.

Retrospective limb
Ascites total protein

Protein concentration G/L

75
50
25
0

p<0,001

Control
Tuberculosis

Fig 9.

Prospective limb
Ascites total protein

Total protein concentration G/L

75
50
25
0

p<0,0001

Control
Tuberculosis

Fig 10.

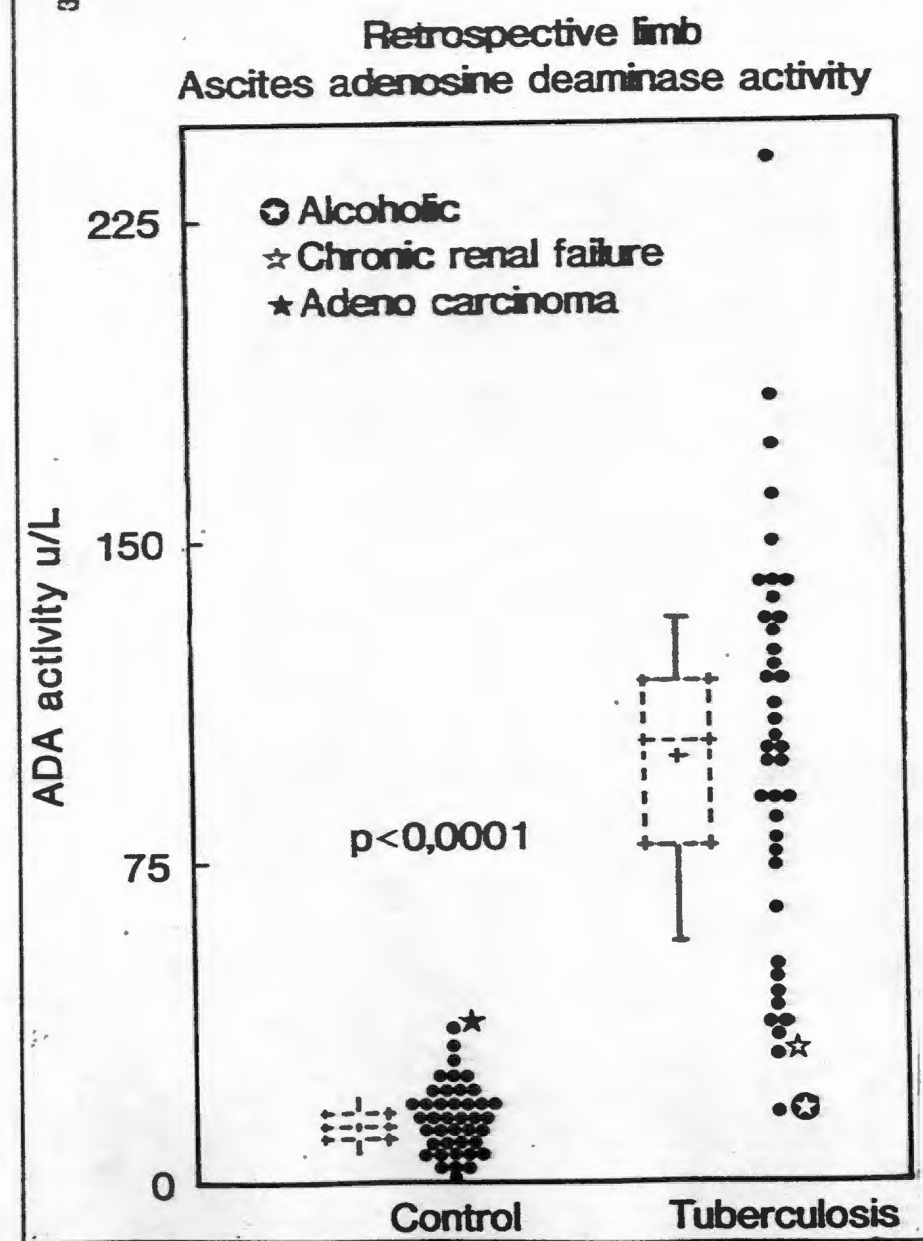
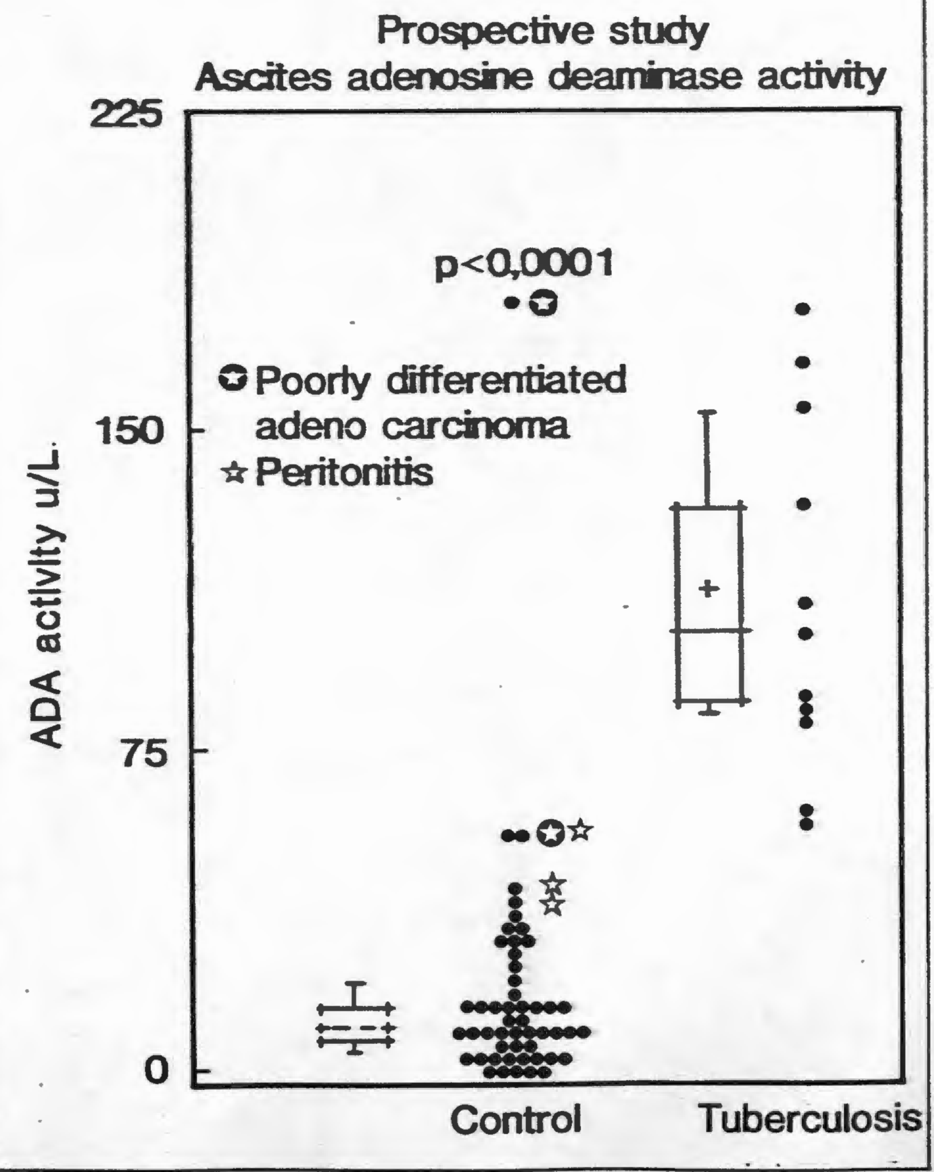


Fig 11.



The descriminatory value of this is shown in table 9.

Table 9
Univariate Logistic Analysis on Ascites Adenosine Deaminase.

	Predicted Negative	Predicted Positive	Total
True Negative	40	1	41
True Positive	2	39	41
Total	42	40	82

Sensitivity: 95.1% Specificity: 97.6% Correct: 96.3%
False Positive Rate: 2.5% False Negative Rate: 4.8%

These values were transformed mathematically to a standard reference range of 0 to 1 for the tuberculosis and control groups For the cases, the probability of being classified as having tuberculosis ranged from 0.01 to 1.0 , and for the controls, the probabiity of being classified as tuberculosis patients ranged from 0.001 and 0.69. *Fig.13* gives the scattergram of these transformed values, and demonstrates the minimal overlap between the two groups. These values represent the probability that a specific ADA value will classify a patient as having tuberculosis. The ascites ADA activity predicted, with a probability aproaching 1, that the patient would have tuberculosis, in the overwhelming majority of those with tuberculosis. The test strongly predicted that the majority of control patients did not have tuberculosis, ie. they were assigned probabilities of having tuberculosis which approached 0. The marked separation between the two groups, of the distribution of the probability values derived from the ascites ADA activity, is an indication of the descriminatory power of the ascites ADA activity.

Probability values greater than 0.5 were used as the cut-off to classify patients into the tuberculosis group and values less than .5 to classify them into the control group. This translates to an ascites adenosine deaminase value of 32.3 IU

Prospective limb

To confirm the accuracy of the model, the prospective data was then used to test this logistic regression procedure. All 11 of the tuberculosis patients were correctly classified. 5 of the 55 control patients were misclassified. (Table 10).

Table 10
Prospective Data. Univariate Logistic Regression Analysis on Ascites Adenosine Deaminase.

