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**Research Article**

**Potential Use of CD4+ and CD8+ T Cell Immunoprofile from Pleural Fluid to Diagnose Tuberculous Effusions**

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**Abstract**

**Introduction**

Tuberculosis (TB) is a global health concern and a leading infectious cause of increased morbidity and mortality, especially in low-resource countries where establishing a diagnosis can be challenging. Tuberculous pleural effusions often occur in pulmonary TB, and in low-resource countries, analysis of the pleural fluid may be of diagnostic value. Evidence suggests that an increase in the ratio of CD4+ to CD8+ (CD4+/CD8+) T lymphocytes may provide diagnostic insight, however evidence is inconclusive.

**Methods**

We sought to characterize CD4+, CD8+ and CD4+/CD8+ T lymphocytes in pleural effusions from patients with and without tuberculosis in order to establish a potential role for CD4+/CD8+ T cell ratio in establishing a diagnosis of TB.

**Results**

CD4+ cells were significantly more abundant in individuals with TB; however the CD4+/CD8+ T cell ratio varied considerably among those with tuberculous effusions. Lower proportions occurred among cases and controls.

**Conclusion**

Analysis of pleural fluid for the quantity of CD4+ and CD8+ T may be useful for establishing a diagnosis of TB in suspicious cases.

**Keywords:** Tuberculous Pleural Effusions; CD4+ and CD8+ T Cell; Immunoprofile; Cytopathological Findings; Histopathology

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Introduction

Tuberculosis (TB) is a major infectious disease with approximately 8.6 million persons developing the disease and 1.3 million deaths annually [1]. Tuberculous pleural effusion (TPE) is a common form of extrapulmonary TB (EPTB), and TPE is the second most frequent manifestation of EPTB [2]. Moreover, pleural effusions are increasingly common in a high TB prevalence areas and should alert the health care provider to the potential presence of TB [3, 4].

The presence of Mycobacterium tuberculosis grown from culture samples are the gold-standard to definitively establish a diagnosis of TB; however, these cultures can take up to 6 weeks for results. The tuberculin skin test can be used to determine exposure, but studies show it is negative in about 33% of patients with a TB effusion. Further the wide-spread use of the BCG vaccine limits the utility of the tuberculin skin test in many low-resource countries.

To establish a diagnosis of TPE, clinicians depend on the ability to culture the acid fast bacilli from a pleural biopsy or from the effusion and / or the demonstration of pleural granulomas [5]. Molecular biology techniques, amplifying the TB genome by PCR, have limited sensitivity, detecting only 25% of the TPE cases [6].

In low resource settings, the pleural effusions provides the most easily accessible diagnostic material, but microbiological and molecular tests have a low sensitivity in the diagnosis of TPE [7]. This is attributed to the fact that the TPE primarily is due to a delayed hypersensitivity reaction [8]. The cellular content of the TB effusion is classically dominated by lymphocytes, this finding is unspecific without T cell immunoprofiling.

Pleural biopsy may improve the diagnostic accuracy to 90% [5], but this option is normally unavailable in low resource settings and unaffordable by the majority of patients in low-resource countries, where TB is common. The biopsy sampling is also associated with a higher co-morbidity compared to thoracentesis alone.

The immunological response during the development and progression of TB pleuritis occurs in three stages. The first stage is meditated by the production of IL-8 and monocyte chemotactic peptide (MCP-1) by activated mesothelial cells which acutely recruit polymorphonuclear neutrophils (PMNs) and monocytes [9]. PMNs and monocytes respond by secreting key cytokines such as TNFα, IL 6 and IL 1. The intermediate stage follows in which CD4+ helper cells and CD8+ cytotoxic cells dominate culminating into a CD4/CD8 ratio of about 4.3 [10]. Finally, inflammation persists resulting in a sustained CD4+/CD8+ cell response, persistent INF-Y release and granuloma formation.

The feasibility and applicability of CD4+ and CD8+ T lymphocytes phenotyping from pleural effusion as an ancillary diagnostic tool for TB in high endemic settings has not been done. Previous work has established that there are distinct differences between the circulating immune profile and the pleural fluid in TB patients[11]. Moreover, flow cytometry is the preferred method for immune profiling, however, due to cost constraints and limited access to expensive equipment, in low resource settings performing immune profiling through the more laborious manual counting is more feasible. In the current study, we used immunocytochemistry analysis and manual lymphocyte counting to assess the feasibility of using CD4/CD8 lymphocyte profile in distinguishing TPE in a case control study involving patients with tuberculous and non-tuberculous pleural effusion.

Materials and Methods

Study design

We performed a descriptive, case control unmatched study during 2011-2012 at Mulago National Referral Hospital (MNRH) Kampala-Uganda, the teaching Hospital of Makerere University and the Huddinge Hospital, Karolinska Institute, Stockholm Sweden. Ethical approval was obtained from Makerere University, College of Health Sciences, School of Medicine Internal Review Board and the Uganda National Council of Science and Technology. Informed consent was obtained from all participating patients or their legal guardians.

