Evaluation of Immune Responses in HIV-infected Patients with Pleural Tuberculosis by the QuantiFERON TB Gold Interferon-gamma assay

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Abstract

Background

Diagnosis of tuberculous (TB) pleuritis is difficult and better diagnostic tools are needed. New blood based interferon-gamma (IFN-γ) tests are promising, but sensitivity could be low in HIV positive patients. The IFN-γ tests has not yet been validated for use in pleural fluid, a compartment with higher level of immune activation than in blood.

Methods

The QuantiFERON TB-Gold (QFT-TB) test was performed on blood and pleural fluid in parallel from 34 patients presenting with clinically suspected pleural TB. Clinical data, HIV status and CD4 cell counts were obtained. Adenosine deaminase activity (ADA) and TB culture in pleural fluid were recorded. The patients were categorized as ‘confirmed TB’, ‘probably TB’ and ‘non-TB’ pleuritis based on TB culture results and clinical and biochemical criteria.

Results

The majority of the patients were HIV infected (74%). The QFT-TB in pleural fluid was positive in 27% and 56% of the ‘confirmed TB’ and ‘probably TB’ cases, respectively, whereas the corresponding sensitivities in blood were 58% and 83%. Specificity of the QFT-TB test in blood and pleural fluid was 100%. Mitogen (positive control) IFN-γ responses in blood were significantly lower in the TB patients compared to the ‘non-TB’ cases (p=0.012) and correlated with CD4 cell count (r=0.600, p=0.028). In blood there was altogether 21% indeterminate QFT-results and the number increased with decreasing CD4 cell counts. In contrast, in pleural fluid there was no correlation between CD4 cell count and indeterminate results (50%).
IFN-γ responses in pleural fluid were high in both TB groups, but lower compared to the IFN-γ measured in the TB antigen (p=0.023) and mitogen samples (p=0.042).

Conclusions

The QFT-TB test could contribute to the diagnosis of culture negative TB pleuritis in certain cases in order to urge initiation of TB therapy. Still, at this stage there is too high numbers of inconclusive results in the HIV positive population to recommend the commercial QFT-TB test for routine use in the diagnostics of pleural TB in a TB/HIV endemic resource-limited setting.
**Background**

Tuberculosis (TB) is globally a major health burden and human immunodefiency virus (HIV) infection is a strong risk factor for the progression from latent infection to active TB. In South Africa 60% of the adult TB cases are HIV positive [1]. TB pleuritis occurs in about 30% of TB patients, the majority of cases in the HIV positive population [2].

The diagnosis of TB pleuritis is generally difficult and the acid fast bacilli [AFB] microscopy method rarely detects the tubercle bacilli, whereas culture is positive in only 40% of cases [3]. Histology of pleural biopsies could offer a sensitivity of up to 80% in immunocompetent patients [4], but expected to be much lower in HIV patients and specificity is generally low. Adenosine deaminase activity (ADA) in pleural fluid is used as a marker of TB pleuritis and a meta-analysis concludes that although variable performance, it is still a considerable good test [5]. However, emphyema, rheumatoid pleurisy and malignancy may give false positive results [6], while immune suppression could give false negative test [7]. Finally, a sensitivity as low as 17% in pleural fluid has been reported for the polymerase chain reaction (PCR) method [8].

HIV infection has changed the nature of the clinical presentation of pleural TB [2]. Patients co-infected with HIV and TB demonstrates stronger pleural reaction than HIV negative persons. Further, T cells from the pleural cavity of patients with TB pleuritis are more activated than those from the peripheral blood and there seems to be a compartmentalisation of TB specific interferon-gamma (IFN-γ) producing cells in the lungs of patients with active TB [9]. T cell responses to TB antigens reside predominantly in the CD4+ T cell subset [10]. A decline in the number of CD4+ T cells and an expansion of activated memory CD8+ T cells characterise chronic HIV infection [11]. Thus, it is of importance to study the immune responses at the local
site of infection in order to improve the understanding of the immunological mechanisms involved in containment and progression of TB in HIV infected patients.

