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# MALIGNANT AND TUBERCULOUS PLEURAL EFFUSIONS: IMMUNOPHENOTYPIC CELLULAR CHARACTERIZATION

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**INTRODUCTION AND OBJECTIVES***:* Tuberculosis and cancer are the main causes of pleural effusion. Pleural involvement is associated with migration of immune cells to the pleural cavity. We sought to characterize the immunophenotype of leukocytes in the pleural effusion and peripheral blood of patients with tuberculosis or malignancy.
**METHODS:** Thirty patients with tuberculosis (14) or malignancy (16) were studied. A control group included 20 healthy blood donors.
**RESULTS:** Malignant phycoerythrin pleural effusions showed higher percentages of CD3, CD4, CD3CD45RO, and CD20CD25 lymphocytes and lower percentages of CD3CD25 and CD20HLA-DR when compared to PB lymphocytes. Compared to PB, tuberculous effusions had a higher percentage of lymphocytes that co-expressed CD3, CD4, CD3CD45RO, CD3TCRαβ, CD3CD28, and CD20 and a lower percentage of CD14, CD8 and CD3TCRγδ-positive lymphocytes. Malignant effusions presented higher expression of CD14 whereas tuberculous effusions had higher expression of CD3 and CD3CD95L. Peripheral blood cells from tuberculosis patients showed higher expression of CD14, CD20CD25 and CD3CD95L. Compared with the control cells, tuberculosis and cancer peripheral blood cells presented a lower percentage of CD3CD4 and CD3CD28-positive cells as well as a higher percentage of CD3CD8, CD3CD25 and CD3CD80-positive cells.
**CONCLUSIONS:** Tuberculous and malignant peripheral blood is enriched with lymphocytes with a helper/inducer T cell phenotype, which are mainly of memory cells. CD14-positive cells were more frequently found in malignant effusions, while CD3-positive cells expressing Fas ligand were more frequently found in tuberculous effusions.

**KEYWORDS:** Pleural fluid; Cancer; Flow cytometry; Monoclonal antibody; Tuberculosis.

## INTRODUCTION

The accumulation of fluid in the pleural space indicates the presence of systemic or local disease. Pleural exudates involve the migration of immune cells to the pleural cavity.[1] Characterization of the cellular composition of such exudates may provide insight into the relevant pathophysiological mechanisms and the functional state of the cells. Tuberculosis and cancer represent the main causes of pleural exudates. In both cases, the pleural fluid is generally lymphocytic, with a predominance of T lymphocytes, particularly CD4-positive T cells.[2]

Considering the compartmentalization of the pleural space, the association between the local and systemic cellular responses should be analyzed. Some investigators have found that, when compared with peripheral blood, exudates have increased numbers of CD4-positive T lymphocytes. However, the immunophenotypic profile of the pleural fluid does not permit discrimination of the causal diagnosis.[2-7]

Different cell populations have been described in tuberculous fluid. Kapila et al.[8] reported a predominance of CD4- positive T lymphocytes with a CD4/CD29 phenotypic

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profile when compared to blood, suggesting that these cells represent memory T cells.[9,10] On the other hand, Dlugovitzky et al.[11] reported an increase in the number of CD25-positive cells (IL-2 receptor-expressing), whereas Faith et al.[12] observed expression of the HLA-DR marker, suggesting the presence of activated T cells.