	Predicted Negative	Predicted Positive	Total
True Negative	50	5	55
True Positive	0	11	11
Total	50	16	66

Sensitivity: 100% Specificity: 90.1% Correct: 92.4%
False Positive Rate: 9.9% False Negative Rate: 0.0%

The same index that was used on the retrospective group, was applied to the prospective group. The tuberculosis patients had values ranging between 5.55 and 33.41. The control group ranged from -6.976 to +34.06. These are represented in the scattergram and boxplot shown in *fig.14*. There is minimal overlap between the tuberculosis and control groups.

The ADA activity was also translated to a reference range between 0 and 1 and these values are shown in *fig 15*. The widely separated distribution of these values again confirms the descriminatory power of this test.

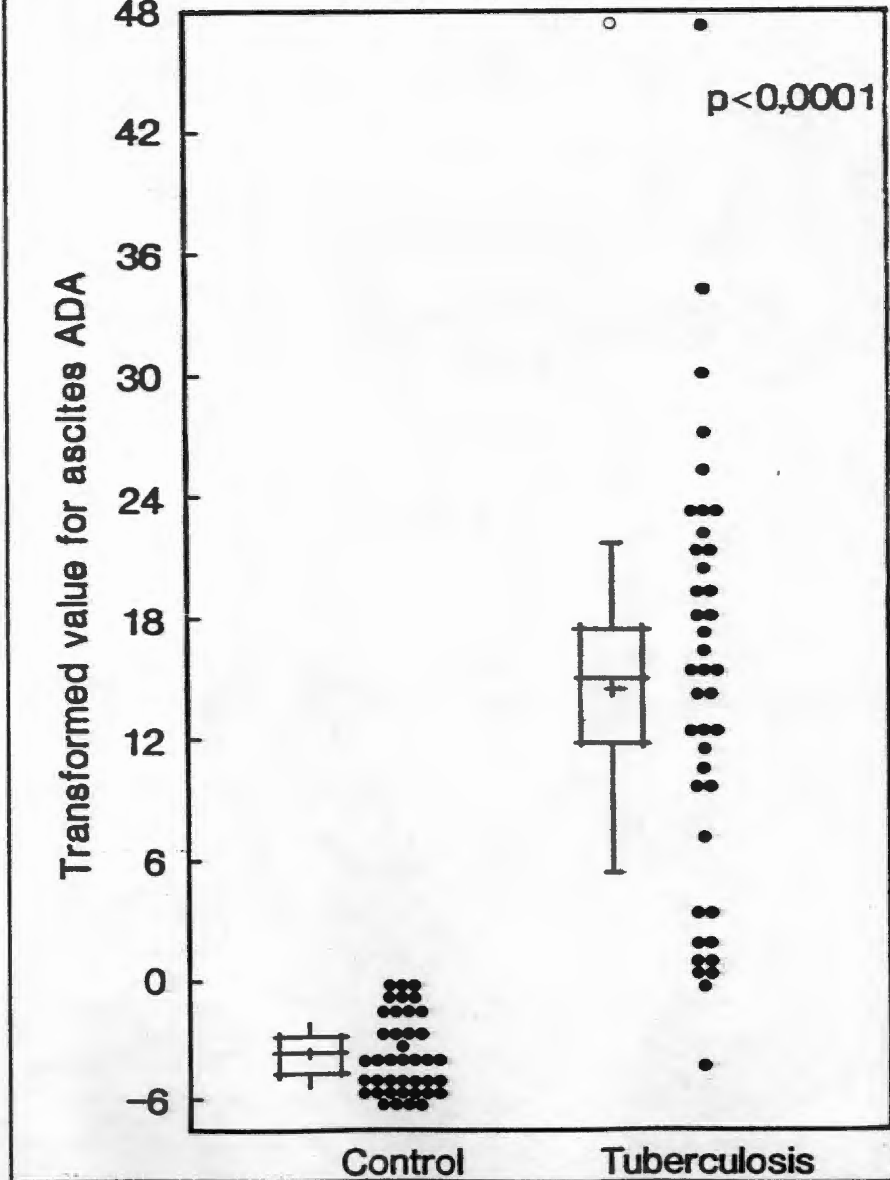


Fig 12
Retrospective group
Univariate logistic regression
of the log. ascites ADA

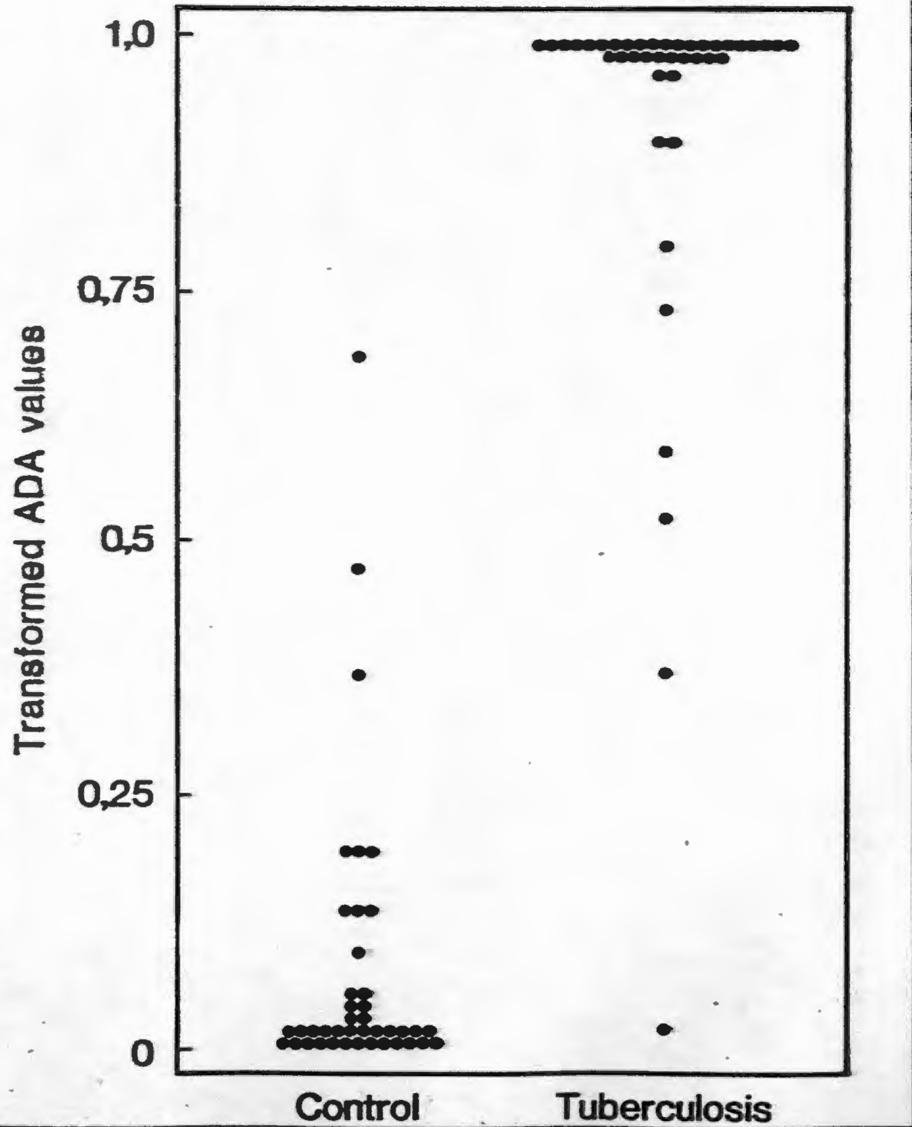


Fig 13.
Retrospective group
Univariate logistic regression on ascites ADA
Values transformed to range 0–1

Fig 14.