TB proteins encoded by the RD-1 gene of *Mycobacterium tuberculosis* (*M. tuberculosis*) are used in commercially available IGRA (Interferon-gamma Release Assays) blood tests [QuantiFERON®-TB Gold In-tube, (QFT-TB) and T spot-TB®] [12-15]. They offer comparable high sensitivities and specificities in the diagnosis of TB in immunocompetent patients, but there is concern about the sensitivity in immunocompromised patients, especially when using the QFT-TB test [16-18]. Thus, IFN-γ based assays may give false negative TB diagnosis in endemic areas with high burden of HIV co-infection where reliable diagnostics tools are needed the most. Several studies have evaluated the new IFN-γ assays in blood from patients with active, including extrapulmonary TB, but few HIV patients or cases of pleural TB have been included and analysis have predominately been performed on blood specimens [14,17,19-22].

The ELISA technique used in the QFT-TB assay could easily be adapted to routine practice and a recent review by Gopi *et al* requests studies of the potential efficacy of IFN-γ assays in the diagnosis of pleural TB [23]. In this study we have quantified TB specific and unspecific immune responses in the pleural cavity of patients with HIV and TB co-infection by using the commercial available QFT-TB test as well as evaluated the utility of this test as a diagnostic tool of TB pleuritis in a high TB and HIV endemic area.
Methods

Study participants
Patients presenting with pleural fluid and clinical suspicion of TB pleuritis admitted to the Dr. George Mukhari Hospital (DGM), Ga-Rankuwa, Pretoria, South Africa in the period 2004-2005 were recruited into the study. The patients were categorized as; (i) ‘confirmed TB’ pleuritis (AFB microscopy or culture positive pleural fluid), (ii) ‘probably TB’ pleuritis (AFB microscopy and culture negative pleural fluid, but ADA ≥30 U/L and clinical symptoms of TB with anti-tuberculous therapy started) and (iii) ‘non-TB’ pleuritis when diagnosed as malignancy or another non-TB condition. The demographic and clinical data, HIV status and CD4 cell count were recorded for each patient (Table 1). Thoracocentesis, but not routinely pleural biopsies (for two patients only), were performed according to clinical practice at the hospital. Peripheral blood was obtained in parallel and before initiation of anti-tuberculosis therapy. None of the patients were treated with antiretroviral therapy.

Specimen preparation and culture
The pleural fluid and sputum specimens were processed according to standard laboratory routines for AFB staining of smears and culture for up to four weeks (BacT-alert, Organon, Teknika). The pleural fluid was also sent for routinely analysis of ADA, using a commercial colorimetric assay kit (Diazyme General Atomics, CA) and for cytological examination. Pleural fluid mononuclear cells (PFMCs) were isolated from approximately 200 ml pleural fluid by density gradient centrifugation (Ficoll histopaque 1077, Sigma), washed and resuspended in RPMI media (1640, L-glutamine and HEPES supl., Sigma) to make a final concentration of 1 x 10^6 cells/ml.
QuantiFERON® TB Gold In-Tube assay

One ml of whole blood and one ml of PFMC suspension were added to each of the three ‘QuantiFERON-TB Gold In-tubes’; TB antigen (ESAT-6, CFP-10 and TB 7.7), positive mitogen control (phytohemagglutinin [PHA]) and negative control (Nil), as provided by the manufacturer (Cellestis Ltd., Victoria, Australia), mixed well and incubated at 37°C for 20 hours. The tubes were centrifuged and 500 µl of the supernatants were harvested and stored at -70°C until the IFN-γ was measured in duplicates by an ELISA reader. The IFN-γ concentrations (IU/ml) were calculated by the ‘QFT-TB analysis Software’. When the value was >15 IU/ml, the maximum concentration that can be estimated from the standard curve, the value was set to 15 IU/ml.