Similarly, several studies have evaluated the lymphocytic profile (pleural fluid and blood) of patients with cancer. These studies generally indicate a predominance of T lymphocytes in the pleural fluid, with increased prevalence of a CD4-positive subpopulation when compared to blood[13-17]. Baxevanis et al.[18] and Mantovani et al.[19] demonstrated a lower percentage of natural killer cells (CD16) in the pleural fluid than in peripheral blood. Only a minority of T lymphocytes were activated, which expressed CD38 and HLA-DR surface markers.[14] These lymphocytes were found to have natural killer activity and very low antitumor cytotoxicity, which is suggestive of depressed immune function[15]. Another factor associated with the lack of a T cell-mediated immune response is the existence of an apoptotic mechanism that, in addition to regulating the homeostasis of cell populations, acts to eliminate self-reactive lymphocytes and negatively regulate the immune response.[20-22] The apoptotic signal is transmitted to mature T cells through various membrane receptors, mainly CD3/TCR, Fas/Apo/CD95, TNF, CTL-4, and CD2. Only activated T cells develop the cellular substrate required for transmission of the apoptotic signal.[23] Thus, the analysis of cell surface markers permits the evaluation of cellular differentiation (CD3, CD4, CD8, CD15, CD20, CD56, TCR$\alpha\beta$, and TCR$\gamma\delta$), cellular activation (CD25, CD45RA, CD45RO, and HLA-DR), and immune co-stimulation (CD28, CD80, CD86 and CD152) in pleural fluid and blood. The aim of this study was to elucidate the pathophysiological mechanisms involved in the genesis of pleural exudates of tuberculous or neoplastic origin.

## PATIENTS AND METHODS

The Ethics Committee of our institution approved this study. Thirty patients with pleural exudates (14 tuberculosis, 16 cancer) with no previous treatment were included in the study. These patients were indicated for thoracocentesis and underwent collection of pleural fluid and blood after they signed an informed consent form. Light's criteria[24] were used for classification of transudate or exudate. The diagnosis of tuberculosis was made based on the presence of compatible clinical and radiological signs associated with a *M. tuberculosis*-positive culture from fluid or pleural fragments, on the presence of caseous granuloma in the pleura, or both. The presence of tumor cells in the pleural fluid or tissue indicated cancer.

Twenty healthy blood donors were included in the study as well (blood control).

### Biochemical examinations and pleural fluid cytology

Biochemical examination of blood and pleural fluid (protein and lactate dehydrogenase) and blood count were performed using automatic devices (Cobas Integra 700, West Sussex, UK; Pentra 120, Horiba ABX, Montpellier, France). The total number of nucleated cells (pleural fluid) was quantified in a Neubauer chamber (Hausser Scientific, Horsham, PA). Slides stained with Leishman panoptic stain were used for qualitative and oncotic cytology.

### Cell preparation and cell viability testing

The mononuclear cell concentrate was obtained by centrifugation of pleural fluid or blood (1500 rpm for 10 min at 25°C), and the final concentration was adjusted to 2 x $10^7$ cells/mm$^2$. Cell viability was evaluated by Trypan blue exclusion (Gibco BRL, Grand Island, NY). Only samples with more than 70% viable cells were used in this study.

#### Flow cytometry

Blood or pleural fluid cells were incubated with monoclonal antibodies conjugated to fluorescein isothiocyanate (FITC) or phycoerythrin (PE). Direct immunofluorescence was used for the analysis of cell surface antigens other than CD95-L. Autofluorescence of cells and nonspecific binding of monoclonal antibodies were corrected for by using isotypic controls. Antigens were quantified by the detection of fluorescence emitted by fluorochrome-labeled monoclonal antibodies in a FACSCalibur flow cytometer (Becton-Dickinson, San Jose, CA) using the CellQuest (Becton Dickinson) program.

Cell populations were identified based on analysis of the cytograms. Cell data were arranged in 2-dimensional cytograms (abscissa: cell size; ordinate: internal cell complexity). Leukocytes were identified by quantification of anti-CD45-labeled cells. Among the CD45-positive population, the group with the lowest internal complexity corresponded to lymphocytes. CD45, CD15- and CD45, CD14-positive cells characterized granulocytes and monocytes/macrophages. T, B, and natural killer lymphocytes were identified based on their staining by CD3, CD20, and CD56 antibodies.

Cell surface markers for activation, co-stimulation, and apoptosis co-expressed by T lymphocytes, B-lymphocytes, or monocytes/macrophages were quantified in cells that were

positive for CD3, CD20, and CD14, respectively, on the abscissa with surface antigens on the ordinate.

