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Morphometric and DNA Image Analysis of Bronchoalveolar Lavage Fluid Macrophages Nuclei in Interstitial Lung Diseases with Lymphocytic Alveolitis

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A B S T R A C T

Lymphocytic alveolitis is a characteristic of diverse interstitial lung diseases (ILD-s), but macrophages are often more numerous cell population in bronchoalveolar lavage fluid (BALF). Aim of this study is to analyze morphometric characteristics of macrophages nuclei in BALF in patients with ILD-s and to detect possible differences allowing distinguishing sarcoidosis from other lymphocytic alveolitis ILD-s. Thirty-one patient with interstitial lung disease who had lymphocytic alveolitis in BALF cell count (17 sarcoidosis and 14 other ILD-s) and nine controls were included in the study. The following patients data were numbered: age, lymphocyte percentage and CD4/CD8 ratio in BALF. Investigated morphometric parameters of macrophages nuclei were: area, outline, maximal radius, minimal radius, length, breadth, form factor (FF), elongation factor (EF) and DNA image cytometry ploidy status determined with Van Velthoven method. Predicted classifications in classification matrix (forward step-wise method in multivariate discriminant function analysis) based on macrophages nuclei length mean, minimum and maximum, breadth SD, FF mean and lymphocyte % were 100% (9/9) correct for control group, 88.235% (15/17) correct for sarcoidosis, and 92.857% (13/14) correct for other lymphocytic alveolitis ILD group. In total, 92.5% (37/40) of the examinees were correctly classified in particular group upon the observed variables.

***Key words**: morphometry, DNA cytometry, bronchoalveolar lavage, macrophages nuclei, lymphocytic alveolitis, interstitial lung disease*

Introduction

Interstitial lung disease (ILD) includes a variety of clinical disorders with different origin. For practical reasons they can be divided in several groups: of known cause (hypersensitivity pneumonitis), idiopathic interstitial pneumonias, granulomatous diseases (sarcoidosis) and rare diseases (alveolar proteinosis)[1]. In the differential diagnosis of the interstitial pulmonary disorders, sarcoidosis, collagen vascular diseases, idiopathic pulmonary fibrosis (IPF) and bronchiolitis obliterans organising pnumonitis (BOOP) are the most common and responsible for about two thirds of all cases[2].

Bronchoalveolar lavage (BAL) is important diagnostic procedure for the cell profile analysis. Lymphocyte increase in differential lung lavage cell count is helpful in differential diagnosis of ILD-s. It is most pronounced in sarcoidosis, hypersensitivity and drug-induced pneumonitis, and less intensive in IPF, BOOP, RB-ILD/DIP (respiratory bronchiolitis associated interstitial lung disease) and collagen vascular diseases. CD4 /CD8 ratio in broncholaveolar lavage fluid (BALF) is helpful in distinction of active sarcoidosis and hypersensitivity pneumonits[1]. However most find its sensitivity and specificity to

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be inadequate for the diagnosis[3]. In about 4–10% of cases, sarcoidosis presents with a CD8 lymphocytic alveolitis and a low CD4/CD8 ratio[4].

Macrophages represent the largest cell population obtained by BAL from the lungs of patients with the most ILD-a. In sarcoidosis macrophages are antigen presenting cells responsible for granuloma formation, but also secrete TNFα, IL-12, IL-15, IL-8 and different growth factors[5]. Their absolute number is often increased in hypersensitivity pneumonitis and macrophages activation is demonstrated by the significant expression of activation markers including CD54, selectins, and costimulatory molecules. Recent data involve macrophages as a cell source of chemokines that drive T-cell recruitment[6]. Macrophages in sarcoidosis are larger than macrophages in healthy controls and posses different ultrastructural characteristics[7]. They are more uniform in appearance than in hypersensitivity pneumonitis and lack foamy characteristics[8]. Recently, three types of macrophages in sarcoidosis have been recognized ultrastructurally, 70% among them with signs of increased activity and cytokine release[9]. Epithelioid cells are rare in BAL fluid and they are a sign of disease activity. There are few morphometric and DNA cytometric studies of epithelioid cells nuclei in histological sections of sarcoid and tuberculoid granulomas. Epithelioid cells in sarcoidosis were more elliptic with smaller outline, area and diameter than epithelioid cells in tuberculosis[10]. Some authors find that most epithelioid cells in sarcoid granuloma of lymph nodes had aneuploid or hypodiploid DNA content[11,12].