According to the manufacturer, the test was considered positive with TB antigen minus Nil ≥0.35 IU/ml and ≥25% of Nil. The test was negative with i) TB antigen minus Nil <0.35 IU/ml or ii) ≥0.35 IU/ml and <25% of Nil if PHA minus Nil ≥0.5 IU/ml. The test was indeterminate with i) TB antigen minus Nil <0.35 IU/ml or ii) ≥0.35 IU/ml and <25% of Nil if PHA minus Nil <0.5 IU/ml. Thus, valid positive and negative controls were a prerequisite for a determinate test.

Pleural fluid supernatants were tested in 1:1, 1:10 and 1:20 dilutions. The mean IU/ml from two parallel samples from the dilution that was within the range of the assay was used to calculate the final results. The values for the pleural fluid were then multiplied with the dilution factor in order to standardise the final results to the number of cells (IU/1 x 10⁶ cells).

Statistics

The statistical analysis was performed by SPSS 14 (SPSS Inc., Chicago, IL, USA). IFN-γ responses are shown as mean, standard deviation and 95% confidence interval (CI) for mean. An independent samples t-test was used to compare differences in IFN-γ responses between HIV positive and HIV negative patients. The Pearson correlation coefficient was computed for
associations between continuous variables. The relationship between IFN-γ responses and HIV and TB status was investigated by linear regression. A P-value of < 0.05 was considered to indicate a statistically significant difference.
Results

Clinical and laboratory characteristics

Thirty-four patients with clinically suspected pleural TB were enrolled in the study and the clinical and laboratory characteristics are shown in Table 1. Chest pain, night sweat and loss of weight were the most common clinical symptoms seen in 85-100% of the patients.

Twelve (35%) of the patients had culture ‘confirmed TB’ pleuritis, whereas 16 patients (47%) were diagnosed as ‘probably TB’ pleuritis based on ADA ≥30 U/L and clinical criteria. Of the remaining six patients classified as ‘non-TB’ pleuritis, five were diagnosed with malignancy by cytology or histology, whereas one HIV case did not receive a final diagnosis.

Among all the suspected and confirmed TB cases 82% (23/28) were HIV positive. The mean CD4 cell count was 104 cells/microL ± 82 (95% CI 45-163) and 230 cells/microL ± 180 (95% CI 131-330) for the ‘confirmed TB’ and ‘probably TB’ patients, respectively (p>0.05), whereas the ‘non-TB’ group (two HIV positive and four HIV negative) had a CD4 cell count of 402 cells/microL ± 322 (95% CI 63 – 740), (p=0.02). However, when adjusted for HIV status there was no significant difference in CD4 cell count between the groups.

ADA was measured in pleural fluid and was one of the criteria for the probably TB diagnosis. The ADA concentrations were comparable in the ‘confirmed TB’ and ‘probably TB’ groups [72 U/L ± 25 (95% CI 54–91) and 68 U/L ± 40 (95% CI 46–89), (p>0.05)], respectively, but significantly higher than in the ‘non-TB’ group [13 U/L ± 5 (95% CI 6–19), (p=0.01)]. The ADA level in pleural fluid from the HIV positive was comparable to the HIV negative patients (62 ±
26 vs 55 ± 63 U/L), (p>0.05) and we found no significant correlation between ADA values and CD4 cell counts.

**QuantiFERON TB Gold results in blood and pleural fluid**

The absolute concentrations of IFN-γ in blood and pleural fluid for the ‘confirmed TB’, ‘probably TB’ and ‘non-TB’ cases are as given in table 2. In blood, the strongest TB IFN-γ responses were found in the ‘probably TB’ group, but both TB groups had higher values above the cut-off of the QFT-TB assay compared to the ‘non-TB’ patients, although this was not significant (p= 0.06). This was also the case when the nil value was subtracted from the TB antigen value in the respective patient (data not shown). For the mitogen control (PHA) IFN-γ responses there was a significant difference both between the ‘confirmed TB’ and ‘probably TB’ groups (p=0.012) and compared to the ‘non-TB cases’, (p=0.012). PHA responses in blood correlated with CD4 cell count (r=0.600, p=0.028).