### Apoptosis

Leukocyte apoptosis was analyzed in blood and pleural fluid using the APO-BRDU kit (Pharmigen, San Diego, CA). Mononuclear cells were separated on a density gradient using the Lymphoprep kit (Nycomed Pharma, Oslo, Norway). A suspension of 2 x $10^6$ cells in a 5-ml aliquot of phosphate-buffered saline was held on ice (15 min). After several washes, the cell sediment obtained by centrifugation was diluted in 50 µl of DNA strand-break labeling solution. Cells were then incubated with 0.1 ml of anti-BrDu (FITC) solution, followed by 0.5 ml PI dye/RNase A solution. Apoptosis was quantified based on the fluorescence emitted by the FITC-labeled anti-BrDu antibody in a FACSCalibur cytometer using the Cell Quest program (Becton-Dickinson, São Paulo, Brazil).

### Statistical analysis

The paired *t* test (normal distribution) and Wilcoxon test (not normal distribution) were used for comparisons of blood and pleural fluid. Leukocyte subpopulations (fluid) were compared using the Student's *t* test or the Mann-Whitney test. Differences in the blood leukocyte subpopulation percentage among groups were determined by analysis of variance (ANOVA), followed by Tukey's multiple comparisons or the Kruskal-Wallis test and Dunn's multiple comparison procedure. The level of significance was set at 0.05, and SPSS 10.0 for Windows statistical software was used for analysis.

## RESULTS

Thirty patients were studied: 14 (12 men and 2 women; mean age: 36.9 years) with pleural tuberculosis and 16 (5 men and 11 women; mean age: 60.4 years) with malignant effusion.

### Immunophenotypic profile of pleural fluid

Four main patterns of cell distribution were identified using 2-dimensional cytograms (abscissa: cell size; ordinate: internal complexity of cells):

Pattern 1: Small cells of low complexity (region usually occupied by lymphocytes in normal blood).

Pattern 2: Cell population described in pattern 1 plus cells with small size and high internal complexity (corresponding to granulocytes in normal blood).

Pattern 3: Populations 1 and 2, plus medium-sized and medium-complexity cells (corresponding to monocytes in normal blood).

Pattern 4: Population 1, plus cells of variable size and complexity (probably corresponding to macrophages and mesothelial cells in fluid).

Pleural fluid from patients with tuberculosis had cells with patterns 1 (9/14; 64.3%) and 3 (5/14; 35.7%), whereas most fluid secondary to cancer had cells with patterns 4 (10/16; 62.5%) and 1 (4/16; 25.0%).

Comparison of cellular components between tuberculous or malignant fluid showed a significant difference in the percentage of T lymphocytes (CD3) and monocytes/macrophages (CD14). Neoplastic effusions had a higher percentage of CD14-positive cells (monocytes/macrophages) than did tuberculous effusions ($p = 0.006$), whereas the percentage of CD3-positive cells (T lymphocytes) was higher in tuberculous effusions ($p = 0.002$). Although the

**Table 1 -** Percentage (mean ± standard deviation) of leukocyte subpopulations in pleural fluid of patients with Tb or cancer (* $p < 0.05$)