Aim of this study is to analyze morphometric and DNA cytometric characteristics of macrophages nuclei in BAL fluid in patients with ILD-s and controls, as well as to detect possible differences allowing differential diagnosis between sarcoidosis and other lymphocytic alveolitis ILD-s.

Materials and Methods

BAL fluid (BALF) was obtained during diagnostic flexible bronchoscopy. Thirty-one patients with interstitial lung disease had lymphocytic alveolitis in BAL fluid cell count (more than 15% of lymphocytes). Among them, 17 had sarcoidosis and in 14 other lymphocytic alveolitis ILD-s were confirmed with standard diagnostic procedures. Among patients with other lymphocytic alveolitis ILD-s, clinical diagnosis was hypersensitivity pneumonitis in 5 patients, 5 patients had diffuse connective tissue diseases, in 2 patients diagnosis was IPF and BOOP in remaining 2 patients. BAL fluid count was normal and interstitial lung disease was not proven with other diagnostic procedures in 9 individuals, so they were considered controls.

Morphometric and DNA image analysis were performed on macrophages nuclei in cytological specimens of BAL fluid. Cytocentrifugation was done with the Shandon Cytospin 4 (Shandon Scientific Ltd, Astmoor, U.K.), speed 1000 rpm, time: 5 min and acceleration rate: low. Number of drops *per* preparation was adjusted according to the total cell count in order to obtain monolayer preparations. The preparations were air-dried and subsequently stained according to the May-Grünwald-Giemsa (MGG) for cell differentials. Archived slides were then restained with Feulgen method for morphometric and DNA image analysis of macrophages nuclei[13]. Random sampling was performed by systemic measurement of cells under microscope. Cells selection started at point inside a region with cellular material evenly distributed, proceeded cell by cell in one field, and continued to the next fields[14]. Light microscopic analysis under oil immersion (x100) was applied and analysis was conducted in one focal nuclear plane. In each case 100 or more nuclei were analyzed, and processed with an image analyzer using SFORM software for digital image analysis (VAMSTEC, Zagreb). Objects contours were marked with special tools, interactive, by mouse selection (Figure 1). Investigated morphometric parameters of macrophages nuclei were simple object parameters as area, outline, maximal radius, minimal radius, length, breadth and complex parameters as form or regularity factor (FF) and elongation factor (EF). Form factor (4π x area/outline^2) describes irregularities compared with a circle, while elongation factor is length to breadth ratio[15]. DNA image cytometric analysis of ploidy status was performed simultaneously as other morphometric measurements. Nuclei of neutrophilic granulocytes were used for internal controls on the same slide as investigated macrophages. DNA content was measured indirectly after quantitative DNA staining with Feulgen. Nuclear IOD (integrated optical density) represents cytometric equivalent of DNA content, rescaling of the IOD values by comparison with IOD values of cells with known DNA content was necessary for quantification of nuclear DNA content of measured cell[16]. DNA image cytometry ploidy status was determined with Van Velthoven method. This method includes near-diploid hyperdiploid and triploid histogram types as aneuploid types. It is superior in describing changes in DNA content of non-malignant cells with high proliferative activity. DNA index (DI) has been cal-