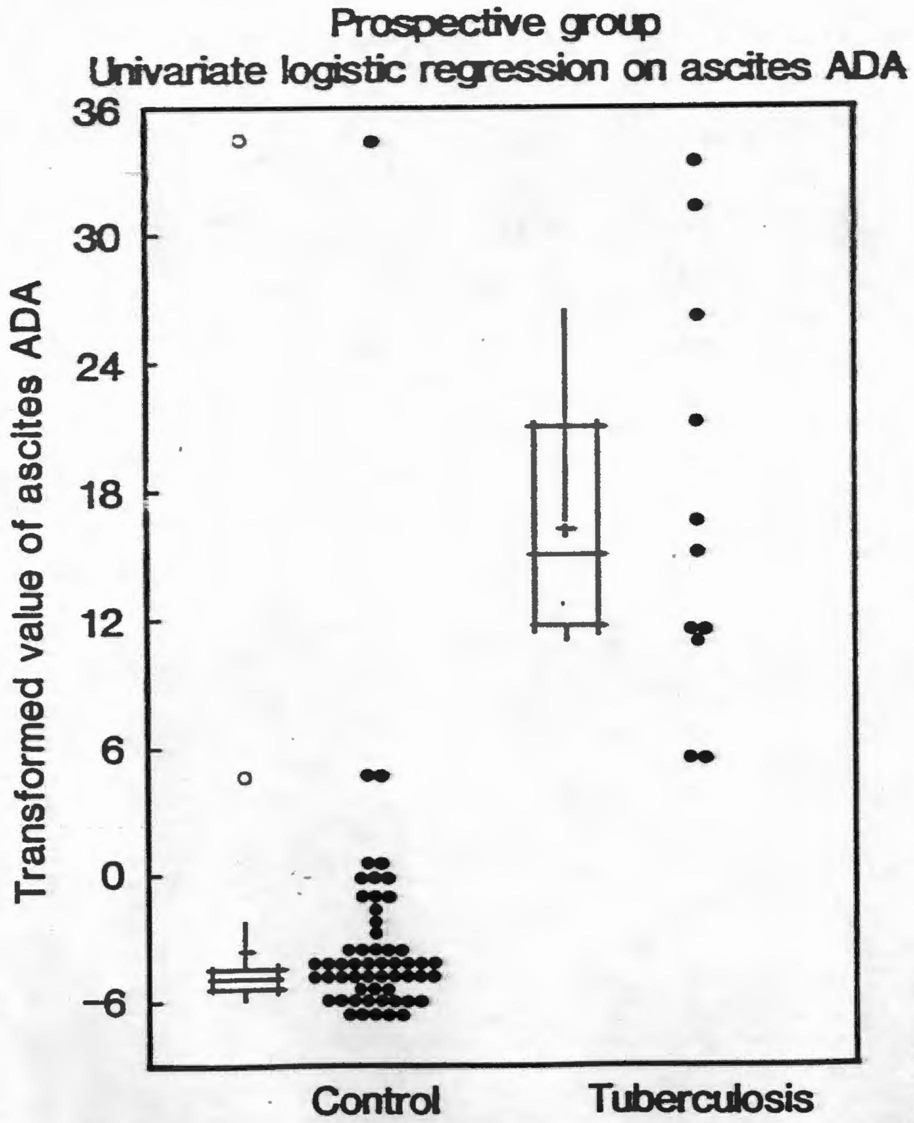
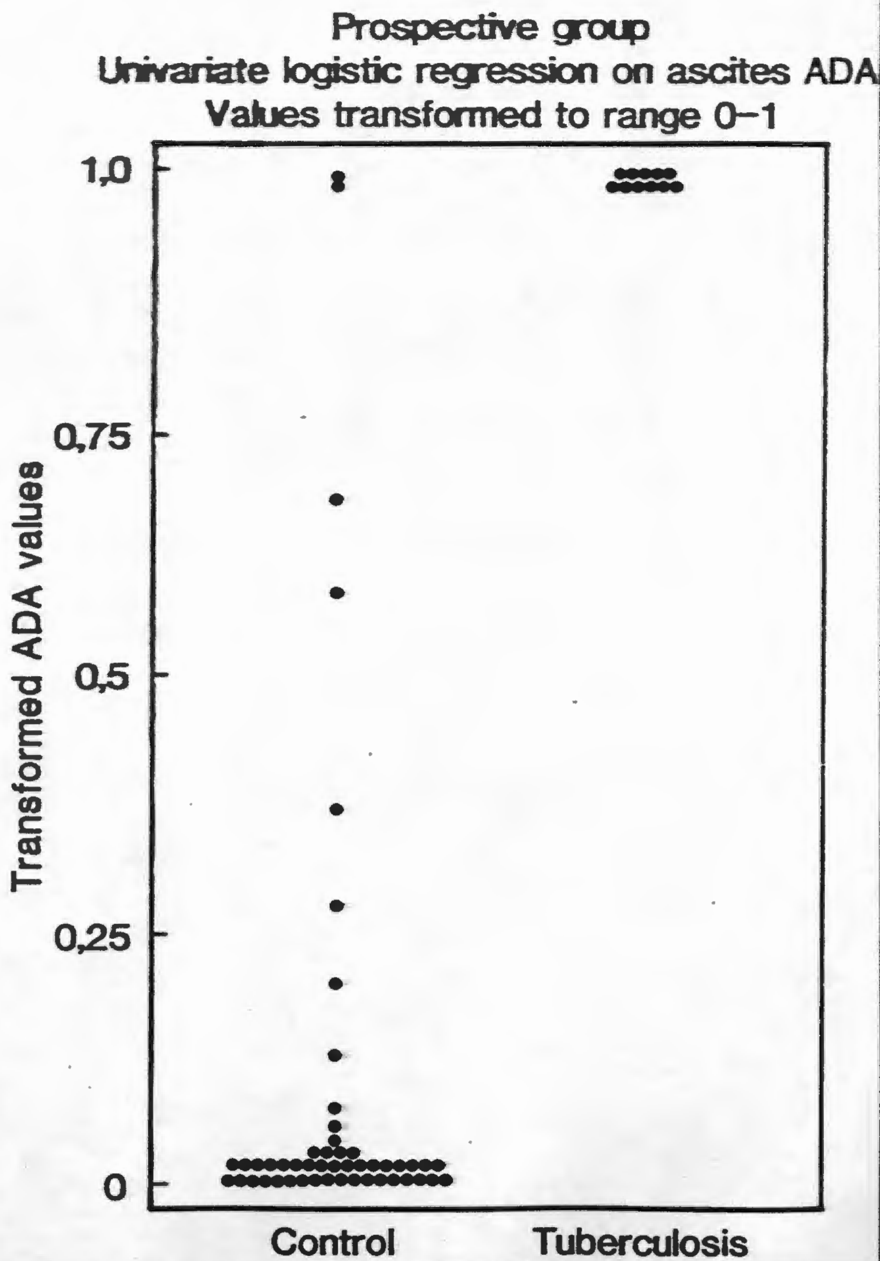


Fig 15.



Unmatched Retrospective Controls

In addition, data on the 13 retrospective control patients who could not used in the above analyses, because they were not race and sex matched was analysed using the univariate descriminant analysis of the ascites adenosine deaminase. Two patients were misclassified in this group. Values ranged from -6.33 to 0.584.

Analysis of false positive cases.

The 5 patients in the prospective group, who had false positive results included 2 patients with poorly differentiated adenocarcinomas and 3 patients with non-mycobacterial peritonitis. One of the latter patients also had pancreatic ascites.

The first patient with adenocarcinoma was a 59 year old caucasian male, who presented with haematuria, a flank mass, and ascites. His ascites adenosine deaminase activity was 54 IU, the ascites LDH was 5500 and fluid white count 2500/ml, with an absolute neutrophil count of 1425/ml. He had no evidence of bacterial peritonitis. Cytology was unequivocally positive at the first diagnostic paracentesis. He never presented a diagnostic problem.

The 2nd patient with adenocarcinoma was a 61 year old coloured female who presented with dyspeptic symptoms and symptoms of anaemia, which was due to iron deficiency. Her ascites adenosine deaminase activity was 190 IU, LDH 63 and cytology was negative. A poorly differentiated gastric carcinoma was found at endoscopy. The ascites cell count was 400/ml, with no polymorphs detected. The fluid was not bloody.

The other 3 patients with false positive results all had peritonitis associated with a surgical condition. The first was a 74 year old coloured man who presented with a 1 week history of irreducible hernia, which was strangulated. E.coli and Entererococcus were cultured from his ascitic fluid. Fluid LDH was 2000 U and cell count 1000/ml, with a predominance of polymorphs. The fluid was not bloody.

The second was a 63 year old black man who had a 1 week history of abdominal pain and swelling. He was found to have a micronodular cirrhosis, haemoperitoneum, and Hafna alvei was cultured from his peritoneal fluid. His fluid adenosine deaminase activity was 54 and LDH 765 U. Cell counts were not done on the fluid. No cause was found for his haemoperitoneum

The 3rd patient was a 53 year coloured man who presented with abdominal pain and swelling. He had diabetes and chronic liver disease due to heavy drinking. The amylase activity in his fluid was 385000, ADA 36 IU, and LDH 800U. His fluid was bloody, and the fluid leukocyte count was 1000/ml. Several cultures of his ascitic fluid were positive for *Candida albicans*

There were no other cases of non-mycobacterial peritonitis, in either the retrospective or prospective groups. Thus all of the patients who had a surgically related non-tuberculous peritonitis had a raised ascites adenosine deaminase. (These were not patients with spontaneous bacterial peritonitis.) In all of these patients, the pre-test probability of tuberculosis was very low, as the true diagnosis was apparent. Thus the falsely raised ADA activity would not have significantly influenced the management of the condition.

In the retrospective limb, only 1 patient had falsely raised ADA activity. He was a 55 year old black man with a 1 month history of abdominal pain and swelling. Cytology revealed adeno-carcinoma cells. ADA was 36 U/l, ascites total protein 20g/l and the fluid was not bloody. The primary site of the tumour was not found.

In the 13 controls who were not race or sex matched, 2 patients had false positive results. The first was a 59 year old white man who had a renal adeno-carcinoma, while the other was a 40 year old white woman with an extra-ovarian adeno-carcinoma of unknown primary site.

Of the 5 false positive cases in the prospective group, the true clinical diagnosis was obvious at the time of admission in 2 and was clear in a third after the first diagnostic paracentesis. This information was not available in the retrospectively studied patients.

Because red blood cells have high adenosine deaminase activity, it was possible that blood stained fluid may falsely raise ADA activity and account for the false positive results. Only the prospective data was analyzed for this, as the information was not available in the retrospective group. Of the 11 patients with tuberculous peritonitis, 3 (27%) had blood stained fluid. Mean ADA in this subgroup was 100U (SD=49.8) which was not significantly different from the mean value in the patients with tuberculosis who had no blood staining of ascites fluid(mean 117U SD=45.7). 9 patients without tuberculosis had bloody peritoneal fluid. 2 of these patients had adenosine deaminase activity greater than 32.3IU and were misclassified. 3 misclassified patients had clear fluid. ADA activity in patients with blood staining (mean=20 U, SD=15.8) were not significantly different from that in patients with clear fluid (mean=14.5 SD=27.0 $p<.55$). Thus the falsely raised ADA activity was not associated with blood stained fluid.