Human subjects

Thirty-five adults suspected of having TB pleuritis were recruited from the Mulago Hospital, Kampala, Uganda. Forty-three control subjects without TB but with pleural effusions from other causes were recruited from the Karolinska University Hospital, Huddinge, Sweden. The diagnosis of TB pleuritis was established when the Ziehl Neelsen stain or Lowenstein-Jensen culture was TB bacilli positive for the effusion or a pleural biopsy, or when granulomas were shown in the pleural biopsy.

Posteroanterior chest x-ray was done in all TB cases. Thoracentesis was performed for all patients with pleural effusion and symptoms of TB, including evening fevers, unintentional weight loss, cough and night sweats. Five milliliters of the pleural fluid was taken for cytology and immunocytochemistry, and a pleural biopsy was obtained. The biopsy was fixed in 4% buffered formalin, dehydrated in graded alcohol, embedded in paraffin, cut and stained with haematoxylin/eosin and Ziehl Neelsen stain.

Immunocytochemistry

The pleural fluid was centrifuged at 1000rpm, making four...
Lymphocytes were always more common. Reactive mesothelial cells and epithelioid cells were scarce, while multinucleated histiocytic giant cells or siderophages were not identified. The Zeihl-Neelsen stain was negative in all effusion smears.

### Table 1. Patients and Controls demographics.

|                | Patients (n=35) | Controls (n=43) | p-value
<table>
<thead>
<tr>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>33 (SD 12)</td>
<td>73 (SD 13)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Gender</td>
<td>Male 20 (57%)</td>
<td>21 (49%)</td>
<td>Age range. 18-60 years</td>
</tr>
<tr>
<td></td>
<td>Female 15 (43%)</td>
<td>22 (51%)</td>
<td>Age range. 45-92 years</td>
</tr>
</tbody>
</table>

### Table 2a. Unadjusted relationship between patient grouping and age with CD4/CD8 ratio*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient</th>
<th>95% CI (β)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient grouping</td>
<td>12.73</td>
<td>2.96-24.56</td>
<td>0.013</td>
</tr>
<tr>
<td>Age</td>
<td>-0.250</td>
<td>-0.510 - 0.24</td>
<td>0.020</td>
</tr>
</tbody>
</table>

*Linear regression.

Both patient grouping and age were significantly associated with CD4/CD8 ratio at unadjusted analysis.

### Table 2b. Adjusted relationship between patients grouping and age with CD4/CD8 ratios*

<table>
<thead>
<tr>
<th>Variable</th>
<th>Regression coefficient (β)</th>
<th>95% CI (β)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patient grouping</td>
<td>9.46</td>
<td>-1.58 - 23.48</td>
<td>0.31</td>
</tr>
<tr>
<td>Age</td>
<td>-0.081</td>
<td>-0.41 - 0.24</td>
<td>0.68</td>
</tr>
</tbody>
</table>

*Linear regression.

At adjusted analysis patient grouping and age were not significant.

Large amounts of mature lymphocytes were seen in only 17/35 (49%) of the cases. The amounts of lymphocytes in the sample, however, correlated only weakly to the presence of CD4+ cells (please report the R value = 0.313 and p value 0.013); a CD4+/CD8+ ratio greater than 7 was seen in 8/17 (47%) lymphocyte rich TPE while the corresponding figure for those with less abundant lymphocytes was 8/18 (44%).

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The proportion of CD4+ T lymphocytes was more often increased in the TPE compared to the non-TB ones (Table 3). When calculating the CD4+/CD8+ ratios for individual effusions (assuming 1% being the lowest proportion of CD8+ cells), the average ratio was 32 in TB group and only 18 in the control group. The variability in the two groups was, however, large (Fig. 1) and none of these mean values differed significantly. A simultaneous infection with HIV, not uncommon in the present setting, might hamper the recruitment of CD4+ cells. Although the HIV status of the patients is unknown, it is interesting that also the effusions with the lowest CD4+ counts were somewhat more common in the TB group.

Table 3. Comparision of absolute count values of immunophenotype T Cells types between patient with Pulmonary tuberculous effusion and controls.

<table>
<thead>
<tr>
<th></th>
<th>TB Effusion Patients</th>
<th>Non TB Effusion Patients (controls)</th>
<th>P Value*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean CD Immunophenotype count</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CD4+ TCells</td>
<td>75.9 (SD28.1)</td>
<td>74.7 (SD17.2)</td>
<td>0.801</td>
</tr>
<tr>
<td>CD8+ T Cells</td>
<td>24.1 (SD28.1)</td>
<td>25.3 (SD17.1)</td>
<td>0.836</td>
</tr>
<tr>
<td>CD4+/CD8+ T cell Ratio</td>
<td>17.3 (SD27.9)</td>
<td>4.5(SD 8.1)</td>
<td>0.013</td>
</tr>
</tbody>
</table>

*Independent sample T test.