The TB IFN-γ responses in pleural fluid were strongest for the ‘probably TB’ group also in this compartment, but both TB groups had IFN-γ values well above the ‘non-TB’ group, although this differences did not reach statistical significance (p>0.05). Whereas negative controls in blood gave low IFN-γ values, in pleural fluid these concentrations were rather high in both TB groups, still almost two-fold lower as compared to the IFN-γ concentrations measured in the TB antigen (p=0.023) and PHA samples (p=0.042). Within the TB patients there was no significant difference in negative control IFN-γ values when the patients were grouped based on HIV status (data not shown). High IFN-γ background values were also found for the ‘non-TB’ group, although at a lower level. However, high IFN-γ in the negative control was only found in the two
HIV positive patients (4.2 and 8.8 IU/1x10⁶ PBMCs) and not in the HIV negative malignancy patients (0.14-0.65 IU/1x10⁶ PBMCs). The various IFN-γ responses in pleural fluid did not correlate to either ADA or CD4 cell count.

By using the definition of cut-off values and valid tests as given by the manufacturer, these results implicated that in pleural fluid 12 (44%) of the overall 27 TB cases tested by the QFT-TB assay were positive, 14 (52%) gave indeterminate results whereas one was negative (4%) as compared to blood where 17 (71%) of the overall 24 tested TB cases were positive, six indeterminate (25%) and one negative (4%). Two of the ‘non-TB’ cases, both HIV positive, had indeterminate QFT-TB tests in pleural fluid, whereas all had conclusive negative results in blood. This gave sensitivities of the QFT-TB test in pleural fluid of 27% and 56% in the ‘confirmed TB’ cases and ‘probably TB’ cases, respectively, whereas the corresponding sensitivities in blood were 58% and 83% (table 3). Although a too small ‘non-TB’ control group to conclude strongly, both the specificity and positive predictive value for the QFT-TB test in blood and pleural fluid were 100% since no false positive tests were found in these patients.

Overall 16 (50%) of the QFT-TB results were conclusive in pleural fluid compared to 23 (79%) in blood. However, this percentage varied according to HIV status and whether TB was confirmed by culture (table 4). There was agreement between the two compartments in 15 of the cases. In contrast, seven patients showed positive QFT-TB test in blood, whereas the results were indeterminate in pleural fluid. In two HIV positive ‘confirmed TB’ patients the QFT-TB was positive in pleural fluid, whereas the results in blood were indeterminate and negative, respectively, because of low mitogen responses. The number of cases with indeterminate QFT-
TB results increased in blood as the CD4 cell count decreased to below 100 cells/microL. In contrast, there seemed to be more conclusive results in pleural fluid with low CD4 cell count.
Discussion

Tuberculous pleuritis is an AIDS defining illness and a common opportunistic infection in endemic areas like South Africa. Kaposi sarcoma and bacterial infections could also cause pleural effusion in HIV infected patients, making diagnosis difficult [24]. The majority of the TB patients in this study had low CD4 cell counts, reflecting the serious implication of this disease in Sub-Saharan Africa. Further, we confirm that low CD4 cell count could also be found in HIV negative TB pleuritis patients [25].

This is to our knowledge the first study evaluating the 2nd generation QFT-TB assay in the diagnosis of pleural TB, testing in parallel blood and pleural fluid from HIV infected patients. IFN-γ based assays have been studied in the diagnosis of extrapulmonary TB [14,17,19], but few studies have included HIV positive patients [16,18,26]. In patients with pleural TB, the tests has predominantly been performed on blood samples and not evaluated for directly use on pleural fluid specimens [20,21]. Finally, there has been inadequate evidence on the validity of IFN-γ assays in immunocompromised individuals, especially in countries where TB is endemic [27].