Neither the total white cell count, neutrophil count or lymphocyte count in the ascites fluid correlated with the adenosine deaminase activity. ($R=-0.002$, $p=0.8$; $R=0.06$ $p=0.6$;and $R=-0.04$ $p=0.75$ respectively). Only the prospective data was analyzed for this, as ascitic fluid white cell counts were not available in the retrospective patients. 3 patients met the criteria for culture negative "neutrocytic" ascites(106), with ascitic fluid polymorph counts greater than 500 cells/mm^3, negative ascitic fluid culture in the ascites fluid, no antibiotic treatment within 30 days, no intra-abdominal source of infection and no pancreatitis. Their ascitic fluid adenosine deaminase activities were 2, 9 and 30 IU respectively. Clearly, this possible variant of spontaneous bacterial peritonitis is not associated with raised adenosine deaminase activity.

To assess whether a false positive ADA result could be due to the presence of pancreatic ascites, all patients with pancreatitis were studied. 5 patients in the prospective group had pancreatic ascites. Only one of these had raised adenosine deaminase activity. This patient had a concurrent candidal infection of the ascitic fluid. The ADA activity in the patient with peritonitis was 36U, while those without peritonitis had mean ADA activity of 12.7U (SD=12.7). No correlation was found between ascites amylase and adenosine deaminase activity. Thus, the false positive ADA result was not due to the presence of pancreatitis.

To assess the correlation of malignant ascites with ADA activity, the data on all patients with malignant ascites were studied.

There were 18 patients in the prospective group, 12 in the matched retrospective group and 6 in the unmatched retrospective group, with malignant ascites. (Total 32). The matched and unmatched retrospective groups are considered together in this analysis, for convenience.

2 of the 18 patients in the prospective group with malignant ascites, had raised adenosine deaminase activity in their ascitic fluid. Only 1 patient out of 12, in the matched retrospective group had a false positive ADA result while 2 out of 6 patients in the unmatched retrospective group, had false positive results.

All of the patients with false positive ADA results related to malignancy, had poorly differentiated adenocarcinomas. 2 were of renal origin, 1 of gastric origin and in 2 patients the primary site was not identified. (Table 11)

Although the false positive ADA results were found in patients with poorly differentiated adeno-carcinomas that arose from gastrointestinal tract, kidney or were from an unknown primary, not all of the patients with poorly differentiated adeno-carcinomas had a false positive ADA result. The correlation

between poorly differentiated adeno-carcinoma and raised ADA did not reach statistical significance. No patient with hepatocellular or ovarian carcinoma had a false positive result.

The ADA did not appear to correlate with the load of malignant cells in the fluid. Cytology was negative in 16 and positive in 9 patients with malignant ascites but normal fluid ADA activity and 2 out of five patients with malignant ascites and raised ADA.

Table 11. Carcinomas Causing Malignant Ascites.

Carcinoma	Prospective Group	Retrospective Group
Ovarian	6	2
Gastric	3 (1*)	5
Renal(AdenoCa)	1 (1*)	2 (1*)
Unknown Primary (AdenoCa)	2	6 (2*)
Hepatocellular	3	2
Pancreatic	0	1
Lymphoma	1	0
Floor of Mouth	1	0
Total	18 (2*)	18 (3*)

* = FALSE POSITIVE
Matched and un-matched control patients included under retrospective group.

Analysis of false negative results.

Analysis of the tuberculous patients who had low ascites ADA activity is as follows:

The first patient was a 41 year old coloured man who had alcoholic cirrhosis. He had been treated for ascites for over 1 year prior to his admission for tuberculosis. He presented with disseminated tuberculosis, and *M.tuberculosis* was cultured from his ascites fluid. His ascitic fluid adenosine deaminase was 12U/l He probably had tuberculous peritonitis due to haematogenous or lymphatic seeding to his pre-existing alcoholic ascites. The seeding may have occured as part of his disseminated disease. He died shortly after contracting disseminated tuberculosis. The pathogenesis of the tuberculous peritonitis in this case may be different to that of the other cases studied, in that the ascitic fluid probably became infected as a result of *M.tuberculosis* spreading haematogenous to his pre-existing alcoholic ascites, during the course of the dissemination of his tuberculosis. This is different from the usual mechanism of infecting the peritoneum, where tuberculous peritonitis results from reactivation of latent abdominal foci of mycobacteria, during periods of diminished host immunity. (qv)

The second patient with a false negative result was a 74 year old black man with chronic renal failure plus alcoholic cirrhosis. Ascites had been present for approximately 1.5 years prior to the onset of symptoms suggestive of tuberculosis. The patient was not undergoing peritoneal dialysis. Ascites fluid adenosine deaminase activity was 30U/l

A further 2 patients are described here. Although they were correctly classified by the discriminant analysis model, they had values close to the range of the non-tuberculous patients. One of these was a 59 year old black female who was employed, not malnourished and a non-drinker. No pre-existing cause of ascites was present. Her adenosine deaminase activity was 33 U/l.

The other patient who was correctly classified, but had values close to the non-tuberculous range was 43 year old coloured female with carcinoma of the colon with multiple liver metastases, and malignant ascites. Malignant ascites preceded the onset of tuberculous peritonitis. Ascites fluid adenosine deaminase activity was 34U/l.

The majority of the patients with low adenosine deaminase activity in their ascitic fluid had an underlying cause for their ascites,

which then became secondarily infected with *M.tuberculosis*.

Of the total of 52 tuberculous patients, (retrospective and prospective groups) there were 19 patients who were alcoholic (one of these patients simultaneously had chronic renal failure), and 21 who were non, or moderate drinkers. A further 2 patients had diabetes mellitus and 3 (1 of whom was simultaneously alcoholic) malignant ascites. In 8 retrospective patients no information about drinking habits was available. The mean ADA activity was 89.5 u/l in the tuberculous group who were alcoholic, and 109.4 in the tuberculous group who were not. This difference was not significant (p0.14). Thus although low ADA activity was found predominantly in patients who had a pre-existing cause for their ascites, the ADA activity was not significantly different overall in the group with pre-existing ascites compared to those without.

This indicates that alcoholism or another disease is extremely common in patients with tuberculous peritonitis, but that ADA activity remains a good screening test for tuberculosis despite the presence of the underlying disease. However, low ADA activities are more commonly, but not exclusively, found in patients with a pre-existing cause for their ascites.

Discussion

Tuberculosis is a major problem in developing countries, including South Africa (1-7). The incidence of extrapulmonary tuberculosis is very high throughout Southern Africa, and is rising (7). The number of cases from the Western Cape is disproportionately high, with the incidence of extrapulmonary tuberculosis being approximately three times higher than that in the rest of South Africa (7). The disease is also becoming a greater problem in western communities because of the spreading AIDS plague (13-16), the increasing population of aged people (17,18) the growing population of homeless people in many first world cities (19) and the immigrant population, from developing countries (8, 29-34).

Tuberculous peritonitis is one of the most important peritoneal diseases. It was the most common cause of ascites in Lesotho, constituting 42% of cases (9), it was the second most common cause of ascites in the series by Nwokolo from Nigeria (10), constituting 23.2% of all cases, and the third most common cause in a series of 8400 laparoscopies performed in Argentina (57).

When undiagnosed and untreated, tuberculous peritonitis has a mortality rate of approximately 50% (107) to 55% (108). Two factors make diagnosis difficult. Firstly, symptoms are vague, signs are non-specific and no biochemical tests is pathognomonic. Secondly tuberculous peritonitis occurs commonly in persons who already have alcoholic cirrhosis (9,19,45,62,109,115) malnutrition with hypoalbuminemia (32), malignant ascites (62), or another cause for ascites, other than tuberculosis.

The difficulty associated with diagnosing tuberculous peritonitis, in rural communities, where TB is at its most prevalent, is further aggravated by cost constraints and the poor access to sophisticated laboratory equipment and skilled laboratory workers. Therefore, any diagnostic test, if it is to have a significant impact on the disease, must be relatively cheap and simple to perform.

Adenosine deaminase activity is easy to measure, and may easily be done in the rural setting. ADA (EC 3.5.4.4) catalyses the conversion of adenosine to inosine, by the removal of ammonia (102). Activity is high in proliferating T-lymphocytes (92-93), particularly in T-lymphocytes and increases with the degree of differentiation of certain subsets of T-cells (93). ADA is also found in high concentration in maturing macrophages/monocytes (94). Thus raised activity of this enzyme is found in the blood (95,97), or any fluid where T-lymphocytes or monocytes/macrophages are proliferating. The immune response to tuberculosis is cell mediated, hence the cell types which release large ammounts of ADA predominate. One may thus anticipate that ADA activity may be be raised in the ascitic fluid in tuberculous peritonitis.

Adenosine deaminase activity has been shown to be increased in tuberculous infections of meninges (95,98), pleura (99-100), and urine (101). We had previously demonstrated that raised adenosine deaminase activity is found in the ascitic fluid of patients with tuberculous peritonitis (110). This was subsequently confirmed in a report on a small number of tuberculosis patients, by Martinez-Vazquez and co-workers (111).

This study was undertaken to establish the sensitivity and specificity of raised ADA activity in ascitic fluid in patients with tuberculous peritonitis, and to delineate potential sources of error, in a large series of patients with tuberculous ascites.

