

Full Length Research Paper

Sputum cellularity in pulmonary tuberculosis: A comparative study between HIV-positive and -negative individuals

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To compare sputum cellularity between HIV-positive and -negative individuals with pulmonary tuberculosis. A cross-sectional study was conducted in patients with pulmonary tuberculosis. Sputum samples were collected and processed within two hours after collection. The absolute number of squamous cells of a total of 400 cells was counted, as well as the absolute number ($\times 10^6$ cells/ml) and percentage of eosinophils, lymphocytes, macrophages and neutrophils and total cellularity and viability were determined. Comparisons of the means of each cell type were held in a significance level of 95% ($p < 0.05$). Pearson's correlation coefficient between the identified cell types was calculated. Results: Assessment was performed in a cohort of 40 subjects, mean age 40 years, 77.5% male, 70% Caucasian, 40% HIV-positive (mean age 35.9 years). Mean percentage viability in the samples was 56.1%. The average value of squamous cells was 58.8. Mean percentages of cells were: 33.7% neutrophils, 1.7% eosinophils, 50.7%, macrophages and 12.3% lymphocytes. The average total cell count was 1.9×10^6 cells/ml. The average CD4⁺ T-cell count in HIV-positive was 95.4 cells/mm³. Association of radiological patterns was present in 72.5% of cases. Pearson's correlation coefficient was 0.08 ($p < 0.01$) between absolute counts of eosinophils and lymphocytes, eosinophils and macrophages and macrophages and neutrophils. Inverse relationship was observed between the percentage of macrophages and neutrophils. There was no statistically significant difference between cell count of HIV-positive and -negative individuals.

Key words: Sputum, tuberculosis, HIV.

INTRODUCTION

Tuberculosis is a chronic infectious disease caused by *Mycobacterium tuberculosis* bacillus (Koch bacillus), whose main characteristic is the preference for lung parenchyma and transmission from person to person, which occurs by inhalation of microorganism infected particles (Brasil. Ministério da Saúde. Coordenação Nacional de DST/AIDS, 1999).

With the advent of the acquired immunodeficiency syndrome (AIDS), recognized in 1981, a profound impact

on the global problem of tuberculosis occurred, which changed its epidemiology and particularly, its control became more difficult. Tuberculosis kills approximately two million people annually and measures to control this disease are vulnerable to early diagnosis, resistance to drugs used for its treatment as well as the socioeconomic conditions of populations at risk (Brasil. Ministério da Saúde. Coordenação Nacional de DST/AIDS, 1999; Duncan et al., 1996).

M. tuberculosis is a facultative intracellular bacterium; its replication process and the way it is carried through the host during the course of infection are not completely defined. It is believed that macrophages are the main

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cells in which the *M. tuberculosis* is found *in vivo*, although dendritic cells may also be infected. *M. tuberculosis* is found in extracellular environment in the stages of lung cavitation. It is not clear how the bacterium adapts specifically to the lungs at the expense of other tissues and how the bacterium survives and grows in phagocytes of macrophages and other cells (Brasil. Ministério da Saúde. Fundação Nacional de Saúde, 2002).

Human immunodeficiency virus (HIV) infection is a leading risk factor for the development of disease in individuals previously infected by the bacillus. While the chance of an infection progressing to TB disease in immunocompetent individuals is 10% over their life span, in HIV-infected individuals that is likely to be 8 to 10% every year. Moreover, it is one of the first and major complications among HIV-infected individuals, appearing before other common infections (Brasil. Ministério da Saúde. Fundação Nacional de Saúde, 2002; Davis et al., 1993).

In 1999, 10.7 million people co-infected with TB/HIV were identified, which represents 0.18% of the world population. In Brazil, of the 40.7 million infected with tuberculosis, about 300 thousand were co-infected with HIV (Brasil. Ministério da Saúde. Coordenação Nacional de DST/AIDS, 1999). TB remains a common disease and is an important differential diagnosis when lung secretions are sent for laboratory examination. Currently, there is little information on sputum cytology of patients with pulmonary tuberculosis (Brasil. Ministério da Saúde. Fundação Nacional de Saúde, 2002).