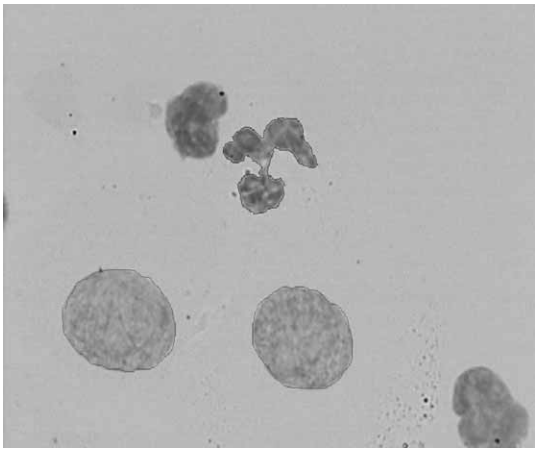

Fig. 1. Marked contours of investigated and control nuclei in morphometric analysis procedure. Feulgen staining, original magnification x1000.

culated as ratio between modal value of the investigated cell peak divided by the modal value of the diploid reference cell peak. Dyploid histograms had a DI value >0.90 <1.15, hyperdiploid >1.16<1.39, triploid >1.40<1.60, hypertiploid >1.61<1.89 and tetraploid >1.90<2.20[17].

Statistical Analysis

Statistical analysis was performed in Statistica for Windows version 6.0 (StatSoft, Inc. Tulsa, OK). Mean, median, standard deviation and minimum and maximum value have been calculated for all morphometric parameters. Mean, standard deviation and 95% confidence interval (95% CI) were used for description of variables and variance analysis was used for comparison between the groups. χ^2-test was used for comparison of variables categories distribution among groups. Variables categories were represented as frequency (%). Classification criteria (diagnostic threshold values) were calculated with discriminant function analysis. Forward step-wise method in multivariate discriminant function analysis was performed because of sample size and complexity.

Results

In the study were included 31 patients with ILD and lymphocytic alveolitis in BAL fluid differential cell count in cytological samples and 9 controls with normal differential cell count. Sarcoidosis was final diagnosis in 17 patients and various ILD entities were diagnosed in 14 patients. The following patients data were included in the study: age, lymphocyte percentage and CD4/CD8 ratio in BAL fluid. Investigated parameters of macrophages nuclei were morphometric parameters: area, outline, maximal radius, minimal radius, length, breadth, form factor (FF), elongation factor (EF) and DNA image cytometry ploidy status determined with Van Velthoven method.

Age range for controls was 32–77 years, ($\overline{X}$±SD 54.67± 15.17), in sarcoidosis patients group range was 23–54 years, ($\overline{X}$±SD 39.18±9.34) and for other lymphocytic alveolitis ILD group range was 27–82 years, ($\overline{X}$±SD 57.21± 17.71). There were statistical significant differences in age means and SD among groups ($p<0.05$). Lymphocyte percentage range was 5.96–17.15, ($\overline{X}$±SD 11.56±7.27) for control group, in sarcoidosis patients 26.67–41.86 ($\overline{X}$±SD 34.26±14.78) and for other lymphocytic alveolitis ILD group range was 35.15–56.57 ($\overline{X}$±SD 45.86±18.55), with statistical significant differences among groups ($p< 0.05$). In control group CD4/CD8 ratio range was 0.8–2.5 ($\overline{X}$±SD 1.69±0.49), in sarcoidosis group 0.7–16.1, $\overline{X}$±SD 6.85±4.37 and for other lymphocytic alveolitis ILD group range was 0.3–6.8 with $\overline{X}$±SD 0.29±26.48. CD4/CD8 ratio showed no significant differences between groups ($p> 0.05$). DNA image cytometry results obtained with Van Velthoven method showed predominant hyperdiplod DNA content (DI>1.16<1.39), 6/9 in control group, predominant triploid DNA content (DI>1.40<1.60), 9/14 in other lymphocytic alveolitis ILD group and 10/17 in sarcoidosis group; with statistical significant differences among controls and investigated groups (χ^2-test, $p< 0.05$). Mean, standard deviation (SD) and 95% confidence interval (95% CI) of investigated morphometric parameters for the groups (controls, sarcoidosis and other lymphocytic alveolitis ILD) have been summarized in Table 1.