| Cells | Tuberculous | | Malignant | | P |
|---|---|---|---|---|---|
| CD15 | 2.3 | (2.5) | 7.0 | (21.7) | 0.967 |
| CD14 | 5.3 | (3.9) | 17.4 | (15.0) | 0.006 |
| CD14CD86 | 99.0 | (1.0) | 81.8 | (37.6) | 0.867 |
| Lymphocytes * | 87.3 | (9.3) | 52.3 | (23.7) | 0.036 |
| CD3 * | 75.2 | (9.0) | 52.3 | (23.7) | 0.002 |
| CD20 | 6.2 | (3.1) | 7.9 | (6.9) | 0.389 |
| CD56 | 2.9 | (1.4) | 2.2 | (1.5) | 0.150 |
| CD20HLA | 99.5 | (0.4) | 91.5 | (25.4) | 0.600 |
| CD20CD25 | 25.6 | (17.3) | 25.8 | (21.3) | 0.972 |
| CD20CD80 | 8.4 | (4.7) | 15.6 | (20.9) | 0.570 |
| CD20CD86 | 13.4 | (11.5) | 7.0 | (8.0) | 0.086 |
| CD3CD4 | 73.0 | (5.9) | 66.6 | (19.8) | 0.234 |
| CD3CD8 | 25.7 | (5.9) | 32.5 | (19.4) | 0.202 |
| CD3CD4/CD3CD8 | 3.1 | (1.1) | 3.3 | (2.6) | 0.780 |
| CD3TCRαβ | 97.6 | (1.8) | 90.7 | (12.9) | 0..67 |
| CD3TCRγδ | 2.9 | (1.7) | 4.3 | (3.5) | 0.182 |
| CD3CD45RA | 32.1 | (15.6) | 23.5 | (20.8) | 0.214 |
| CD3CD45RO | 73.2 | (11.6) | 66.1 | (26.8) | 0.349 |
| CD3HLA | 19.7 | (10.0) | 19.7 | (12.4) | 0.999 |
| CD3CD25 | 22.7 | (12.6) | 22.8 | (15.7) | 0.990 |
| CD3CD28 | 88.6 | (15.1) | 73.0 | (29.4) | 0.076 |
| CD3CD80 | 1.5 | (1.3) | 2.5 | (2.3) | 0.160 |
| CD3CD95 | 79.7 | (14.1) | 66.2 | (33.9) | 0.162 |
| CD3CD95L | 55.7 | (41.0) | 26.5 | (26.5) | 0.061 |
| CD3CD152 | 1.6 | (1.3) | 2.1 | (1.5) | 0.421 |

percentage of pro-apoptotic T lymphocytes (CD3, CD95L+) appeared to be higher in tuberculous effusions, the statistical significance was marginal (p = 0.061) (Table 1).

**Comparison of cells in pleural fluid and blood**

The following cell populations were found to be elevated in tuberculous effusions: total lymphocytes (p < 0.001), TCD3 (p < 0.001), CD3CD4 (p < 0.001), CD3CD45RO (p = 0.001), CD3TCRαβ (p = 0.009), CD3CD28 (p = 0.003), and CD20 (p < 0.001). The CD4/CD8 ratio was also higher in pleural fluid (p < 0.001) (Table 2). In malignancy, a higher percentage of the following cell types and subtypes were observed in the pleural fluid: total lymphocytes (p < 0.001), CD3 lymphocytes (p < 0.001), CD3CD4 lymphocytes (p = 0.001), CD3CD45RO lymphocytes (p = 0.002), CD20CD25 lymphocytes (p = 0.006), and CD3TCRαβ lymphocytes (p = 0.028). The CD4/CD8 ratio was also higher in the pleural fluid (p = 0.039) (Table 3).

These results demonstrate that lymphocytes represent the predominant cell population in malignant effusions and that these lymphocytes are mainly helper/inducer T cells (CD4) with a memory cell phenotype (CD45RO). The same profile was observed in effusions from patients with tuberculosis.

**Comparison of cells in peripheral blood: tuberculosis versus cancer**

No significant differences were observed for most surface markers. However, a higher percentage of CD14 (p = 0.012), CD3CD95L (p = 0.005), and CD20CD25 cells (p = 0.006) were observed in the tuberculosis group (Table 4).

When we compared blood cell subpopulations between patients with tuberculosis or cancer and the control group,

**Table 2 -** Percentage (mean ± standard deviation) of leukocyte subpopulations in peripheral blood and pleural fluid of patients with tuberculous pleural effusions (* p < 0.05)