Radiological alterations of patients with TB/HIV co-infection depend on peripheral blood CD4⁺ T-cell count. When the CD4 count is below 200 cells/mm³, most manifestations of TB are atypical. At the beginning of HIV infection, or when the CD4⁺ T-cell count is above 200 cells/mm³, tuberculosis presents a radiologic form similar to that in immunocompetent patients, with typical reactivation pattern and with areas of alveolar consolidation at the apex, posterior segments of the upper lobes and superior segments of the lower lobes, often associated with cavitation (Boiselle et al., 2002; Haramati and Jenny-Avital, 1998; Shah et al., 1997; Keiper et al., 1995; Post et al., 1995; Naidich and McGuinness, 1991; Pitchenik and Rubinson, 1985; Pizzichini et al., 1996). In patients who are in advanced stages of HIV infection, with CD4⁺ T-cell count below 200 cells/mm³, significant radiographic differences are documented, compared to immunocompetent patients, such as mediastinal and/or hilar lymph nodes and in some cases, no radiographic alterations (Haramati and Jenny-Avital, 1998; Shah et al., 1997; Keiper et al., 1995; Naidich and McGuinness, 1991; Pitchenik and Rubinson, 1985). Tuberculosis infection in immunocompetent individuals begins primarily as a non-specific inflammatory reaction, progressing to a typical granulomatous reaction that limits *M. tuberculosis* spread.

In immunosuppressed individuals with low CD4⁺ T-cell count, granuloma formation does not occur (Botasso et al., 2007).

Although primarily cellular immunity is involved, other defects are also identified and play important role in the morbidity of HIV infection. T lymphocytes are critical for the activation of B lymphocytes and subsequent production of immunoglobulins, which is compromised by the primary disorder of cellular immunity. These systemic alterations are concomitant to the local alterations. Subsystems of different T lymphocytes are involved in immune response against *M. tuberculosis*. Interferon-gamma production by cells appears to be fundamental for disease control. Th1 cytokine response type is predominant in patients with mild and moderate forms of pulmonary tuberculosis, while Th2 type cytokine production prevails in more severe disease. Studies show that patients with cavitary tuberculosis revealed the presence of IL-4 produced by Th2 system. In contrast, Th1-type cytokines are found in cases of non-cavitary disease (Botasso et al., 2007).

The macrophages present CD4 antigens and can be directly infected by HIV. In these cases the process of chemotaxis is also disturbed, resulting in decrease or even absence of granulomatous reaction. In addition, there is a decrease in chemotaxis of polymorphonuclear cells (Davis et al., 1993). Therefore, alterations in sputum cellularity occur concurrently to alterations in peripheral blood CD4⁺ T-cells, reflecting on the manifestations of respiratory diseases in this specific group of patients, both in the radiological manifestations and in the tissue reactions where there is no granuloma formation due to decreased immunity (Davis et al., 1993).

During a recent infection with HIV, when the function of the immune system is relatively intact, sputum examination of smear-positive for tuberculosis predominates. In contrast, patients with advanced HIV infection with significant immunosuppression often present negative sputum examination results and disease disseminates. Although the correlation between the significance of sputum examination and decrease in the immune system function (drop in the number of CD4⁺ T-cells) in HIV-positive patients with pulmonary tuberculosis is well documented, the relationship between sputum examination and local immune response in the lungs is not clear (Mwandumba et al., 2008).

The study carried out by Belda and collaborators found the following results for sputum cellularity in healthy patients: cell viability was 89.7%, the proportion of eosinophils was 1.1%, neutrophils 64%, macrophages 86.1%, lymphocytes 2.6%, metachromatic cells 0.04% and epithelial cells 4.4%. Female gender and atopy are associated with a significant elevation of eosinophils, male-to-female ratio was 0.3% and between atopic and non-atopic patients was 0.4% (Belda et al., 2000).

There is little information on sputum cytology in pulmonary tuberculosis. A study carried out by Tani and

collaborators showed that alveolar macrophages are always present, while neutrophils are present in 97.9% of samples, usually in large numbers. Lymphocytes were found in 84.9% and eosinophils in 8.9%, usually in small numbers. Epithelial cells were found in 56.1% of samples, usually appearing in groups with oval and elongated nucleus, along with a large vacuolated cytoplasm. Multinucleate giant cells were present in 40% of samples, usually in small numbers and often associated with epithelial cells. Respiratory epithelium cells showed changes in 20% of samples, which include grouped columnar cells with hyperchromatic nucleus. Squamous metaplasia was observed in 19% of samples (Tani et al., 1987).