In the retrospective limb, 63 patients with tuberculous ascites were identified over a 5 year period, but only 41 had adenosine deaminase measured. This is a large increase in patients with tuberculous ascites, as only 60 cases were identified in the 10 year period prior to that (2). Although 2 patients from a different hospital were included in this study, the increased incidence is probably real. This is supported by epidemiological data (7). The increase may partially be due to in-

creased prevalence of the disease, as has been demonstrated by these epidemiological studies (7). In addition, more people from rural areas (eg Transkei), who take ill in the rural area, may be specifically traveling to Cape Town to seek medical help. Thus the denominator in the incidence equation may be spuriously enlarged (7).

The sex ratio of the tuberculous patients was approximately equal, similar to several previous studies (2,31,35,45-46,62,112), although other studies have reported either male (9,32), or female (113) preponderance. The variation of sex ratio probably reflects the local social forces and customs of that population, e.g. in certain communities on the Indian subcontinent, the women are responsible for caring for the sick, including the tuberculotics. The women would thus have increased exposure to the TB bacillus.

The median age of the patients studied retrospectively was 43 (mean 44.12) years, and that of the prospectively studied group 51 years (mean 48.54). This is similar to the studies of Menzies (9) Gilinsky (2), and Sherman (70), who studied adult populations only. Those studies that included the pediatric population (30-32,44,48,107;112) generally reported mean ages in the mid 30's. Studies on adult populations from the Indian subcontinent (35) and Iran (8), reported median ages of 33 and 29.5 years respectively. This probably reflects a different pattern of disease in these areas.

79% of the tuberculosis patients were black and the other 21% of mixed race. There were no caucasians with tuberculous peritonitis in this series. The high percentage of blacks is particularly significant because the majority of patients attending Groote Schuur Hospital are of mixed race or caucasian. Blacks accounted for <40% of admissions during the period of the study. The high prevalence of tuberculosis in blacks and to a lesser extent, Asiatics and coloureds, is well recognised (1). This is probably due to a complex interaction of host and environmental factors. Socio-economic factors such as malnutrition, stress, overcrowding, poor access to medical facilities, delay in seeking treatment, (which leads to greater dissemination of the disease), and many other socio-economic factors, may play an important role in the increased prevalence of the disease, in the black population. In addition, HLA-BW15 has been associated with an increased risk of tuberculosis in a North American Black population (114), indicating the potential importance of host factors, in the increased incidence of the disease in blacks.

The tuberculosis was conclusively proven in all of the retrospective cases. AFB were detected in the ascites of 4 patients (9.8%) confirming the low sensitivity of this examination. In 9 patients caseating granulonmas containing acid fast bacilli were found, and caseating granulomas negative for acid fast bacilli were found in a further 13 (31.7%) of the patients (Total 22).

Cultures were positive in the ascites of 35 of the 41 patients (85%) studied retrospectively. 1 patient with AFB in his ascites had negative cultures. The sensitivity of culture was probably exaggerated, in the retrospective group, as a positive culture was one of the methods used to identify cases. This would have introduced a bias towards positive cultures. In the prospective group, cultures were only positive in 7 of the 11 cases (63% sensitivity). This sample was not biased towards positive cultures, and is probably the more accurate sensitivity rate for culture, at Groote Schuur Hospital. The highest sensitivity for culture reported in the literature was 83% (35). 1 litre of ascites fluid was cultured in each case. Culturing large volumes of fluid may increase sensitivity of fluid culture in detecting tuberculosis. In an earlier study from Groote Schuur Hospital (2), the rate of positive cultures was only 11.6%, for ascites. Culture rates reported in the literature vary considerably. In the series by Sarin, not a single culture was positive. Khoury (32) reported 40% positivity, Karney 50% (45), Sherman 69% (70), and Menzies 70% (9) positivity in their cases of tuberculous peritonitis. These differences probably re-

flect variations in volumes of fluid cultured, care with transport and handling and laboratory expertise. False positive results may occasionally occur, possibly due to laboratory contamination.

In the retrospective group, the haematological and biochemical parameters measured included haemoglobin, white cell count, sedimentation rate, serum total protein, serum albumin and ascites total protein and adenosine deaminase activity. Serum albumin ($p < 0.03$), ascites total protein ($p < 0.0001$) and ascites adenosine deaminase ($p < 0.0001$) were found to be significantly different. These parameters were then considered together in a multivariate linear discriminant analysis. Results of the serum albumin or ascites total protein were missing from 5 tuberculous and 1 control patient. Thus the linear discriminant analysis model was applied to 36 tuberculous and 40 control patients. The overall accuracy of this discriminant analysis model was 95.9% with a sensitivity of 94.4% and a specificity of 97.5%. When the prospective data was tested using this model, similar results were obtained. Although sensitivity was high, at 100%, specificity dropped to 84.6%. Overall accuracy was 92%.

This discriminant analysis gives excellent discrimination between tuberculous and control patients. The drop in specificity was expected, as retrospective data almost invariably give optomistic results. The clinicians did not have access to the adenosine deaminase results, and hence, the results were not biased.

Univariate analysis of the data, using logistic regression, and compensating for variations in the adenosine deaminase variances by log transformation, revealed that the contribution of the serum albumin and ascites total protein to the overall descrimination, was very small. Serum albumin alone was accurate in discriminating between the two groups only 55% of the time which is only marginally better than chance. Ascites total protein fared only slightly better with an overall accuracy of 75% (False positive rate = 28.2% false negative rate = 21.6%).

Therefore, ascites adenosine deaminase was analysed on its own. Logarithmic transformation was done to compensate for differences in variances between the two groups. Univariate logistic regression was done, and the best fit for the data found to be :

$$LOG[P/(1-P)] = -6.976 + 0.216(ADA)$$

The overall accuracy of ADA activity alone, in discriminating between tuberculous ascites and non-tuberculous controls, using this analysis, was 96.3%. Specificity was 97.6% and sensitivity 95.1%.

When this model was applied to the prospective data, as a test of its accuracy, it gave similar results. Specificity was 90.1% sensitivity 100% and overall accuracy 92.4%. The model was also applied to data collected retrospectively on 13 control patients who were not analysed in the retrospective limb, because they were not race or sex matched with the tuberculosis group. Only 2 of these controls were misclassified.

This confirms the validity of the test, in 52 patients with tuberculous peritonitis and 111 controls, and gives an accurate reflection of the discriminatory power of the test. In the study by Martinez-Vazquez, 10 tuberculous and 55 controls were studied. The adenosine deaminase activity was 108.5 U/l (range 72.5-148 U/l) in patients with tuberculous ascites, which is similar to that found in our patients. 2 of his control patients with malignancy, had adenosine deaminase activity greater than 40 U/l.

The data in the present study indicate that ascites adenosine deaminase activity, is a powerful discriminator between tuberculous and non-tuberculous cases, with sensitivity and specificity greater than 90%. When the ADA activity is mathematically converted to a scale of probability between 0 and 1, the distribution of the probabilities (*figs 13-14*) indicates that when the ascitic fluid adenosine deaminase activity is high, the probability is overwhelmingly great that the patient has tuberculosis. However, when the ADA activity

is low, the probability that the patient has tuberculosis is correspondingly low. There is no grey area of intermediate probability. There is also very little overlap between the groups.

ADA is produced by proliferating lymphocytes, especially T-cells, as well as maturing macrophages (92-94). It is thus presumably raised in ascites fluid in tuberculous peritonitis secondary to the type 4 immune response, which is characterrized by the proliferation of these cells. The high accuracy of this test, demonstrated above, is probably due to the fact that few organisms or conditions, apart from *M.tuberculosis*, elicit this type of immune response. However, this type of immune response is not specific for tuberculosis, and any condition giving rise to a type 4 response (eg cryptococcol infection), may potentially give rise to high ascites fluid adenosine deaminase activity. These conditions are rare in our hospital setting, compared to tuberculous infections, hence the specificity probably depends on the relative prevalence of conditions giving rise to a type 4 response.

In the western communities where *M.avium-intracellulare* and *M.tuberculosis* are common pathogens in AIDS patients, the value of this test still has to be assessed. The attenuated immune response in these patients may affect the accuracy of the ADA as a test for mycobacterial infections.

Because the measurement of adenosine deaminase activity is relatively simple and cheap, and does not require sophisticated equipment, this test is accessable to even the most rural laboratory. This is of great importance, because of the very high prevalence of tuberculosis in these areas.

The high accuracy of the test makes it valuable for screening for tuberculous peritonitis. It should be done in all patients with ascites, even if there is an pre-existing cause for the ascites, such as cirrhosis, because tuberculous infections often occur in association with other diseases, especially alcoholic cirrhosis (qv). Undiagnosed tuberculous peritonitis carries a very high mortality in these patients.

An important difference between this study and that of Martinez-Vasquez (111) was in the range of adenosine deaminase activity found in tuberculous peritonitis. In our study, ADA activity ranged from 12 to 250 U/l, with 1 patient each having values of 30, 33, 34, 37 and 39 U/l, while tuberculous patients in the Martinez-Vasquez study all had values greater than 72.5 U/l. This difference is almost certainly due to the small number of patients studied, in the latter publication. In the 11 patients studied prospectively, the values ranged from 58 U/l to 187 U/l. These results are similar to those of Martinez-Vasquez (111), and indicate how studies with small numbers of subjects may be misleading.

In the prospective group, 32.7% (18/55 patients) of the control patients had ascites due to alcoholic cirrhosis. In all the tuberculous patients, the alcoholism rate was 43% (19/44), although not all of these patients had documented cirrhosis.