| Cells | Pleural fluid | | Blood | | P |
|---|---|---|---|---|---|
| CD15 * | 2.3 | (2.5) | 62.4 | (8.0) | 0.000 |
| CD14 * | 5.3 | (3.9) | 10.7 | (3.0) | 0.003 |
| CD14CD86 | 99.0 | (1.0) | 99.3 | (0.8) | 0.236 |
| Lymphocytes * | 87.3 | (9.3) | 23.1 | (6.8) | 0.000 |
| CD3 * | 75.2 | (9.0) | 13.9 | (4.6) | 0.000 |
| CD20 * | 6.2 | (3.1) | 2.0 | (1.0) | 0.000 |
| CD56 | 2.9 | (1.3) | 4.4 | (2.5) | 0.109 |
| CD20HLA | 99.5 | (0.4) | 99.8 | (0.2) | 0.657 |
| CD20CD25 | 25.6 | (17.3) | 19.3 | (18.6) | 0.309 |
| CD20CD80 | 8.4 | (4.7) | 9.8 | ( 9.1) | 0.875 |
| CD20CD86 | 13.4 | (11.5) | 22.4 | (16.2) | 0.071 |
| CD3CD4 * | 73.0 | (5.9) | 50.9 | (19.0) | 0.000 |
| CD3CD8 * | 25.7 | (5.9) | 45.0 | (13.7) | 0.000 |
| CD3CD4/CD3CD8 * | 3.1 | (1.1) | 1.3 | ( 0.8) | 0.000 |
| CD3TCRαβ * | 97.6 | (1.8) | 93.0 | ( 5.4) | 0.009 |
| CD3TCRγδ * | 2.9 | (1.7) | 8.5 | ( 5.3) | 0.001 |
| CD3CD45RA * | 32.1 | (15.6) | 58.8 | (11.7) | 0.000 |
| CD3CD45RO * | 73.2 | (11.6) | 62.5 | (10.4) | 0.001 |
| CD3HLA | 19.7 | (10.0) | 22.0 | (10.0) | 0.554 |
| CD3CD25 | 22.7 | (12.6) | 27.2 | (8.4) | 0.153 |
| CD3CD28 * | 88.6 | (15.1) | 70.6 | (12.5) | 0.003 |
| CD3CD80 | 1.5 | (1.3) | 1.0 | (1.0) | 0.133 |
| CD3CD95 | 79.7 | (14.1) | 74.7 | (16.4) | 0.220 |
| CD3CD95L | 55.7 | (41.0) | 50.9 | (34.7) | 0.530 |
| CD3CD152 | 1.6 | (1.3) | 1.3 | (1.4) | 0.277 |

**Table 3 -** Percentage (mean ± standard deviation) of leukocyte subpopulations in peripheral blood and pleural fluid of patients with neoplastic pleural effusions (*p < 0.05)

| Cells | Pleural fluid | | Blood | | P |
|---|---|---|---|---|---|
| CD15 * | 1.5 | (1.5) | 66.7 | (8.2) | 0.002 |
| CD14 | 20.1 | (17.0) | 7.4 | (2.2) | 0.754 |
| CD14CD86 | 99.5 | (1.1) | 95.2 | (10.5) | 0.237 |
| Lymphocytes * | 76.2 | (17.4) | 20.5 | (7.1) | 0.000 |
| CD3 * | 60.2 | (18.9) | 12.3 | (5.1) | 0.000 |
| CD20 | 7.6 | (7.2) | 2.9 | (2.3) | 0.061 |
| CD56 | 2.3 | (1.5) | 3.2 | (1.7) | 0.268 |
| CD20HLA * | 98.1 | (4.4) | 99.1 | (1.8) | 0.033 |
| CD20CD25 * | 23.2 | (19.3) | 6.9 | (6.3) | 0.006 |
| CD20CD80 | 19.4 | (24.9) | 17.1 | (30.3) | 0.849 |
| CD20CD86 | 8.2 | (8.5) | 15.2 | (19.5) | 0.237 |
| CD3CD4 * | 66.0 | (22.0) | 47.8 | (13.4) | 0.001 |
| CD3CD8 | 32.9 | (21.4) | 44.8 | (17.9) | 0.119 |
| CD3CD4/CD3CD8 * | 3.6 | (3.1) | 1.4 | (0.9) | 0.039 |
| CD3TCRαβ * | 97.3 | (2.1) | 91.9 | (7.8) | 0.028 |
| CD3TCRγδ | 3.8 | (1.5) | 6.3 | (5.4) | 0.217 |
| 002CD3CD45RA * | 29.5 | (23.4) | 53.2 | (17.9) | 0.002 |
| CD3CD45RO * | 72.7 | (21.7) | 56.0 | (21.0) | 0.002 |
| CD3HLA | 19.9 | (13.5) | 18.6 | (12.9) | 0.837 |
| CD3CD25 * * | 24.6 | (14.6) | 30.9 | (15.6) | 0.048 |
| CD3CD28 | 80.5 | (23.9) | 72.0 | (11.6) | 0.166 |
| CD3CD80 | 2.0 | (1.7) | 0.7 | (0.6) | 0.055 |
| CD3CD95 | 78.6 | (16.3) | 79.3 | (13.6) | 0.905 |
| CD3CD95L | 19.5 | (21.9) | 16.2 | (22.1) | 0.713 |
| CD3CD152 | 2.0 | (1.2) | 5.2 | (12.2) | 0.666 |