TABLE 1
MEAN VALUE, STANDARD DEVIATION AND 95% CONFIDENCE INTERVAL OF INVESTIGATED MORPHOMETRIC PARAMETERS FOR THE GROUPS

Morphometric parameter	Controls (n=9)	ILD patients (n=31)	
		Sarcoidosis (n=17)	Other LA-ILD (n=14)
Area (μm²)	85.696±9.699	96.777±6.358	102.577±8.298
	78.241–93.151	93.508–100.047	97.786–107.368
Outline (μm)	37.801±1.812	40.901±1.844	41.070±2.333
	36.408–39.194	39.953–41.849	39.723–42.417
Minimal radius (μm)	4.198±0.285	4.105±0.251	4.205±0.294
	3.978–4.417	3.977–4.235	4.035–4.375
Maximal radius (μm)	6.202±0.312	6.957±0.374	7.177±0.550
	5.963–6.442	6.764–7.149	6.860–7.495
Length (μm)	12.030±0.633	13.441±0.681	13.889±1.047
	11.543–12.517	13.090–13.791	13.285–14.494
Breadth (μm)	9.255±0.565	9.496±0.347	9.706±0.354
	8.821–9.690	9.318–9.674	9.501–9.910
Elongation factor (EF)	1.315±0.047	1.439±0.085	1.458±0.122
	1.279–1.351	1.396–1.483	1.388–1.529
Form factor (FF)	0.752±0.027	0.737±0.047	0.771±0.033
	0.731–0.772	0.713–0.760	0.752–0.791

ILD – interstitial lung disease, LA-ILD – lymphocytic alveolitis interstitial lung disease

TABLE 2
COMPARISON BETWEEN INVESTIGATED PARAMETERS IN GROUPS (VARIANCE ANALYSIS)

Parameters	Ly%	Age	EF (Elongation factor)	FF (Form factor)	Area	Outline	Min radius	Max radius	Length	Breadth
Statistical descriptions with p-values <0.05	Mean SD	Mean SD	Mean SD Max	SD Min Max	All	All	SD Min Max	All	All	Mean SD Min Max

Mn – mean value, SD – standard deviation, Max – maximum value, Min – minimum value, All – mean, median, standard deviation, maximum and minimum values

TABLE 3
HIGHEST MEAN VALUES FOR EVERY MORPHOMETRIC PARAMETER IN GROUPS ACCORDING TO STATISTICAL DESCRIPTIONS

	Area	Outline	Minimum radius	Maximum radius	Lenghth	Breadth	EF	FF
Controls			Min					Min Max
Sarcoidosis	SD Max	SD Max	SD Max	SD Max	SD max	SD Max	SD Max	SD
Other LA- ILD	Mn Md Min	Mn Md Min	Mn Md	Mn Md Min	Mn Md Min	Mn Md Min	Mn Md Min	Mn Md

Mn – mean, Md – median, SD – standard deviation, Max – maximum value, Min – minimum value, EF – elongation factor, FF – form factor, LA-ILD – lymphocytic alveolitis interstitial lung disease