Osler was one of the first to document the association between cirrhosis and tuberculosis (115) This association of tuberculous peritonitis with other diseases has previously been noted in several studies. Karney and co-workers (45) found significant underlying disease in 19 of their 30 cases of tuberculous peritonitis, with chronic alcoholism accounting for 13, renal failure, silicosis, myelofibrosis, cardiac failure, schizophrenia and mental retardation for the others. Menzies (9) also found that alcoholism was extremely common in in his series of tuberculous peritonitis reported from Lesotho. 55% of his 50 tuberculosis cases were labeled as alcoholic. Burack and Hollister (109) documented cirrhosis in 20 of their 47 patients with tuberculous peritonitis, Sochocky (62) noted cirrhosis in 5 and systemic lupus erythematosis in 1 of his 100 cases. Klimach and co-workers (112), reported on a group of 109 cases of gastrointestinal tuberculosis, of predominantly Asian origin where the incidence of alcoholism is low. 4 patients had diabetes mellitus, 3 patients were on steroids, one had aplastic anaemia, and one

"preleukemia." Nafeh and co-workers (59) in a laparoscopic study from Egypt found cirrhosis in 6 out of 28 patients with tuberculous peritonitis. In this series, all the patients with cirrhosis were then considered separately. 30% of the cirrhotic patients were found to have co-existent tuberculosis. This underlines the strong association between the tuberculosis and cirrhosis.

The high prevalence of underlying disease makes it mandatory to screen every patient with ascites of any cause, for tuberculosis. Ascites adenosine deaminase activity measurement is ideal for this, as it is a sensitive, specific, and relatively simple and safe test.

The patients with false positive results formed a fairly homogeneous group. In all, 111 control patients were studied. 1 patient in the race and sex matched retrospective group, 2 in the unmatched retrospective group and 5 in the prospective group had adenosine deaminase activity in ascites fluid greater than 32.3 U/l. 5 of these 8 patients with false positive results had poorly differentiated adeno-carcinomas, while the other 3 had bacterial peritonitis secondary to a surgical condition. The ADA was generally marginally raised in the false positive patients, although 1 patient had ADA activity of 190 U/l.

The adeno-carcinoma was of renal origin in 2 cases, of unknown extra-ovarian origin in 2 and a gastric carcinoma in 1 patient. They were all poorly differentiated adeno-carcinomas. There were a total of 36 patients with adeno-carcinomas in the control groups, of whom 5 had raised ADA activity. The mean adenosine deaminase activity in the group with carcinoma was 15.9 U/l (SD = 12.2) which was not different from the other controls who did not have adeno-carcinoma (mean = 11.2 SD = 11.8, $p = 0.1$).

It is unclear why only a small proportion of all adeno-carcinomas give rise to increased adenosine deaminase activity. The raised adenosine deaminase activity may be due to release from the proliferating carcinomatous cells, or to the host response to the carcinoma. Gastrointestinal and splenic tissue is particularly rich in ADA (116). The ADA occurs in multiple molecular forms. The small forms exhibit high biological activity, while larger forms have lower or no biological activity. The small forms are complexed into larger forms, in the presence of a protein of apparent molecular weight of 200 000 daltons (118). It has also been shown that certain tumours express different levels of immunoreactive adenosine complexing protein (ADCP), and that ADCP is specifically found only in mature intestinal columnar cells (117). This suggests that adenosine deaminase may be released in less mature proliferating adeno-carcinomas due to deficiency of the adenosine complexing protein.

Apart from the 5 patients with false positive results related to adeno-carcinoma, there were 3 patients who had false positive results related bacterial peritonitis, which occured in the setting of a surgical abdomen. None of these patients met the criteria for spontaneous bacterial peritonitis. Only 2 of the control patients were diagnosed as having spontaneous bacterial peritonitis. They had ADA activities of 6 and 19U/l respectively. A further 3 patients met the criteria for culture negative neutrocytic ascites, in this series. None had raised ADA. This is in keeping with the larger experience of Martinez-Vasquez, where ADA ranged from 0-6 U/l in 8 patients with spontaneous bacterial peritonitis.

It is unclear why patients with peritonitis associated with an acute "surgical" abdomen developed raised ADA activity, as opposed to patients with spontaneous bacterial peritonitis, who did not. It may relate to the intensity of the inflammatory response in the acute abdomen. Alternatively, it may be due to the dilutional effect of the pre-existing ascites, in patients with spontaneous bacterial peritonitis, as this only occurs where there is pre-existing ascites (122).

Whatever the reason, this is an important finding, as an acute surgical abdomen will not be confused with a tuberculous abdomen, hence will not cause diagnostic difficulty. Occasionally patients with tuberculous peritonitis

do present with an acute abdomen after a short period of symptoms (112). This is more common with gastrointestinal than peritoneal involvement. Even these patients will not cause diagnostic difficulty, as they will generally undergo laparotomy and have the diagnosis made that way.

Several factors were examined that could possibly account for the falsely raised adenosine deaminase activity. There was no correlation of ADA level with peritoneal blood staining. Of the 5 evaluable control patients with raised ADA, 2 had and 3 did not have bloody fluid. ADA levels in control patients with blood stained fluid, was not significantly different from control patients with clear fluid. (mean = 20, SD = 15.8, for patients with blhood stained fluid, vs mean =14.5, SD=27, for clear fluid, p=0.55), nor were the ADA levels higher in tuberculous patients with blood stained fluid compared to tuberculous patients with clear fluid. (Mean = 100U/l, SD = 49.5 for blood stained fluid, vs 117 U/l SD=45.7, for clear fluid, p= 0.2).

Similarly, neither total ascites white cell count, neutrophil or lymphocyte count correlated with ascites adenosine deaminase activity. Because one patient with a false positive result had pancreatic ascites together with a Candidal peritonitis, all other patients with pancreatitis were analysed. There were 5 control patients with pancreatic ascites. Apart from the patient with simultaneous infection, none had raised ADA activity. The mean ADA was 12.75 U/l SD=12.7).

There was no apparent race or sex predilection for false positive results. Only the prospective data was analysed, because the retrospective data was selected for race and sex. 1 of 12 whites (8.3%), 2 of 26 coloureds (7.7%) and 2 of 17 (11.8%) black controls had false positive results.

In summary, this study has shown that ascites adenosine deaminase activity is raised in tuberculous peritonitis and that this accurately distinguishes between tuberculosis and non-tuberculous patients. The accuracy remains high in cases of dual disease, where tuberculosis is superadded to an underlying disease which has caused ascites. It also appears to be accurate in distinguishing spontaneous bacterial peritonitis from tuberculous peritonitis. False positive results do occur but are uncommon. They were seen, in this study, in patients with poorly differentiated adeno-carcinomas of gastrointestinal and renal origin and in cases where the primary site of carcinoma was not found. False positive results may also occur in the presence of peritonitis associated with a "surgical" abdomen, but have not been observed in a small number of cases of spontaneous bacterial peritonitis.

Because of the common association of tuberculous peritonitis with other diseases, adenosine deaminase activity should be routinely measured in all cases of ascites, irrespective of supposed etiology. If positive, the adenosine deaminase activity may serve as a guide to further investigation, eg laparotomy or laparoscopy. This approach would reduce the number of patients exposed to these highly invasive procedures, and hence should reduce the morbidity mortality associated with the diagnostic process, by preselecting the patients most likely to benefit from laparoscopy or laparotomy.

A positive ADA result will also alert the clinician to the possibility of tuberculosis, in subjects with dual disêase, where tuberculosis had not previously been suspected.

References

1.Benatar SR. Tuberculosis in the 1980s, with particular reference to South Africa. S Afr Med J 1982;62:359-64.

2.Gilinsky NH, Marks IN, Kottler RE, Price SK. Abdominal tuberculosis. A 10 year review. S A Med J 1983; 64: 849-857

3.Novis BH, Marks IN. Gastrointestinal and peritoneal tuberculosis: A study of cases at Groote Schuur Hospital 1962-1972. S Afr Med J 47 1973;47:365-72.

4.Ibrahim EM,AnimJT,Al-Idrissi,Al-Mohaya S. Abdominal tuberculosis a frequent diagnostic challenge. Ann Trop Med Parasitol. 1985;79:163-7

5.Deville de Goyet C, Kleeberg HH. Comparison of tuberculous case finding in a high prevalence area. S Afr Med J 1974;48:2582-7

6.South African Medical Research Council Tuberculosis Research Institute. Annual report 1977-8. Parrow Valley,CP:SAMRC,1978.

7.Department of National Health (Collie A.) Extrapulmonary tuberculosis. Epidemiological Comments 1987 September, 14(9): 1-21

8.Bastani B, Shariatzadeh MR, Dehdashti F. Tuberculous peritonitis - Report of 30 Cases and Review of the literature. Q J Med New series 1985; 56(221):549-557

9.Menzies RI,Alsen H,Fitzgerald M, Mohapeloa RG. Tuberculous peritonitis in Lesotho. Tubercle 1986; 67:47-54.

10.Nwokolo C. Ascites in Africa. Br Med J 1967;1:33-37.

11.Farer LS, Lowell AM, Meador MP. Extrapulmonary tuberculosis in the United States.. Am J Epidemiol. 1979; 109 (2): 205-217.

12.Wells AD, Northover JMA, Howard ER. Abdominal tuberculosis: still a problem today. J Roy Soc Med 1986;79:149-53

13.Sunderam G, McDonald RJ,Maniatis T, Oleske J,Kapila R, Reichman LB. Tuberculosis as a manifestation of the aquired immunodeficiency syndrome. JAMA 1986;256(3):362-6

14.Saltzman BR, Motyl MR, Friedland GH, McKitrick JC, Klein RS. Mycobacterium tuberculosis bacteremia in the aquired immunodeficiency syndrome. Case report. JAMA 1986;256(3):390-1.