**Table 4 -** Percentage (mean ± standard deviation) of leukocyte subpopulations in peripheral blood of patients with tuberculous and neoplastic pleural effusions as compared to the control group

| Cells | Malignant | | Tuberculous | | Control | | P | |
|---|---|---|---|---|---|---|---|---|
| CD15 | 66.7 | (8.2) | 62.4 | (8.1) | 58.6 | (13.9) | 0.176 | |
| CD14 * | 7.4 | (2.2) | 10.7 | (3.0) | 7.7 | (3.4) | 0.012 | (2x1; 2x3) |
| CD14CD86 | 95.2 | (10.6) | 99.3 | (0.5) | 99.3 | (0.5) | 0.143 | |
| Lymphocytes | 20.5 | (7.1) | 23.1 | (6.8) | 27.6 | (9.6) | 0.074 | |
| CD3 * | 12.3 | (5.1) | 13.9 | (4.6) | 20.0 | (9.4) | 0.014 | (3x1) |
| CD20 | 2.9 | (2.3) | 2.0 | (1.1) | 3.1 | (1.6) | 0.207 | |
| CD56 | 3.3 | (1.7) | 4.4 | (2.5) | 3.5 | (1.6) | 0.289 | |
| CD20HLA | 99.1 | (1.8) | 99.8 | (0.3) | 99.8 | (0.3) | 0.411 | |
| CD20CD25 * | 7.0 | (6.3) | 19.3 | (18.6) | 7.0 | (2.9) | 0.006 | (2x1; 2x3) |
| CD20CD80 | 17.1 | (30.4) | 9.8 | (9.1) | 5.2 | (4.6) | 0.391 | |
| CD20CD86 * | 15.2 | (19.6) | 22.4 | (16.2) | 8.5 | (11.9) | 0.004 | (2x3) |
| CD3CD4 * | 47.8 | (13.4) | 50.9 | (14.0) | 64.4 | (8.0) | 0.001 | (3x1; 3x2) |
| CD3CD8 * | 44.8 | (17.8) | 45.0 | (13.7) | 32.3 | (7.3) | 0.007 | (2x3; 1x3) |
| CD3CD4/CD3CD8 * | 1.4 | (0.9) | 1.3 | (0.8) | 2.1 | (0.7) | 0.008 | (3x2; 3x1) |
| CD3TCRαβ | 91.9 | (7.8) | 93.0 | (5.4) | 89.8 | (19.1) | 0.740 | |
| CD3TCRγδ * | 6.3 | (5.4) | 8.4 | (5.3) | 4.7 | (2.6) | 0.049 | (2x3) |
| 002CD3CD45RA * | 53.2 | (17.9) | 58.8 | (11.8) | 39.7 | (11.9) | 0.001 | (2x3; 1x3) |
| CD3CD45RO | 56.0 | (21.0) | 62.5 | (10.3) | 59.2 | (13.7) | 0.563 | |
| CD3HLA * | 18.6 | (12.9) | 22.0 | (10.0) | 12.4 | (6.0) | 0.023 | (2x3) |
| CD3CD25 * * | 30.9 | (15.6) | 27.2 | (8.5) | 14.7 | (4.9) | 0.001 | (1x3; 2x3) |
| CD3CD28 * | 72.0 | (11.6) | 70.6 | (2.5) | 84.9 | (7.7) | 0.001 | (3x2; 3x1) |
| CD3CD80 * | 0.7 | (0.6) | 1.0 | (0.9) | 0.2 | (0.2) | 0.001 | (2x3; 1x3) |
| CD3CD95 | 79.3 | (13.6) | 74.7 | (16.5) | 76.7 | (9.6) | 0.704 | |
| CD3CD95L * | 16.2 | (22.1) | 50.9 | (34.7) | 13.2 | (9.5) | 0.005 | (2x1; 2x3) |
| CD3CD152 * | 5.2 | (12.2) | 1.3 | (1.4) | 0.5 | (0.4) | 0.022 | (2x3) |