Statistical descriptions of values (mean, median, SD, minimum, maximum) that were significantly different between controls, sarcoidosis and other lymphocytic alveolitis ILD groups for each morphometric parameter have been shown in Table 2. Parameters: area, outline, maximal radius and length were statistically significant ($p<0.05$) among groups according to statistical descriptions. Additionally, all statistical descriptions of nuclei areas, outlines, maximal radius, length, elongation factor and form factor median and standard deviation were the lowest in control group compared with ILD-s groups. All mean values and medians of investigated morphometric parameters were highest in other lymphocytic alveolitis ILD group, but there were differences in the highest values in SD, minimum and maximum of all investigated parameters, most of them were in sarcoidosis group, except the least value of minimal radius minimum and form factor (FF) minimum and maximum values in control group (Table 3). Variables obtained with discriminant forward step-wise analysis were used as classification functions for investigated groups, including morphometric parameters of macrophages nuclei, as well as percent of lymphocytes in BALF cell count. Morphometric parameters of macrophages nuclei used for classification matrix were length mean, minimum and maximum, breadth standard deviation and form factor (FF) mean. Their characteristics were summarized in Table 4. The classification matrix shows the number of cases that were correctly classified on the diagonal of the matrix and those that were misclassified. Predicted classifications in classification matrix based on macrophages nuclei length mean, minimum and maximum, breadth SD, FF mean and lymphocyte % were 100% (9/9) correct for control group, 88.235% (15/17) correct for sarcoidosis, as well as 92.857% (13/14) correct for other lymphocytic alveolitis ILD group (Table 5). In total, 92.5% (37/40) of the examinees were correctly classified in particular groups with observed variables.

Discussion

The present study demonstrates statistical significant differences in morphometric characteristics of macrophages nuclei in controls, sarcoidosis and other ILD groups with lymphocytic alveolitis.

Classification matrix based on macrophages nuclei length mean, minimum and maximum, breadth standard deviation (SD), form factor (FF) mean and percentage of lymphocytes was 92.5% correct for all groups, 100% correct for control group, 88.235% correct for sarcoidosis, and 92.857% correct for other lymphocytic alveolitis ILD group.

Bronchoalveolar lavage in the interstitial lung diseases is considered helpful in sarcoidois, hypersensitivity pneumonitis, alveolar proteinosis, detection of dust particles and asbestos bodies, or identification of malignant cells[8,18]. Macrophages play an important role in the de-

TABLE 4
MEAN VALUE AND STANDARD DEVIATION OF DISCRIMINANT PARAMETERS USED AS CLASSIFICATION FUNCTIONS FOR GROUPS

Parameters	Controls (n=9)	ILD patients (n=31)	
		Sarcoidosis (n=17)	Other LA-ILD (n=14)
lymphocytes (%)	11.56±7.27	34.26±14.78	45.86±18.55
Length Mean (μm)	12.030±0.633	13.441±0.681	13.889±1.047
Length Max (μm)	15.713±1.148	20.896±1.492	20.510±2.177
Length Min (μm)	8.866±0.593	9.607±0.620	10.116±0.847
Breadth SD (μm)	1.013±0.102	1.271±0.163	1.117±0.065
Form factor (FF) Mean	0.752±0.027	0.737±0.047	0.771±0,033

SD – standard deviation, ILD – interstitial lung disease, LA-ILD – lymphocytic alveolitis interstitial lung disease

TABLE 5
CLASSIFICATION MATRIX FOR GROUPS (MULTIVARIATE DISCIMINANT FUNCTION ANALYSIS, FORWARD STEP-WISE METHOD)

	Percent Correct	Controls	Sarcoidosis	Other LA-ILD
Controls	100.000	9	0	0
Sarcoidosis	88.235	0	15	2
Other LA-ILD	92.857	0	1	13
Total	92.500	9	16	15

Rows: observed classifications, columns: predicted classifications; LA-ILD – lymphocytic alveolitis interstitial lung disease