15.Stoneburner RL, Kristal A. Increasing tuberculosis incidence and its relationship to aquired immunodeficiency syndrome in New York City. International conference on aquired immunodeficiency syndrome (AIDS), Atlanta, April 1985.

16.Pitchenik AE, Cole C, Russel BW, Fischl MA, Spira TJ, Snider DE, Tuberculosis, atypical Mycobacteriosis and the aquired immunodeficiency syndrome among Haitian and non-Haitian patients in South Florida. Ann Int Med. 1984;101:641-45.

17.Stead WW, Lofgren JP, Warren E, Thomas C. Tuberculosis as an endemic and nosocomial infection among the elderly in nursing homes. N Engl J Med 1985; 312:1483-7

18.Mackay AD, Cole RB. The problems of tuberculosis in the elderly. Q J Med 1984;53:497-510.

19.McAdam J, Brickner PW,Glicksman R, Edwards D, Fallon B, Yanowitch P. Tuberculosis in the SRO/homeless population. IN Brickner PW, Sharer LK, Conanan B, Elvy A,Savarese M, eds. Health care for homeless people. New York. Springer, 1985:155-75.more invasive procedures,

20.Goedert JJ, Weiss SH, Biggar RJ, Landesman SH, Weber J, Grossman RJ, Guroff MR. Lesser Aids and tuberculosis (Letter). Lancet 1985; ii: 52.

21.Biggar RJ. The AIDS problem in Africa. Lancet 1986; i: 79-82.

22.Alvarez S, McCabe WR. Extrapulmonary tuberculosis revisited: a review or experience at Boston City and other hospitals. Medicine (Baltimore) 1984;63:25-55.

23.Festenstein F. Tuberculosis in hospital doctors. B Med J 1984;289:1327-8

24.Lange P, Mortensen J, Viskum K. Tuberculosis.in a developed country. Acta Med Scand 1986; 219:481-7

25.Stead WW. Pathogenesis of a first episode of chronic tuberculosis in man: Recrudescence of residuals of the primary infection or exogenous reinfection? Am Rev Respir Dis 1975;111:573-77.

26.Nardell E, McInnes B, Thomas B, Weidhaas S. Exogenous reinfection with tuberculosis in a shelter for the homeless. N Eng J Med. 1986; 315(25):1570-5.

27.Nice CM Jr. The pathogenesis of tuberculosis. Dis. Chest. 1950; 17:550-560.

28.Pettengell KE, Simjee AE, Larsen C, Garb M. Gastrointestinal tuberculosis in patients with pulmonary tuberculosis. S Afr Med J 1987;72:65

29.Medical Research Council Tuberculosis and Chest diseases Unit. National survey of tuberculosis notifications in England and Wales 1978-9. Br Med J 1980;281:895-98.

30.Palmer KR, Patil DH, Basran GS, Riordan JF, Silk DBA. Abdominal tuberculosis in urban Britain- a common disease. Gut 1985; 26:1296-1305.

31.Lambrianides AL, Ackroyd N Shorey BA. Abdominal tuberculosis. Br.J.Surg. 1980; 67: 887-89.

32.Khoury GA, Payne CR, Harvey DR. Tuberculosis of the peritoneal cavity. Br. J. Surg. 1978; 65: 808-11.

33.Cook GC. Tuberculosis-Certainly not a disease of the past. Editorial. Q J Med 1985; 56(221): 519-21.

34.Grange JM, Aber VE, Allen BW. et al Comparison of strains of mycobacterium TB from British, Ugandan and Asian immigrant patients: a Study in Bacteriophage typing, susceptability to hydrogen peroxide and sensitivity to thiophen-2 carbonic acid hydrazide. Tubercle 1977;58:207-15

35.Singh MM, Bhargava AN, Jain KP. Tuberculous peritonitis. An evaluation of Pathogenetic Mechanisms, Diagnostic Procedures and Therapeutic Measures. N Eng J Med. 1969; 281:1091-4

36.Sherlock S. Diseases of the liver and biliary system. 1985;p117-34 Blackwell Scientific Publications 7th ed .

37.Fastaia J,Dumont AE, Pathogenesis of ascites in mice with peritoneal carcinomatosis. J Natl Cancer Inst 1976; 56(3): 547

38. Coates G, Bush RS, Aspin N. A study of ascites using lymphoscintigraphy with ^{99m}Tc-sulfur colloid. Radiology. 1973: 107:577-583.

39.Garrison RN, Galloway RH, Heuser LS. Mechanism of malignant ascites production. J.Surg.Res. 1987; 42: 126-132.

40.Wolfe JHN, Behn AR, Jackson BT. Tuberculous peritonitis and the role of diagnostic laparoscopy. Lancet 1979;I:852-3

41.Rodriguez de Lopo C, San Migual Jogler S, Pons Romero F. Laparoscopic diagnosis of tuberculous ascites. Endoscopy 1982; 14:178-9.

42.Stites DP, Stobo JD, Fudenberg HH, Wells JV. Basic and clinical immunology 5th ed.1984. Lange Medical publications. Los Altos California.

43. West JB (Ed). Physiological basis of medical practice 11th ed. Williams and Wilkins Baltimore London 1985:375-89.

44.Bhansali SK. Abdominal tuberculosis. Experience with 300 cases. Am J Gastro. 1977; 67(4) 324-337.

45.Karney WW, O'donoghue JM, Ostrow JH, Holmes KK, Beaty HN. The spectrum of tuberculous peritonitis. Chest, 1977; 72: 310-315.

46.Orrego H,Israel Y, Blendis LM. Alcoholic liver disease: information in search of knowledge? Hepatology 1981; 1:267.

47.Snider DE. The tuberculin skin test. Am.Rev.Resp.Dis. 125(supp) 1982; 108-18.

48.Vyravanathan S, Jeyarajah R. Tuberculous peritonitis: a review of thirty cases. Postgad Med J 1980; 56:, 649-51.

49.Witte CL Witte MH Dumont AR. Protein content of lymph and edema fluid in congestive heart failure. Circulation 1969; 60:623-30.

50.Di Bisceglie AM, Schamroth CL. Transudative ascites with a high protein content. S Afr Med J 1985; 67:941-2

51.BoyerTD, Kahn AM, Reynolds TB. Diagnostic value of ascitic fluid Lactic Dehydrogenase, Protein, and WBC levels. Arch Int Med. 1978; 138:1103-1105.

52.Sampliner RE,Iber FL. High Protein ascites in patients with uncomplicated cirrhosis. Am J Med Science 1974;267:275-9

53. Binford CH, Meyers WM, & Connor DH, Krieg RE, & Dooley JR. Section 6 Diseases caused by mycobacteria. In Binford CH, and Connor DH. Pathology of tropical and extraordianry diseases Armed Forces Institute of Pathology. Washington DC 1976:205-243.

54.Millard M. Pathology of the lung, pleura and mediastinum: In Anderson WAD, Kissane JM (Eds) Pathology 7th ed CV Mosby St Loius 1977; 1101-5.

55.Kelling G. Uber oesophagosckopie, gastroskopie, und zolioskopie. Munch Med Wochenschr. 1902; 49: 21-4 as reported in ref(kane, and krejs)

56.Geake TMS Spitaels JM. Moshal MG, Simjee AE. Peritoneoscopy in the diagnosis of tuberculous peritonitis. Gastrointest endosc 1981;27(2): 66-68.

57.Jorge AD. Peritoneal tuberculosis. Endoscopy 1984; 16:10-12.

58.Wolfe JHN Behn AR, Jackson BT. Tuberculous peritonitis and the role of diagnostic peritoneoscopy. Lancet 1979 I:852-3.

59.Nafeh MA Shahwan MM, Mohammed SS. Rashwan NM. Endoscopic diagnosis of ascites in Assiut province, Upper Egypt. Endoscopy 1983; 15:347-9

60.Kane MG, Krejs GJ. Complications of diagnostic laparoscopy in Dallas: a 7 year prospective study. Gastrointest endosc. 1984; 30:237-40

61.Gilinsky NH, Voigt MD, Bass DH, Marks IN. Tuberculous perforation of the bowel. S Afr Med J

62.Sochocky S. Tuberculous peritonitis. A review of 100 cases. Am Rev Resp Dis. 1967; 95:398-401

63.Piedrahita P, Bitterfield WC. Abdominal exploration as a diagnostic procedure. Am J Surg. 1976;131:181-206.

64.Powell Jackson P, Greenway B, Williams R. Adverse effects of exploratory laparotomy in patients with unsuspected liver disease. Br J Surg. 1982; 69:449-51.

65.Donohoe RF, Schnider BI Gorman J. Needle biopsy of the peritoneum. Arch Int Med (Chicago) 1959; 103:739-42.