We found that in this group of South African patients with predominantly advanced HIV disease the overall sensitivity of the QFT-TB test in blood was 71% compared to only 44% in pleural fluid. The sensitivity in blood is comparable to that found in other studies of extrapulmonary TB in HIV negative patients, reporting sensitivities of IGRA-tests between 70-90% [14,17,19]. In a study of South African children with TB, the sensitivity of the ELISPOT assay was reduced from 83% in HIV negative to 73% in HIV positive children [18]. Our QFT-TB results are slightly superior to that reported by Sohn et al using the 1st generation QFT-TB test based on PPD in 28
suspected HIV negative TB pleuritis patients, giving sensitivities of 60% in blood and 44% in pleural fluid [20]. Assuming lower sensitivities if immunocompromised patients were included in their study, our data supports that the 2nd generation QFT-TB is more sensitive not only in blood [15], but also in pleural fluid than the original QFT-TB test. In comparison, only around 40% of our patients were confirmed TB by culture, which is comparable to other studies of HIV negative patients [3]. Our ‘non-TB’ control group is unfortunately too small to allow reliable specificity data and strong conclusions are further limited by the findings of indeterminate results in pleural fluid for two of the ‘non-TB’ patients. Still, no false positive QFN-TB results were found in any of the compartments, indicating that the QFT-TB seem to be a reasonable good ‘rule-in’ test.

For the patients with ‘confirmed TB’ the sensitivity of QFT-TB was only 58% in blood and 27% in pleural fluid, whereas corresponding sensitivities were 83% and 56% for the culture negative ‘probably TB’ patients. It is reported that positive culture are more common in advanced HIV patients compared to HIV negative TB pleuritis patients [28], possibly explained by spread of M. tuberculosis from the lungs into the pleural space increasing the mycobacteria concentration. We found a correlation between CD4 cell count and PHA responses in blood. Thus, the trend, although not statistical significant, towards a lower CD4 cell count in the ‘confirmed TB’ group giving more indeterminate results due to low PHA responses could explains this difference in sensitivities. For two of the patients with positive test in pleural fluid, QFN-TB in blood was not conclusive. Keeping in mind that advanced HIV patients suffer from various opportunistic pulmonary infections making precise diagnosis difficult, testing QFN-TB in pleural fluid could
at certain occasions help diagnosing culture negative TB pleuritis and contribute to decision making in the clinical setting.

In blood we found 25% indeterminate results in the TB group due to weak PHA IFN-γ responses. The percentage of indeterminate results in blood increased as the CD4 cell count decreased, most likely caused by T cell anergy, typically seen in advanced HIV infection [29]. However, several patients had CD4 cell counts in the range of 50-100 cells/µL, but still positive QFT-TB tests both in blood and pleural fluid. In contrast, non-conclusive results could also be obtained in blood from HIV patients with rather high CD4 cell count, indicating that also the quality of the individual’s general and TB specific immunity is a prerequisite for a conclusive test. Recent studies of patients on immunosuppressive therapy [17] and with HIV infection [16] have also reported high numbers of indeterminate results. All together, these data point to the fact that inconclusive results from blood are more common in immunocompromised patients and need to be taken into consideration in the clinical setting.

In contrast, in pleural fluid we found no correlation between blood CD4 cell count and PHA IFN-γ responses, and in this compartment TB and PHA responses were generally strong. Rather, indeterminate results were caused by high levels of IFN-γ measured in the negative control samples. It has been shown that patients with pleural TB who are co-infected with HIV have a higher rate of pleural inflammation than that observed in non-HIV-infected persons [30]. This could explain the high IFN-γ responses from in vitro unstimulated pleural T cells leading to inconclusive results, as seen in this study. Background responses were not correlated to peripheral blood CD4 cell count and were especially seen in HIV patients with CD4 cell counts
at the upper range for this cohort, supporting previous studies reporting high levels of chronic immune activation in HIV patients from early disease [31]. A compartmentalization of immune cells in HIV patients with an expanded population of activated memory CD8+ T cells in lymphoid tissue [31] as well as in the lungs [32] are seen from early stages of HIV infection. A marked increase in memory T cells has also been found in HIV negative patients with tuberculous pleurisy [33]. These T cells produce IFN-γ and could contribute to the high background, masking the TB specific immune responses in pleural fluid, especially in the HIV positive patients [30]. For the two HIV patients in the ‘non-TB’ group the QFT-TB was inconclusive, also due to high IFN-γ concentrations in the negative control samples, still at a much lower level than seen in the TB groups. In contrast, the HIV negative ‘non-TB’ patients with malignancy had all very low IFN-γ background levels, as earlier reported [34].