velopment of ILD-s. They are activated and produce cytokines. Sarcoidosis has been characterized by the production of TNFα, IL-12, IL-15, and IL-8, in hypersensitivity pneumonitis macrophages produce TNFα, IL-1 and IL-8. Regulatory cytokines, such as IL-10, are also secreted and may play a regulatory role, soluble TNF receptors are inhibitors of TNF and can block TNF bioactivity[1,19]. Differences in macrophages size and foamy appearance of cytoplasms are visible in light and electron microscope[7–9]. In the study of Burkhardt et al.[9], 70% of macrophages nuclei in sarcoidosis were indented. Cellular BALF profile of a drug-induced hypersensitivity reaction appeared to be similar to the hypersensitivity pneumonitis profile, with lymphocyte increase and foamy cytoplasms[20]. Studies of Drent and colleagues proposed the computer model and showed that predominant cells obtained by BALF may differentiate between three common interstitial lung diseases: sarcoidosis, hypersensitivity pneumonitis and idiopathic pulmonary fibrosis, that cover about 80–90% of ILD[20,21]. Updated version was helpful to distinguish disorders of a bacterial infectious origin and three mentioned ILD[20]. The data needed for the model were BAL volume recover, the total and differential cell count, patients gender, age and smoking status[20–22]. Our study for the first time proposes a model based on morphometric parameters of macrophages nuclei in cytological smears of BALF in differential diagnosis of sarcoidosis and other lymphocytic alveolitis ILD groups. Furthermore, morphometric analysis in our study revealed statistical significant differences with variance analysis among controls, sarcoidosis and other lymphocytic alveolitis ILD groups in nuclei area, outline, maximal radius and length. Highest maximum values and standard deviations in all morphometric parameters except form factor have been found in sarcoidosis. Form factor is complex shape factor, it is 1 in perfect round shapes, less than 1 as shape is more elliptic or irregular. Increase in standard deviation (SD) of nuclei area is the exact criterion of variations in nuclei size[15]. Form factor in our study had highest standard deviation in macrophages nuclei in sarcoidosis that reflects variability in nuclei shape. This finding is opposite to relatively uniform appearance of macrophages in sarcoidosis usually described in light microscope, referred to more visible cytoplasmic appearance[7,8]. In the study of Tosi and colleagues differences in shape of nuclear profiles of epithelioid cell in sarcoidosis and tuberculosis were best demonstrated by form factor[10].

Elongation factor as length to breadth ratio could sort out elongated, slender nuclei, as those in epithelioid cells. Elongation factor showed highest maximum values and standard deviations in sarcoidosis group, but highest means and medians of elongation and form factors were in non-sarcoidosis group. Combination of standard deviations, means and medians of elongation and form factors of macrophages nuclei could reflect finding of epithelioid like cells in sarcoidosis. More clinical data and morphometric parameters combinations are needed to confirm these observations.

Apoptosis impairment, probably reduction, has been involved in pathogenesis of sarcoidosis and other ILD-s[23–25]. Cells undergoing apoptosis are smaller with irregular contours[26,27]. Detection of these cells on morphological basis is possible, but BALF cells could posses degenerative changes similar to apoptotic. Observed morphome-

tric differences in macrophages nuclei among groups in our study could be, partially, a result of pathogenesis differences.

Lymphocyte increase in differential lung lavage cell count is most pronounced in sarcoidosis, hypersensitivity and drug-induced pneumonitis, and less intensive in IPF, BOOP, RB-ILD/DIP and collagen vascular diseases[28]. Lymphocyte percentage range in our study showed statistical significant differences among sarcoidosis and other lymphocytic alveolitis ILD-s group, more pronounced in later, probably due to hypersensitivity pneumonitis group. CD4/CD8 ratio is helpful in diagnosis of sarcoidosis and hypersensitivity pneumonitis, but not characteristic[3–5]. We found similar results, with no significant differences. BAL cellular analysis is also dependent on the quality of the obtained lavage specimen. BAL cellularity is influenced by the technical preparation of BAL recovery. When using cytocentrifugation, counting in a circular pattern around the center area is required, as cells, especially lymphocytes might not equally distribute over the cytocentrifuged spot[4]. This factor can be eliminated by using a standardized protocol as in our study.