we observed a significant decrease in the percentage of CD4 T lymphocytes in the disease groups (p = 0.001). Despite the reduced percentage of CD4 lymphocytes in patients with cancer, the T cells of these patients coexpressed some activation markers, such as CD25 and CD80. In the tuberculosis group, CD95L and CD152 were increased in addition to these T-cell activation markers. Coexpression of T-cell activation markers suggests that these lymphocytes underwent the first stages of antigen activation.

### Determination of apoptosis

The apoptosis data are expressed as the percentage of positive mononuclear cells in the fluid and blood of patients with tuberculosis or cancer and in the blood of control subjects. Apoptotic cells in the pleural fluid were only observed in 6 (20.0%) of the 30 patients studied (5 with tuberculosis: 5/14; 35.7%, and one with cancer: 1/16; 6.2%). The percentage of apoptotic mononuclear cells ranged from 0.2 to 32.9% in the pleural fluid and from 1.7 to 27.0% in the blood (Table 5); in the control group this percentage ranged from 0.2 to 7.9% (Table 6).

## DISCUSSION

Tuberculosis and cancer are among the main causes of exudative pleural effusions. Although the effusions are predominantly lymphocytic in both cases, many studies have evaluated the subpopulations, activation state, and relationship with a peripheral blood cellular profile.[3-5,18,19]

Separate analysis of the pleural fluid showed that only CD3 and CD14 cell subpopulations quantitatively differed between tuberculosis and cancer. CD3 cells predominate in tuberculosis, whereas CD14 cells are more highly expressed in cancer. It should be emphasized that the increase in CD14-positive mononuclear cells in neoplastic effusions

**Table 5 -** Percentage of BrdUTP-positive mononuclear cells in pleural fluid and peripheral blood of patients with tuberculous (1–14) or neoplastic (15–30) pleural effusions

| Patient | Diagnosis | Pleural fluid | Blood |
|---|---|---|---|
| 1 | Tuberculosis | 30.27 | 26.66 |
| 2 | | 32.88 | 1.75 |
| 3 | | 0.93 | --- |
| 4 | | 0.57 | --- |
| 5 | | 0.47 | --- |
| 6 | | 0.70 | --- |
| 7 | | 30.25 | 6.45 |
| 8 | | 1.26 | --- |
| 9 | | 0.64 | --- |
| 10 | | 0.70 | --- |
| 11 | | 29.86 | 15.27 |
| 12 | | 1.30 | --- |
| 13 | | 20.64 | --- |
| 14 | | 0.32 | --- |
| 15 | Malignant | 1.10 | --- |
| 16 | | 0.83 | --- |
| 17 | | 0.36 | --- |
| 18 | | 0.74 | --- |
| 19 | | 0.92 | --- |
| 20 | | 3.05 | 1.89 |
| 21 | | 0.62 | --- |
| 22 | | 0.53 | --- |
| 23 | | 0.70 | --- |
| 24 | | 0.76 | --- |
| 25 | | 0.76 | --- |
| 26 | | 0.23 | --- |
| 27 | | 0.62 | --- |
| 28 | | 1.91 | --- |
| 29 | | 23.58 | 8.06 |
| 30 | | 0.73 | --- |