Figure 1. Percentage of CD4+ cells in effusions caused by TB and controls. The variability is large, although CD4+/CD8+ ratios > 7 is more common in TB effusions. NB, also the lowest values are more common in the TB group.

The Receiver Operating Characteristic (ROC) plots show a total Area under Curve (AUC) value of 0.59 (Fig. 2). In the interval with sensitivity values exceeding 0.66 (CD4+/CD8+ ratios<2), the plot follows the diagonal closely, i.e., these values don’t contribute to the diagnosis. The curve, however, deflects from the diagonal for cases with CD4+/CD8+ ratio above 2, and a plot limited to cases with such ratios gives an AUC = 0.67. These plots an optimal show an optimal cut off at a CD4+/CD8+ ratio of 7, when the test detects 53% of the TB cases with a specificity of 0.76.

Discussion

The finding of a high CD4+/CD8+ ratio was more common among effusions caused by TB. In high endemic areas, a lymphocytic pleural fluid with rare mesothelial cells has a high probability of being due to TB. This is in agreement with earlier results [12], describing that such hypercellular pleural fluids associated with mycobacterial pleuritis when >50% of the inflammatory cells are lymphocytes, with no admixture of eosinophilic granulocytes and <10% of nucleated cells being mesothelial.

A multitude of diseases may however, cause a pleural effusion with influx of phagocytic cells and chronic mononuclear inflammatory cells in the pleural cavity, orchestrated by cytokines [13]. The dominant T cell phenotype in both the TB and non-TB effusions is the CD4+ cells. There is, however, a significant difference between the TB and non-TB effusion in cases with the higher CD4+/CD8+ T cell ratios [14, 15]. This predominance of CD4+ cells indicates that they play an important role in the immunological reactions following exposure to *M tuberculosis* antigen and formation of a pleural effusion. There is a possible confounding relationship between the patient groupings and age variables since the values of both regression coefficients changed they were adjusted for using linear regression. Including age in the model changed the regression coefficient for the patients grouping by 25% which makes it
a confounder of that relationship. This concurs with findings from previous studies in which the distribution of CD4/CD8 ratio was significantly affected by age[16].

Homing and compartmentalization of the CD4+ cells has been demonstrated at sites of TB infection[17]. Thus in broncho-alveolar lavage from patients with advanced stages of pulmonary TB disease, there is a higher proportion of CD4+ cells relative to the CD8− cells, resulting in increased CD4+/CD8− ratios [18]. Simultaneously, the peripheral blood from these TB patients contains a lower proportion of CD4+ cells.

The CD4+ T cells are vital to the mounting of an effective immune response to TB, although in more advanced stages of the disease, CD8− T cells play an important role as well [19]. Such stages of the disease will therefore have lower CD4+/CD8− T cell ratios. This is also in agreement with results obtained in previous studies in which CD4+ T cell were found to be significantly higher in TB compared to malignant effusions [20, 21]. The difference in CD4+ cell count can therefore be included in the diagnostic strategy to identify a TB effusion especially in settings with constrained resources. The analysis is fast compared to culture which takes about 2-6 weeks [22] and is able to detect Mycobacteria tuberculosis in effusion in only 20-25% of cases [7].

The migration of CD4+ cells into the pleural fluid correlates with adenosine deaminase activity(ADA), a commonly employed biomarker in diagnosis of TB effusions [23]. However ADA activity is also raised in a number of other diseases like malignancy, bacterial diseases, empyema and collagen vascular diseases [23]. Therefore ADA has but limited diagnostic value in TB effusions [24, 25].

The limitations of the study include the fact that the ages of the controls were different from those of the TB effusion patients. The controls and the patients do not share the same environment and race. Further multiple variables including age, sex, individual genetic configuration, immunodeficiency virus infection and nutrition may influence individual immunological reaction to TB antigen. As people age, T cell responds less quickly to antigens [16]. These confounders have not been adjusted for apart from age and race. Previous exposure to TB antigen may be important in hypersensitivity reaction associated with TPE. The patients and control come from high and low TB prevalence regions respectively. This may influences the magnitude of the hypersensitivity reaction to TB antigen.

In conclusion analysis of the CD4+ and CD8+ T cell immunoprofile can be a screening tool, indicating and establishing TB as the cause for a pleural effusion with lymphocytic dominance. The levels of CD4+ cells are, however, not pathognomonic for TB, and the analysis can merely be use as a guide to do further tests like PCR and culture to diagnose the infection in a clinically, especially in a low resource setting.

Conflict of interest statement

The authors declare no conflict of interest or competing interest in executing and publishing this research.

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References