A recent meta-analysis concludes that IFN-γ is a sensitive and specific test for the diagnosis of tuberculous pleurisy [35]. However, in HIV endemic areas one should be aware of the problem with high background signals in HIV patients causing difficulties interpreting the results. Further, the cut off value for a positive QFT-TB test could be different in pleural fluid where immune responses are at a higher ‘set-point’ than in blood, especially in HIV positive patients. Still, we used the criteria that the actual TB antigen value should be >25% of ‘nil’ for each case in order for the test to be conclusive. The TB antigen IFN-γ responses were also significantly higher than the respective negative controls, indicating that the actual TB antigen value was above background signals when a conclusive result was given. We have tested QFT-TB on separated cells from pleural fluid although direct analysis of the fluid would be an easier approach in a clinical setting. However, we observed that there was a large variation in
concentration of cells as well as strength of responses between the various patients, possibly decreasing the sensitivity of the assay if concentration of cells was not performed.

Based on our results, one could argue that testing with IFN-γ assays only in blood, offering a better sensitivity than in pleural fluid, could be satisfactory also for TB pleuritis patients. In a high TB endemic country as South Africa one would expect a high number of patients with positive blood IFN-γ assays test because of latent TB making interpretation difficult. Still with associated pleural effusion, the diagnosis of active TB would be most likely. Thus, testing blood from HIV positive patients with QFT-TB could be useful improving management of patients as culture results takes time to obtain and pleural fluid sampling not always possible to perform. In support of this, Connell *et al.* have reported two perinatal TB cases where culture was positive only after six weeks, but QFT-TB results were available within 48 hours resulting in adequate and successful treatment for the patients [36].

Most studies comparing ADA and IFN-γ in the diagnosis of TB pleuritis have shown IFN-γ to be superior [37,38]. Some use ADA cut-off values at 45 U/L to exclude false positive tests [6]. In our study five TB patients had ADA in the range of 30-45 U/L, and we found that a positive QFT-TB both in blood or pleural fluid contributed to the TB diagnosis in patients with negative culture and ADA values close to cut-off values. We found no correlation between ADA and CD4 cell count or IFN-γ responses. Whether CD4 cell count impacts on the value of ADA in the diagnosis of tuberculous pleuritis in advanced HIV-1 infection warrant further studies in larger TB confirmed cohorts.
Conclusions

Although the commercial QFT-TB test is not validated for use in pleural fluid, our study indicates that the test could in certain cases contribute to the diagnosis of TB pleuritis in the HIV positive population in order to urge initiation of TB therapy. The test could especially be helpful in culture negative patients with low CD4 cell counts where diagnosis is particular difficult. Still, there is a compartmentalization of TB specific and unspecific immune responses between pleural fluid and blood, especially in HIV positive patients, as reported in our study. Thus, further and larger studies are needed to determine appropriate cut-off values as well as the usefulness and cost benefit of this test before it could be recommended for use in the diagnostics of pleural TB in a TB/HIV endemic resource-limited setting.

Competing interests

The authors have no financial or non-financial competing interests to declare.
Author’s contributions:

Kamaldeen Baba: has participated in initiation and design of the study, analysis and interpretation of data and drafting of the manuscript.

Steinar Sørnes: has participated in experimental work and revising of the manuscript.

Anwar A. Hoosen: has participated in initiation and design of the study, interpretation of data and revising of the manuscript.

Jacob M. Lekabe: has participated in experimental work and revising of the manuscript.