This study was carried out to compare sputum cellularity between HIV-positive and HIV-negative individuals with pulmonary tuberculosis.

MATERIALS AND METHODS

A cross-sectional study was performed at Hospital Nereu Ramos, in Florianópolis, Santa Catarina, in which all patients over 14 years with pulmonary tuberculosis admitted between November 2008 and February 2009 were analyzed. Patients who had pulmonary comorbidity were excluded from the study, as well as those who refused to sign the Term of Free and Informed Consent, or those who were unable to produce sputum spontaneously. HIV-infected patients were considered those who had positive serology for HIV, patients with pulmonary tuberculosis and those with identification of *M. tuberculosis* in respiratory samples (bronchoalveolar lavage and/or lung and pleural biopsies).

Chest x-rays were classified according to alteration patterns in alveolar consolidation, interstitial, pleural effusion, mass, nodule, cavitation, mediastinal and/or hilar lymph nodes and their associations. Siemens X-ray device was used for postero-anterior and lateral chest radiography, using 120 Kv and 3 to 6 mAs.

Sputum samples were collected at morning and before breakfast. The sputum samples were processed within two hours after collection. An aliquot of sputum was treated with approximately 4 volumes of DTT (dithiothreitol) plus 4 volumes of PBS (phosphate buffer) and filtered after homogenization. Twenty microlitres of filtrate were mixed with 20 μ l of 4% trypan blue. This mixture was placed in a Neubauer chamber where the absolute number of cells ($\times 10^6$ cells/ml) and the number of live and dead cells in a clear field microscope with a 400x magnification were counted. The percentage of live and dead cells was resulting from the equation: (number of live cells/total number of cells) \times 100. The presence of alveolar macrophages was determined by differential leukocyte counting in the filtrate of the sputum sample. The filtrates were concentrated by citospin technique and the slides were stained by the May-Grünwald/Giemsa method and viewed in clear field microscope with a 1000x magnification. The number of squamous epithelial cells in a total of 400 cells and the number of eosinophils, lymphocytes, macrophages and neutrophils on differential count of 100 cells was determined. The absolute number and percentage of each cell type was calculated based on the total number of cells (Pizzichini et al., 1996; Botasso et al., 2007).

Each participant was registered in a form of inclusion and agreed to participate by signing a Term of Free and Informed Consent.

Database development and statistical analysis were performed using SPSS version 16.0[®] software. Data were summarized as percentage or mean, as indicated, and comparisons of means of each cell type were performed by Student's t-test, with a significance

level at 95% ($p < 0.05$). The Pearson's correlation coefficient for the identified cell types was also calculated.

The research project was submitted to the Ethics Committee and Human Research at Unisul and approved under code number 09.005.4.01.III.

RESULTS

Forty consecutive individuals, 31 (77.5%) male, were evaluated. Regarding ethnicity, 28 (70%) were Caucasian. Mean age was 40 years (SD \pm 12), ranging from 22 to 69 years.

Of the participants, 16 (40%) were HIV positive and 24 (60%) were negative. Mean age of HIV-positive individuals was 35.9 years (SD \pm 7.16) and mean age of HIV-negative individuals was 42.8 years (SD \pm 13.8). There was no statistically significant difference between mean age of HIV-positive and HIV-negative individuals ($p > 0.05$).

Mean percentage of viability in sputum samples was 56.1% (SD \pm 31%). The average value of squamous cells in a total of 400 cells assessed was 58.8 (SD \pm 157.7).

Mean value of cells ($\times 10^6$ cells/ml \pm SD) and mean percentage (\pm SD) in sputum samples were: neutrophils 0.9 ± 1.4 (33.7 \pm 3.2%), eosinophils 0.03 ± 0.08 (1.7 \pm 2.8%), macrophages 0.8 ± 1.3 (50.7 \pm 20.3%), lymphocytes 0.2 ± 0.3 (12.3 \pm 11.8%) and total cells 1.9 ± 2.5 .