**Table 6 -** Percentage of BrdUTP-positive mononuclear cells in the peripheral blood of healthy blood donors (control)

| Control | Blood |
|---|---|
| 1 | 3.62 |
| 2 | - |
| 3 | - |
| 4 | 5.94 |
| 5 | 5.72 |
| 6 | 3.86 |
| 7 | 5.60 |
| 8 | 7.92 |
| 9 | 0.74 |
| 10 | 1.02 |
| 11 | 4.46 |
| 12 | 4.32 |
| 13 | 2.94 |
| 14 | 2.74 |
| 15 | 1.68 |
| 16 | 0.20 |
| 17 | 0.20 |
| 18 | 3.98 |
| 19 | 7.90 |
| 20 | 2.86 |

was not associated with any change in this cell population in the peripheral blood. Previously, Gjomarkaj et al.[25] studied mononuclear cells (CD14) derived from neoplastic effusions and showed that these cells coexpress the HLA-DR antigen (activation marker), produce interleukin 1β and tumor necrosis factor α (TNF α), and stimulate the proliferation of allogenic T lymphocytes. These findings suggest that these cells are involved in the tumor-associated inflammatory reaction.

Although the percentage of apoptotic T lymphocytes appeared to be higher in tuberculosis, the statistical significance of this difference was marginal. This result might be explained by the size of the sample (30 cases); however, the number of antigens analyzed and the complexity of preparations also limited the analysis.

As the pleural cavity represents a separate compartment, it is important to establish the relationship between local phenomena and systemic repercussions. When we compared the cellular composition of pleural fluid and blood, we observed a relative increase in CD4-positive T lymphocytes in the pleural fluid from patients with tuberculosis and patients with cancer (higher in tuberculosis). This increase of CD4-positive T lymphocytes in the pleural fluid has been previously reported and is probably related to the predominance of this cell type over CD8-positive T cells or to reductions in the latter that have been observed in tuberculosis.[8,9,12,15-18,26-29]

The number of CD3CD45RO cells (activated memory T cells) was higher in the pleural fluid. This finding, together with the reduced CD3CD45RA population (naive T cells), suggests that this phenotype is related to the process of cell activation. No significant differences between fluid and blood were observed for other markers (CD95L, CD152, or HLA-DR).

Despite the importance of apoptosis in pathological processes, few investigations have been conducted on pleural effusions. In our study, the blood and fluid of only 6 (20%) patients showed elevated percentages of apoptotic cells when compared to the blood of healthy subjects. Most (83.3%) samples with an elevated percentage of apoptotic cells were tuberculous exudates. To investigate the possible association between cells undergoing apoptosis and specific immunophenotypes, we evaluated the correlation of apoptosis with surface marker expression (CD14, CD3CD95, CD3CD95L, CD3CD28, CD3HLA-DR, CD3CD45RO, and CD3CD45RA), but no correlation could be established. However, cell apoptosis in these fluids might be related to signaling pathways that involve other molecules, such as Bcl-2 and Bax, or to a deficiency in phagocytotic mechanisms[30,31]. Further studies of pleural effusions are necessary to understand the pathophysiological mechanisms responsible for increased apoptosis.

The increased percentage of CD3CD95L cells in the blood of patients with tuberculosis as compared to controls

and patients with cancer might reflect the host response to mycobacterial infection. These bacteria escape destruction by macrophages by preventing the fusion of phagosomes and lysosomes, thereby impairing the bactericidal action of lysosomal components.[32,33] Organisms use multiple mechanisms to prevent mycobacterial infection, such as the activation of a Th1-type response. Once activated, these lymphocytes express Fas ligand (CD95L) on their membrane to eliminate a variety of cells, including macrophages, that express the Fas molecule (CD95), resulting in the destruction of cells infected with mycobacteria.[31] Our finding of CD95L expression by lymphocytes might be related to this mechanism.

In the present study, we have evaluated various cellular apoptosis markers to characterize the cellular phenotypes of patients with tuberculous or neoplastic pleural effusions. In addition, we used the total cell content of the pleural fluid in our evaluations. This approach minimizes sample manipulation and reduces the loss of leukocyte subpopulations.

## CONCLUSION

Tuberculous and malignant pleural effusions are enriched in lymphocytes with a helper/inducer T cell phenotype, which are mainly memory cells. CD14-positive cells were more prevalent in malignant effusions, while CD3-positive cells expressing Fas ligand were more prevalent in tuberculous effusions.

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