Mathew J. Mpe: has participated in initiation of the study, collection of samples and revising of the manuscript.

Nina Langeland: has participated in initiation and design of the study, interpretation of data and revising of the manuscript.

Anne Ma Dyrhol-Riise: has participated in initiation and design of the study, analysis and interpretation of data and drafting of the manuscript.

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References


Table 1. Clinical characteristics of patients enrolled (n=34)

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number</th>
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<tbody>
<tr>
<td><strong>Gender</strong></td>
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<tr>
<td>Females</td>
<td>12</td>
</tr>
<tr>
<td>Males</td>
<td>22</td>
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<tr>
<td>Age: median (range)</td>
<td>39 (20-70)</td>
</tr>
<tr>
<td><strong>Clinical symptoms: n (%)</strong></td>
<td></td>
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<tr>
<td>Chest pain</td>
<td>34 (100)</td>
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<tr>
<td>Productive cough</td>
<td>10 (29)</td>
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<tr>
<td>Fever</td>
<td>16 (47)</td>
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<tr>
<td>Shortness of breath</td>
<td>16 (47)</td>
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<tr>
<td>Night sweat</td>
<td>29 (85)</td>
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<tr>
<td>Loss of weight</td>
<td>31 (91)</td>
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<tr>
<td>Lymphadenopathy</td>
<td>27 (79)</td>
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<tr>
<td>Oral thrush</td>
<td>25 (74)</td>
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<tr>
<td>Chest x-ray infiltrates: n (%)</td>
<td>2 (6)</td>
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<tr>
<td>Culture positive TB: n (%)</td>
<td>12 (35)</td>
</tr>
<tr>
<td>Total TB cases</td>
<td>28 (82)</td>
</tr>
<tr>
<td>Non-TB cases</td>
<td>6 (18)</td>
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<tr>
<td>HIV-1 positive patients – n (%)</td>
<td>25 (74)</td>
</tr>
<tr>
<td>CD4 cell count HIV positive: mean (SD)(^a)</td>
<td>113 (80)</td>
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<tr>
<td>CD4 count &gt; 200 cells/microL</td>
<td>2 (8%)</td>
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<tr>
<td>CD4 count 100-200 cells/microL</td>
<td>7 (28%)</td>
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<tr>
<td>CD4 count &lt; 100 cells/microL</td>
<td>13 (52%)</td>
</tr>
<tr>
<td>CD4 cell count HIV negative: mean (SD)</td>
<td>490 (200)</td>
</tr>
</tbody>
</table>

\(^a\) CD4 cell count was not available for 3 patients.
Table 2. Interferon-gamma (IFN-γ) responses (TB antigen, PHA and nil) in blood (n=29) and pleural fluid (n=32)\(^a\)

<table>
<thead>
<tr>
<th></th>
<th>TB antigens</th>
<th>Positive control (PHA)</th>
<th>Negative control (nil)</th>
</tr>
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<tbody>
<tr>
<td><strong>Blood (IU/ml)</strong></td>
<td></td>
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<tr>
<td><strong>Confirmed TB pleuritis (n=12)</strong></td>
<td>2.89 ± 3.73 (0.52-5.27)</td>
<td>4.07 ± 6.70 (-0.19-8.32)(^b)</td>
<td>0.41 ± 0.55 (0.06-0.75)</td>
</tr>
<tr>
<td><strong>Probably TB pleuritis (n=12)</strong></td>
<td>4.53 ± 3.55 (2.27-6.78)</td>
<td>1.04 ± 1.14 (0.31-1.77)(^c)</td>
<td>0.37 ± 0.43 (0.09-0.64)</td>
</tr>
<tr>
<td><strong>Non-TB pleuritis (n=5)</strong></td>
<td>0.16 ± 0.09 (0.04-0.28)</td>
<td>9.51 ± 6.72 (1.25-15)</td>
<td>0.11 ± 0.04 (0.06-0.16)</td>
</tr>
<tr>
<td><strong>Pleural fluid (IU/ 1x10(^6) PFMCs)</strong></td>
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<tr>
<td><strong>Confirmed TB pleuritis (n=16)</strong></td>
<td>26.20 ± 48.38 (-6.30-58.71)(^d)</td>
<td>22.4 ± 28.31 (2.15-42.65)</td>
<td>15.88 ± 17.59 (4.06-27.70)</td>
</tr>
<tr>
<td><strong>Probably TB pleuritis (n=11)</strong></td>
<td>41.84 ± 46.50 (17.06-66.61)(^d)</td>
<td>29.79 ± 36.53 (10.32-49.26)</td>
<td>21.86 ±29.06 (6.37-37.34)</td>
</tr>
<tr>
<td><strong>Non-TB pleuritis (n=5)</strong></td>
<td>2.77 ± 3.08 (-1.05-6.59)</td>
<td>4.72 ± 2.79 (1.25-8.18)</td>
<td>2.90 ± 3.67 (-1.67-7.46)</td>
</tr>
</tbody>
</table>