Mean CD4⁺ T-cell count in HIV-positive individuals was 95.4 ± 62.8 cells/mm³, with a minimum of 13 and maximum of 224 cells/mm³.

Mean value and percentage of cells in sputum samples of HIV-positive and HIV-negative individuals are shown in Table 1.

There was a statistically significant difference in absolute counts of squamous cells when compared between HIV-positive and -negative individuals ($p < 0.028$). There was no statistically significant difference between the other cell counts when compared between HIV-positive and -negative individuals ($p > 0.05$).

With regard to radiological alterations, the association between patterns was present in 29 (72.5%) of cases (10 HIV-positive and 19 HIV-negative), alveolar injury in 32 (80%) of cases (13 HIV-positive and 19-negative), interstitial lesion in 15 (37.5%) of cases (8 HIV-positive and 7 -negative), cavitation in 10 (25%) of cases (4 HIV-positive and 6 -negative), pleural effusion in 9 (22, 5%) of cases (3 HIV-positive and 6 -negative), atelectasis in 4 (10%) of cases (1 HIV-positive and 3 -negative), nodules in 3 (7.5%) of cases (HIV-negative) and pneumothorax, adenomegaly and mass in 1 (2.5%) of cases, respectively (HIV-negative).

The average values and percentage of cells in sputum samples in accordance with the radiological alterations are shown in Table 2.

Pearson's correlation coefficient between the cell values in sputum samples is shown in Table 3.

Table 1. Mean value and percentage of cells in sputum samples of HIV-positive and -negative individuals.

	HIV-positive		HIV-negative	
	n (±DP)	% (±DP)	n (±DP)	% (±DP)
Viability	-	54.4 ± 33.5	-	57.2 ± 29.9
Squamous Cells* (in 400 cells)	116.1 ± 241.1	-	20.5 ± 21.4	-
Neutrophils [#]	1.1 ± 1.7	34.3 ± 28	0.7 ± 1.1	33.4 ± 20.1
Eosinophils [#]	0.03 ± 0.1	2.2 ± 3.6	0.04 ± 0.1	1.4 ± 2
Macrophages [#]	0.6 ± 1.2	50.6 ± 28.4	0.9 ± 1.3	50.8 ± 13.1
Lymphocytes [#]	0.2 ± 0.3	10.6 ± 9.6	0.2 ± 0.3	13.4 ± 13.1
Total Cells [#]	1.9 ± 2.8	-	1.8 ± 2.4	-

* p 0.028 [#] x 10⁶/ml.

Table 2. Mean value and percentage of cells in sputum samples according to the radiological patterns.