DNA cytometry has been reserved for malignant changes, because malignant cells and cells with rapid division have shorter cell cycle than normal cells. Normal cells with high proliferative activity as in gastric mucosa and breast epithelia have nuclear DNA content amounts distributions in diploid-tetraploid regions. Hyperplastic processes showed a polymodal distribution of DNA content values with few aneuploid values[29]. Burkhardt find that 30% of macrophages nuclei in sarcoidosis had densified heterochromatin as result of increased metabolic activity[9]. DNA image cytometry results of macrophages in our study analyzed with Van Velthoven method showed predominant triploid DNA content in groups with ILD-s and predominant hyperdiplod DNA content in control group, with statistical significant differences among investigated groups. Macrophages in controls had predominant aneuploid/hyperdiploid DNA content possibly due to activation during transformation in BAL fluid. This finding was typical for benign cells with high proliferative activity, as previously reported for gastric mucosa cells, breast epithelial cells and macrophages and epithelioid granuloma cells in sarcoidosis[9,11,12,29]. Macrophages in other lymphocytic alveolitis ILD-s have been also characterized with proliferative activity and aneuploid/triploid DNA content can be expected.

Our study showed possible differentiation of healthy controls and patients with lymphocytic alveolitis ILD-s, and furthermore classification of ILD-s to sarcoidosis and other lymphocytic alveolitis ILD groups on the basis of macrophages nuclei morphology in BALF. Morphometric nuclear parameters determined by forward step-wise method in multivariate discriminant function analysis were length (mean, minimum and maximum value), breadth – standard deviation (SD) and form factor (FF) – mean value. These parameters together with percentage of lymphocytesl in BALF cell count allowed 92.5% overall correct classification of examinees, 100% for controls, 88.235% for sarcoidosis, and 92.857% correct classification for other lymphocytic alveolitis ILD group.

Conflict of Interest

No financial or other potential conflict of interest exist among the authors.

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MORFOMETRIJSKA I DNA IMAGE ANALIZA JEZGARA MAKROFAGA U BRONHOALVEOLARNOM LAVATU BOLESNIKA SA BOLESTIMA PLUĆNOG INTERSTICIJA I LIMFOCITNIM ALVEOLITISOM

S A Ž E T A K

Makrofagi su u većine bolesti plućnog intersticija, kao i u limfocitnom alveolitisu, najbrojnija stanična populacija u bronhoalveolarnom lavatu. Cilj rada bio je uz morfometrijsku analizu jezgara makrofaga u bronhoalveolarnom lavatu bolesnika sa bolestima plućnog intersticija procijeniti mogućnosti razlikovanja sarkoidoze od drugih bolesti intersticija koje se prezentiraju limfocitnim alveolitisom. U ispitivanje je uključen trideset i jedan bolesnik sa bolestima plućnog intersticija i limfocitnim alveolitisom u bronhoalveolarnim lavatu (17 bolesnika sa sarkoidozom i 14 bolesnika sa drugim bolestima) i 9 kontrolnih ispitanika. Bilježeni su dob bolesnika, postotak limfocita i omjer CD4 i CD8 T limfocita u bronhoalveolarnom lavatu. Analizirani su morfometrijski parametari jezgara makrofaga: površina, opseg, najveći polumjer, najmanji polumjer, duljina, širina, faktor oblika (FF), faktor izduženosti (EF), te DNA indeks određen metodom po Van Velthovenu. Multivariatnom diskriminativnom analizom uz korištenje stupnjevitog elimininiranja identificirane su varijable: prosjek, minimalne i maksimalne vijednosti duljine, SD širine, prosjek faktora oblika (FF) i prosjek postotka limfocita. Ove varijble omogućuju razlikovanje pojedinih skupina: potpuno razlikovanje (9/9) kontrolne skupine, 88,235% (15/17) razlikovanje skupine bolesnika sa sarkoidozom i 92,857% (13/14) skupine sa drugim bolestima plućnog intersticija i limfocitnim alveolitisom. Identificirane varijable omogućuju točno klasificiranje 92,5% (37/40) ispitanika.