Data are presented as mean (absolute values) ± SD. 95% confidence intervals are listed in brackets.

\(^a\) It was not possible to obtain blood from five of the patients and pleural fluid from two of the patients.

\(^b\) \(p < 0.05\) compared with 'probably TB' and 'non-TB' groups.

\(^c\) \(p < 0.05\) compared with 'confirmed TB' and 'non-TB' groups.

\(^d\) \(p < 0.05\) compared with positive control (PHA) and negative control.
Table 3. Results of the QuantiFERON-TB® Gold assay in peripheral blood (n=29) and pleural fluid (n=32)\textsuperscript{a}

<table>
<thead>
<tr>
<th></th>
<th>Total TB cases</th>
<th>Confirmed TB</th>
<th>Probably TB</th>
<th>non-TB</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>QFT-TB positive test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>17/24 (71%)</td>
<td>7/12 (58%)</td>
<td>10/12 (83%)</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>12/27 (44%)</td>
<td>3/11 (27%)</td>
<td>9/16 (56%)</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td><strong>QFT-TB negative test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>1/24 (4%)</td>
<td>1/12 (9%)</td>
<td>0/12 (0%)</td>
<td>5/5 (100%)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>1/27 (4%)</td>
<td>0/11 (0%)</td>
<td>1/16 (6%)</td>
<td>3/5 (60%)</td>
</tr>
<tr>
<td><strong>QFT-TB indeterminate test</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>6/24 (25%)</td>
<td>4/12 (33%)</td>
<td>2/12 (17%)</td>
<td>0/5 (0%)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>14/27 (52%)</td>
<td>8/11 (73%)</td>
<td>6/16 (38%)</td>
<td>2/5 (40%)</td>
</tr>
</tbody>
</table>

\textsuperscript{a}The nominator varies according to the number of patients tested by the assay in each group. It was not possible to obtain blood from five of the patients and pleural fluid from two of the patients.
Table 4. Conclusive results of the QuantiFERON-TB® Gold assay in peripheral blood and pleural fluid stratified by TB and HIV-1 status.

<table>
<thead>
<tr>
<th></th>
<th>Confirmed TB</th>
<th>Probably TB</th>
<th>non-TB</th>
<th>HIV-1 positive</th>
<th>HIV-1 negative</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>Total HIV-1</td>
<td>CD4 &gt; 200</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>CD4 100-200</td>
<td>CD4 ≤ 100</td>
</tr>
<tr>
<td>Peripheral blood</td>
<td>8/12 (67%)</td>
<td>10/12 (83%)</td>
<td>5/5 (100%)</td>
<td>15/21 (71%)</td>
<td>2/2 (100%)</td>
</tr>
<tr>
<td>Pleural fluid</td>
<td>3/11 (27%)</td>
<td>10/16 (63%)</td>
<td>3/5 (60%)</td>
<td>10/24 (48%)</td>
<td>0/2 (0%)</td>
</tr>
</tbody>
</table>

The nominator varies according to the number of patients tested by the assay in each group.