	Viability % (±DP)	Squamous cells		Neutrophils		Eosinophils		Macrophages		Lymphocytes		Total cells	
		n (±DP)	% (±DP)	n (±DP)	% (±DP)	n (±DP)	% (±DP)	n (±DP)	% (±DP)	n (±DP)	% (±DP)	n (±DP)	% (±DP)
Association	Yes	54.4 ± 30.2	60.2 ± 182.3	1 ± 1.5	35.4 ± 24.5	0.03 ± 0.1	1.9 ± 3.1	0.8 ± 1.4	49.8 ± 19.7	0.2 ± 0.3	11.7 ± 10.1	2 ± 2.8	2 ± 2.8
Standards	No	60.4 ± 34.3	55 ± 62.6	0.7 ± 1	29.4 ± 20	0.03 ± 0.1	1.3 ± 1.6	0.7 ± 1.1	53 ± 22.5	0.2 ± 0.3	13.9 ± 15.8	1.6 ± 1.9	1.6 ± 1.9
Alveolar	Yes	53.8 ± 31.2	59.3 ± 174	1 ± 1.4	34 ± 23.3	0.03 ± 0.1	1.7 ± 3	0.9 ± 1.4	51.3 ± 19.9	0.2 ± 0.3	11.7 ± 10.7	2.1 ± 2.7	2.1 ± 2.7
	No	65 ± 30.6	56.9 ± 66.7	0.4 ± 1	32.8 ± 24.6	0.03 ± 0.1	1.6 ± 1.8	0.2 ± 0.2	48.3 ± 23.2	0.2 ± 0.3	14.8 ± 15.9	0.8 ± 1.3	0.8 ± 1.3
Interstitial	Yes	57.8 ± 36.5	122.9 ± 246.7*	0.9 ± 1.7	33.5 ± 23.6	0.03 ± 0.1	3.1 ± 3.7*	0.5 ± 1	46.5 ± 22.1	0.2 ± 0.3	15.6 ± 11.7	1.6 ± 2.7	1.6 ± 2.7
	No	55 ± 28	20.3 ± 28.5*	0.9 ± 1.1	33.9 ± 23.5	0.03 ± 0.1	0.8 ± 1.5*	1 ± 1.4	53.2 ± 19.2	0.1 ± 0.3	10.3 ± 11.6	2 ± 2.5	2 ± 2.5
Cavitation	Yes	47.4 ± 23	33.6 ± 58	0.7 ± 1.3	30.9 ± 24.6	0.03 ± 0.1	2 ± 4.4	0.8 ± 1.4	57.5 ± 17.3	0.1 ± 0.1	11.3 ± 10.3	1.7 ± 2.5	1.7 ± 2.5
	No	59 ± 33.1	67.2 ± 179.2	0.9 ± 1.4	34.7 ± 23.1	0.04 ± 0.1	1.6 ± 2	0.8 ± 1.2	48.4 ± 21	0.2 ± 0.3	12.7 ± 12.4	1.9 ± 2.6	1.9 ± 2.6
Pleural effusion	Yes	69.6 ± 19.8	12.3 ± 16.5	1.2 ± 1.2	39.6 ± 31.1	0.1 ± 0.1	1.3 ± 1.9	1.1 ± 1.5	47.3 ± 25.4	0.2 ± 0.4	5.2 ± 4.8*	2.5 ± 2.7	2.5 ± 2.7
	No	52.1 ± 32.8	72.3 ± 177.3	0.8 ± 1.4	32.1 ± 20.7	0.03 ± 0.1	1.8 ± 3	0.7 ± 1.2	51.7 ± 19	0.2 ± 0.3	14.4 ± 12.4*	1.7 ± 2.5	1.7 ± 2.5
Atelectasis	Yes	69 ± 4.4	17.5 ± 31.1	1.1 ± 1.9	39 ± 30.1	0.0002 ± 0.0003	0.5 ± 1	0.5 ± 0.4	49.5 ± 27.5	0.1 ± 0.2	8.8 ± 11.5	1.8 ± 2.3	1.8 ± 2.3
	No	54.6 ± 32.4	63.4 ± 165.6	0.9 ± 1.3	33.2 ± 22.8	0.04 ± 0.1	1.8 ± 2.9	0.8 ± 1.3	50.8 ± 19.8	0.2 ± 0.3	12.7 ± 11.9	1.9 ± 2.6	1.9 ± 2.6
Nodule	Yes	37 ± 34	3.7 ± 5.5	1.3 ± 2.3	39 ± 39	0 ± 0	0 ± 0	0.4 ± 0.5	42.3 ± 20.2	0.1 ± 0.1	18.3 ± 23.3	1.8 ± 2.9	1.8 ± 2.9
	No	57.6 ± 30.7	63.2 ± 163.3	0.8 ± 1.3	33.3 ± 22.3	0.04 ± 0.1	1.8 ± 2.8	0.8 ± 1.3	51.4 ± 20.4	0.2 ± 0.3	11.8 ± 10.8	1.9 ± 2.6	1.9 ± 2.6
Pneumothorax	Yes	67	1	4*	79*	0	0	1	19	0.1	2	5.1	5.1
	No	55.8 ± 31.4	60.3 ± 159.5	0.8 ± 1.3*	32.6 ± 22.3*	0.03 ± 0.1	1.7 ± 2.8	0.8 ± 1.3	51.5 ± 19.9	0.2 ± 0.3	12.6 ± 11.8	1.8 ± 2.5	1.8 ± 2.5
Adenomegaly	Yes	67	1	4*	79*	0	0	1	19	0.1	2	5.1*	5.1*
	No	55.8 ± 31.4	60.3 ± 159.5	0.8 ± 1.3*	32.6 ± 22.3*	0.03 ± 0.1	1.7 ± 2.8	0.8 ± 1.3	51.5 ± 19.9	0.2 ± 0.3	12.6 ± 11.8	1.8 ± 2.5*	1.8 ± 2.5*

* p < 0.05.