66.Levine H Needle biopsy diagnosis of tuberculous peritonitis. Am Rev resp dis. 1968; 97:889-894

67.Levine H (Letter) Needle biopsy diagnosis of tuberculous peritonitis. Am Rev resp dis. 1968; 98: 519

68.Sarin LR, Mehta SR, Sharma SK. Diagnosis of abdominal tuberculosis: A critical evaluation of various techniques with particular reference to peritoneal biopsy. Ind J Med Science. 1964;18:319-28

69.Jain SC, Misra SM, Misra NP, Tandon PL: Peritoneal biopsy in ascites. J Ind Med Assoc 1964; 43: 219

70.Sherman S, Rohwedder JJ, Ravikrishnan KP, Weg JG. Tuberculous enteritis and peritonitis. Report of 36 general hospital cases. Arch Int Med 1980;140; 506-508

71.Arloing S Courmont P.Sur la recherche et la valeur cliniquede l'agglutination du bacille de Kochpar le serum sanguin de l'homme. C R Acad Sci (Paris) 1898;127:425-8. as reported in Nicholls AC ref. 4

72.Besredka A. Ueber die fixationsreaktion bei tuberculose, der meerschweinschen. Kanichen und menschen.Z Immun Forschg. 1914;21:77

73.Daniel TM, Debanne SM. The sero diagnosis of tuberculosis and other mycobacterial diseases by enzyme linked imunosorbant assay. Am Rev Resp Dis 1987; 135: 1137-51

74.Mitcheson DA, Aber VR, Ahmed FJ Allen BW, Devi S. Evaluation of a serological test for tuberculosis. Br Med J 1977;1:1383-4.

75.Nicholls AC, Horsfield K. Thorax 1976;31:289

76.Winters WD, Cox RA. Serodiagnosis of tuberculosis by radioimmunoassay. Am Rev resp dis.1981;124:582-5

77.Minden P, Mc Clatchly JK, Cooper R, Bardana EJ, Farr RS. Science 1972;176:57-8

78.Bardana EJ, Mc Clatchly JK, Farr RS, Minden P. Universal occurrence of antibodies to tubercle bacilli in sera from tuberculous and non-tuberculous individuals. Clin exp Immunol 1973;13:65-77.

79.Brooks , Choudhary G, Craven RB, Alley CC, Liddle JA. Edman DC, Conerse JD. Electron capture gas chromatography detection and mass spectrum identification of 3-(2'-ketohexyl)indoline in spinal fluids of patients with tuberculous meningitis. J Clin Microbiol 1977;5:625-8.

80.Mardh PA, Larson L Hoiby N, Engbaek HC Odham G. Tuberculostearic protein as a diagnostic marker in tuberculous meningitis. Lancet 1983;1:367

81.French GL, Teoh R, Chan CY, Humphries MJ, mCheung SW, O'Mahony G. Diagnosis of tuberculous meningitis by detection of tuberculostearic acid in cerebrospinal fluid. Lancet 1987 ii:117-9.

82.Sada E, Ruiz-palacios GM, Lopez-Vidal Y, Ponce de Leon S. Detection of mycobacterial antigen in cerebrospinal fluid of patients with tuberculous meningitis by enzyme linked imunoabsorbant assay. Lancet 1983;ii:651-2

84.Kadival GV, Mazarelo TBMS, Chaparas SD. Sensitivity and specificity of enzyme linked imunoabsorbant assay in the detection of antigen in tuberculous meningitis cerebrospinal fluids. J Clin Microbiol 1986; 23:901-4

85.Bal V, Kamat RS, KamatJ KandothP. Enzyme linked imunoabsorbant assay for mycobacterial antigens. Ind J Med Res 1983; 78:477-83.

86.Krambovitis E Mcillmurray MB, Lock PE Hendrickse W Holzel H. Rapid diagnosis of tuberculous meningitis by latex particle agglutination. Lancet 1984; ii:1229-31.

87.Chandramuki A, Allen PRJ, Keen M, Ivanyi J. Detection of mycobacterial antigen and antibodies in the cerebrospinal fluid of patients with tuberculous meningitis. J Med Microbiol 1985; 20:239-47.

88.Kadival GV, Samuel AM, Mazarelo TBMS, Chaparas SD. Radioimmunoassay for detecting Mycobacterial tuberculosis antigen in cerebrospinal fluids of patients with tuberculous meningitis. J Inf Dis 1987; 155:608-11.

89.Baig MMe, Pettengell KE, Simjee AE, Sathar MA, Voster BJ. Diagnosis of tuberculosis by detection of mycobacterial antigen in pleural effusions and ascites. S Afr Med J 1986; 69:101-2.

90.Chawla TC Sharma A Kiran U, Bhargava Shriniwas DK, Tandon BN. Tubercle 1986; 67:55-60.

91.Kalckar H. J Biol Chem. 1947; 167:461

92.Giblett ER, Anderson HE, Cohen F, Pollara B, Meuwissen HJ. Lancet 1972 ii:1067

93.Shore A Dosch H-M Gelfand EW. Role of adenosine deaminase in the early stages of T cell maturation. Clin Exp Immunol 1981;44: 152-5.

94.Minkowski MD, Bandiera A. Different functional subsets of cultured T cells express characteristic levels of adenosine deaminase activity. Cellular Immunol 1985; 95: 380-91

95.Fischer D, Van der Weyden MB, Snyderman R. A role for adenosine deaminase in human monocyte maturation. J Clin Invest 1976; 58: 399-407

96.Donald PR, Malan C, Van Der Walt A, Schoeman JF. The simultaneous determination of cerebrospinal fluid and plasma adenosine deaminase activity as a diagnostic aid in tuberculous meningitis. S Afr Med J 1986;69:505-7.

97. Galanti B, Guisti G. Minerva Med 1968; ii(59):5867

98.Muller Beissenhirtz von W, Keller H. Die bestimmung der adenosinedeaminase im serum. Prufung diagnosticher moglichkeiten. Deutsch Med Wochenschr 1966; 91:159-68

99.Malan C,Donald PR, Golden M, Taljaard JJF. Adenosine deaminase levels in cerebrospinal fluid in the diagnosis of tuberculous meningitis. J Trop Med Hyg 1984; 87:33-40

99.Maritz FJ, Malan C Le Roux I. Adenosine deaminase estimations in the differentiation of pleural effusions S Afr Med J 1982; 62:556-8

100.Pettersson T Ojala K, Weber TH. Adenosine deaminase in the diagnosis of pleural effusions. Acta Med Scand 1984; 215: 299-304

101.Van Heerden IJ, Du Plessis DJ. Adenosine deaminase in urine- a marker for urinary tract tuberculosis.(let) S Afr Med J 1986;70:121

102.Giusti G. Adenosine deaminase. In Bergmeyer HU, ed. Methods of enzymatic analysis, vol 2. 2nd ed. New York academic Press, 1974: 1092-99.

103. Chaney AL, Marbach EP. Clin Chem. 1968;8: 130

104. Berthelot M. Repertoire de chimie applique. 1859; 1: 284. As reported in (93).

105.Kohler H, Benz EJ. Clin Chem. 1962;8:133

106.Runyan BA, Hoefs JC. Culture-negative neutrocytic ascites: A variant of spontaneous bacterial peritonitis. Hepatology 1984; 4(6):1209-1211.

107.Dineen P, Homan WP, Grafe WR. Tuberculous peritonitis. 43 years experience in diagnosis and treatment. Ann Surg. 1976; 184(6): 717-22.

108.Barrow DW. Tuberculous peritonitis . Southern Med.J 1943;36: 646 as reported in (63)

109.Burack WR, Hollister RM Tuberculous peritonitis: A study of 47 cases encountered by a general medical unit in 25 years. Am.J.Med.1960; 28:510-23.

110.Voigt MD, Kalvaria I, Isaacs S, Kirsch RE. (Abstract) Dept. Medicine Conference: October 1986.

111.Martinez-Vazquez JM, Ocana I, Ribera E, Segura RM, Pascual C. Adenosine deaminase activity in the diagnosis of tuberculous peritonitis. Gut 1986; 27:1049-53.

112.Klimach OE, Ormerod LP. Gastro-intestinal tuberculosis: A retrospective review of 109 cases in a district general hospital. Q J Med. 1985; 56(221): 569-78.

113.Das P, Shukla HS. Clinical diagnosis of abdominal tuberculosis. Br J Surg. 1976; 63: 941-46.

114.Al-Arif LI, Goldstein R, Affronti LF, Janicki BW. HLA-BW15 and tuberculosis in a North American black population. Am Rev resp dis.1981;123:356-8.

115. Osler WA. Principles and practice of Medicine, 7th Ed. Appleton-Century Crofts, New York. 1909.

116.Van Der Weyden MB, Kelley WN. Human adenosine deaminase. Distribution and properties. J.Biol Chem. 1976; 251(18): 5448-56.

117.Ten Kate J, Wijnin J Th, Boldewijn J Meera Khan P, Bosman FT. Immunohistochemical localization of adenosine deaminase complexing protein in intestinal mucosa, and in colorectal adeno-carcinomas as a marker of tumor cell heterogeneity. Histochem J. 1985; 17:23-31.

118.Svendsen JH, Mikkelsen AL, Siemssen OJ. Peritonitis due to genital tuberculosis. Ann Chir Gynaecol 1985; 74(4): 180-2

12 SEP 1988

119.Reynolds TB, Campra JL. Ascites in liver disease. In Berk JE, Haubrich WS, Kalser MH, Roth JL, Schaffner F. (Eds): Bockus Gastroenterology, .Vol. 5 The Liver 4th ed 1985:3121-3125 WB Saunders Philadelphia

120.British Thoracic and Tuberculosis Association. Tuberculosis among immigrants related to length of residence in England and Wales. Br Med J. 1975;3:698-9.

121.Jaffe HW Bregman DJ, Selik RM: Aquired Immunodeficiency syndrome in the United States: The first 1000 cases. J.Inf.Dis.1983;148:339-345.

122.Crossley JR, Williams R.:Spontaneous bacterial peritonitis. Gut 1985; 26:325-8