Table 3. Pearson's correlation coefficient between mean value and percentage of cells in sputum samples.

	Viability (%)	Squamous cells (n)	Neutrophils (n)	Neutrophils (%)	Eosinophils (n)	Eosinophils (%)	Macrophages (n)	Macrophages (%)	Lymphocytes (n)	Lymphocytes (%)	Total cells
Viabilidade (%)	1	-0.2	0.2	0.1	0.1	0.2	0.02	-0.5**	0.3	0.01	0.2
Squamous cells (n)	-0.2	1	-0.1	-0.2	-0.02	0.2	-0.2	0.1	-0.03	0.2	-0.2
Neutrophils (n)	0.2	-0.1	1	0.6**	0.4*	-0.04	0.6**	-0.5**	0.4*	-0.2	0.9**
Neutrophils (%)	0.2	-0.2	0.6**	1	0.1	-0.3	0.1	-0.7**	0.2	-0.3	0.4*
Eosinophils (n)	0.1	-0.02	0.4*	0.1	1	0.4*	0.5**	-0.3	0.8**	0.1	0.6**
Eosinophils (%)	0.2	0.2	-0.04	-0.3	0.4*	1	-0.01	-0.1	0.2	0.2	0.02
Macrophages (n)	0.02	-0.2	0.6**	0.1	0.5**	-0.01	1	0.1	0.3*	-0.3	0.9**
Macrophages %	-0.5	0.1	-0.5**	-0.7**	-0.3	-0.1	0.1	1	-0.5**	-0.2	-0.3*
Lymphocytes n	0.3	-0.03	0.4*	0.2	0.8**	0.2	0.3*	-0.5**	1	0.4*	0.5**
Lymphocytes %	0.01	0.2	-0.2	-0.3	0.1	0.2	-0.3	-0.2	0.4*	1	-0.2
Total cells	0.2	-0.2	0.9**	0.4*	0.6**	0.02	0.9**	-0.3*	0.5**	-0.02	1

* p < 0.05. ** p < 0.01.

DISCUSSION

The results show that the population studied was predominantly male, coinciding with those found by Tani et al. in a study on sputum cellularity in patients with pulmonary tuberculosis (Tani et al., 1987). Male pre-dominance among patients with pulmonary tuberculosis is also described in several Brazilian studies showing that the assessed sample is in accordance with the epidemiological profile of the disease described in our country (Brito et al., 2004; Cruz et al., 2008; Silveira et al., 2007). Mean age of participants in this study was 40 years with standard deviation of 12 years, results which are very close to those found by Cruz and collaborators, in which the mean age was 41.08 and standard deviation was 14.32 years (Cruz et al., 2008). Silveira and collaborators observed similar results, obtaining a mean age of 49 years (Silveira et al., 2007). There was no statistically significant difference between mean age of HIV-positive and -negative individuals. The national epidemiological profile of

the two conditions, AIDS and tuberculosis, is very similar with regard to age and gender (Brasil. Ministério da Saúde. Coordenação Nacional de DST/AIDS, 1999; Brasil. Ministério da Saúde. Fundação Nacional de Saúde, 2002).

Serology testing for HIV was positive in 40% of participants in this study. HIV infection is one of the factors responsible for increasing the number of tuberculosis cases. The risk for these individuals to develop from TB infection to TB disease is 8 to 10% every year (Brasil. Ministério da Saúde. Fundação Nacional de Saúde, 2002; Davis et al., 1993). A study conducted by Santos and collaborators, comparing data between 2001 and 2003 with data between 1991 and 1993, in a city of the state of Santa Catarina, has shown an increase in the number of cases of HIV/AIDS coinfection in the period (Santos et al., 2005).

The average percentage of cell viability in sputum samples was 56.1%, indicating that, on average, the samples were of good quality. According to Pizzichini and collaborators, samples with more than 40% of viable cells are considered

of good quality, that is, from the lower respiratory tract (Pizzichini et al., 1996). Moreover, samples with less than 50% viability and with high contamination from squamous cell hamper studies that require precise cell counts and great accuracy in cell identification (Efthimiadis et al., 1997.).

In this study, the differential cell count showed a predominance of alveolar macrophages (50.7%), followed by neutrophils (33.7%), lymphocytes (12.3%), and eosinophils (1.7%). Efthimiadis and collaborators reported similar results concerning cellular predominance in healthy individuals, however, with a proportional number of lymphocytes significantly lower than that described here (1.4%), as well as eosinophils (0.6%) (Efthimiadis et al., 1997.). Similar results were described by Spanevello and collaborators, in which the proportion of lymphocytes (1%) and eosinophils (0.6%) was also lower than that described in this study (Spanevello et al., 2000). These results demonstrate the lymphocyte characteristic of immune response involved in pulmonary tuberculosis (Botasso et al.,

2007; Mwandumba et al., 2008).

A lymphocyte CD4⁺ T-cell count of 200 cells/mm³ is considered the cut-off point between individuals who will have the typical or atypical form of pulmonary tuberculosis, because this value determines the acquired immunodeficiency degree from the disease, that is, values below 200 cells/mm³ indicate an advanced immunosuppression (Curvo-Semedo et al., 2005). The average found for the CD4⁺ T-cell count in HIV-positive individuals in this study was 95.38 ± 62.8 cells/mm³, indicating that patients had a very compromised immunity.

For average values of cells found in sputum samples from individuals with and without HIV, there was a statistically significant difference in the absolute count of squamous cells, with higher values in HIV-positive individuals ($p < 0.05$). There is a probability that this figure results from a higher contamination at collection of the sputum sample of these patients (Efthimiadis et al., 1997). There was no statistically significant difference between the other cell counts when compared between HIV-positive and HIV-negative individuals ($p > 0.05$). HIV-positive patients have CD4⁺ T-cell count reduced in peripheral blood, but this fact does not seem to occur in the immune response that occurs in the lung tissue. Patho-physiology features of tuberculosis in this specific group of patients may then be a consequence of qualitative alterations of lymphocyte immune response and not quantitative alterations as suggested in peripheral blood. In this study, lymphocytes found in sputum samples were not typified, so there is no way to know to which lymphocyte subpopulation they belong. The type of lymphocyte involved in immune response determines its quality; further studies with this methodology are needed to confirm or refute this hypothesis (Botasso et al., 2007; Mwandumba et al., 2008; Deveci et al., 2006; Nicod, 2007).

Radiological alterations in patients were distributed in various patterns. Pattern association was found in 72.5% of cases. Curvo-Semedo and collaborators reported the coexistence of 30% between consolidation, cavitation and lymph node (Curvo-Semedo et al., 2005). Cavitation was present in 25% of cases. Curvo-Semedo's study reported that cavitation occurs in approximately 50% of patients. Lower results found in this study can be due to the number of HIV-positive patients with low lymphocyte CD4⁺ T-cell count in peripheral blood, which do not form either cavitation or granules (Boiselle et al., 2002; Haramati and Jenny-Avital, 1998; Shah et al., 1997; Keiper et al., 1995).

Adenomegaly was reported in 2.5% of cases, which corroborates the findings by Curvo-Semedo et al. (2005), who describe that mediastinal or hilar lymph nodes are rarely found in post-primary disease, occurring in approximately 5% of cases.

The percentages of eosinophils were different in individuals with interstitial lesion, being higher when the lesion occurred ($p < 0.05$). This may be due to the quality

of immune response in patients with this type of injury when compared to other radiographic alterations (Boiselle et al., 2002; Haramati and Jenny-Avital, 1998; Shah et al., 1997; Keiper et al., 1995). Studies with larger sample sizes and methodology addressed to this topic in particular must be performed to confirm or refute this hypothesis. Other differences between the cellular values found and the type of radiological alterations, although statistically significant, may not be highlighted due to the small number of patients with these alterations (adenomegaly, pneumothorax, nodules and atelectasis).

Pearson's correlation coefficient was 0.08 ($p < 0.01$) between eosinophil and lymphocyte absolute counts, indicating the concomitant increase in the two cell types in the inflammatory response of pulmonary tuberculosis.

The same trend was observed between eosinophils and macrophages and between macrophages and neutrophils. Inverse relationship was observed between the percentage of macrophages and neutrophils.

Further studies with larger samples should be performed to confirm or refute the numerical trends presented here.

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