Lung lavage cell profiles in diffuse lung disease

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Review

Lung Lavage Cell Profiles in Diffuse Lung Disease

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A B S T R A C T

The standard armamentarium of tests that are used by pulmonologist are laboratory tests, pulmonary function tests, different radiological techniques (conventional chest-X rays, HRCT scans, etc) and pathohistological analyses of biopsies. The minimally invasive bronchoalveolar lavage (BAL) procedure, in addition to methods earlier mentioned, is an important diagnostic instrument that can facilitate the diagnosis of various diffuse lung diseases (DLD). BAL fluid white blood cell profiles are analyzed, malignant cells looked for, and in certain circumstances particular stains are performed to detect yet other cell types. Additionally, BAL can play a very important role in the diagnosis of respiratory tract infections. All these analyses are usually readily performed in a moderately equipped cytological laboratory.

Key words: *bronchoalveolar lavage, diffuse lung disease, cell profiles*

Introduction

By simple definition bronchoalveolar lavage (BAL) denotes putting saline in the lung via flexible bronchoscope, then removing as much as possible to examine cells and non-cellular components.

BAL was introduced into clinical practice almost 40 years ago. Since, it has gained universal acceptance as a minimally invasive procedures that enables sampling of respiratory tract contents of the distal parts of the lungs, the parenchyma. Analysis of bronchoalveolar lavage fluid (BALF) aids in the diagnosis and management of various diffuse lung disease. In this article we will focus on significance of the evaluation of the cellular component of the lung lavage fluid in the recognition and diagnose of DLD, the task completed in the cytological laboratory. The search for the incriminating microbes is also very important and is performed on the regular basis. It is to note that numerous other analysis, especially of the acellular components of the BAL fluid, are also possible, but they are usually not done except in the research projects. Examination of BAL cells or acellular components of BAL via gene microarray technology or proteomic analysis may allow BAL to assume a more prominent role in diagnosis and management of lung disease in the near future.

Bronchoscopy with BAL fluid analyses is indicated if the patient is fit for the procedure and the clinical and high-resolution computed tomography (HRCT) findings suggest a condition in which BAL is likely to be diagnostic or significantly increases diagnostic confidence. Even, if the BAL is not diagnostic, it can provide findings that are inconsistent with suspected diagnoses and help focus attention on prosecution of alternative diagnoses.

Procedures

Bronchoscopy and bronchoalveolar lavage

By the aid of bronchoscope it is not possible to inspect the parenchyma, but the precise and detailed inspection of the bronchial mucosa, and additional techniques, like bronchoalveolar lavage (BAL) and transbronchial lung biopsy, enable us to gain enough information about the pulmonary parenchyma.

The lung lavage fluid is obtained during fiberbronchoscopy, usually along with other procedures like transbronchial lung biopsy, transtracheal and transbronchial fine needle aspiration biopsy of lymph nodes, catheter aspiration, and others.

Lung lavage is a procedure which enables to obtain cells and non-cellular components from the peripheral, bronchoalveolar compartment of the lung.

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Recommendations for performing and analyzing BAL have been published by both the European Respiratory Society and the American Thoracic Society[1,2]. Since, some recommendations have been reconsidered. For instance, traditionally the middle lobe and lingula are lavaged in patients with DLD because they are easily accessible and the probability to gain satisfactory return of the fluid is greatest. The almost ubiquitous usage of HRCT images to target areas of active pathological process, especially the ground glass pattern areas, changed this approach because lavaging these areas increases the likelihood of obtaining diagnostic, retrieved BAL fluid[3,4]. Also, it is well known that the amount of the returned lavage fluid is of great interest. But, if the patient is lavaged in order to confirm malignant cells, Pneumocystis jiroveci or other infection, the amount is not that important. In the case of DLD, a good representation of the alveolar space should be obtained. It has been shown that patients with greater than 5% retrieved fluid have a higher diagnostic yields[5], but ideally the percentage for patients with suspected DLD would be at least 39%.

BAL fluid should be processed and analyzed to examine cellular components and to detect infectious agents.

Examination of cellular components.

The examination of the inflammatory cell population of lung lavage fluid is the most important and most beneficial of all the analyses of lung lavage fluids, especially in differentiation between diverse diffuse lung diseases. One has to take into consideration that there are several limitations, several factors that may affect the cellular population of the lavage sample. Different laboratories use different steps in this process, which concerns the preparation of the cells for different cell count, cellular staining, the number of cells on which the counting has been performed, and last, but not the least important are the skills of the reader of the slides. The standard procedure of examination of cellular populations of lung lavage fluid, routinely performed at the Cytological Laboratory at the University Hospital for Lung Disease »Jordanovac«, Zagreb, will be described.

It is very important to process the lung lavage fluid immediately in order to preserve the cell viability, because in the contrary it becomes unsuitable for analysis.

But prior to the laboratory processing it is mandatory to asses the macroscopic feature of the lavage fluid. In non-smokers the fluid is slightly turbid and in the smokers more turbid and grayish. If the patient has been exposed to coal particles or gunpowder the lavage fluid is dark in appearance, and the sedimentation of particles to the bottom of the container is observed. In the cases of acute alveolar hemorrhage each consecutive retrieved fraction of lavage fluid becomes more hemorrhagic. This is in contrast with the bleeding from the central airways when the first fraction is the most bloody. In case of chronic alveolar hemorrhage, when the alveoli are filled with siderophages, the lavage color will be orange-brownish. This is very important finding and demands further processing with Pearl's blue iron staining. Thick, white lavage fluid which sometimes aggravates the aspiration through the bronchoscope may indicate the possibility of pulmonary alveolar proteinosis; in the case of alveolar proteinosis, with periodic acid-Schiff reagents the lavage fluid turns red.

At the beginning of the processing of the lavage fluid small amount of the fluid is separated for bacterial culture. Routine microbiological analysis includes nonspecific bacterial culture, direct fluorescent microscopy for acid resistant bacilli, and mycobacterial culture. If necessary the processing is broadened and the material can be cultivated for anaerobic bacteria and fungi, gene probes for mycobacteria and atypical bacteria (*Chlamydia pneumoniae*, Legionella, etc.) can be added, as well as staining with Kwik-Diff to detect Pneumocystis jiroveci.

The total cell count in lung lavage fluid is determined by hemocytometer[6] or by counting the cells in the Bürker-Tierck canister.

Following the filtration and removal of the mucus, the fluid is centrifuged for 5 minutes, in the cytocentrifuge, at the speed of 1000 rpm. Thus the cellular component is separated from the lavage supernatant. Supernatant is suitable for biochemical and immunological analysis, and various extracellular components like albumin, enzymes, enzyme inhibitors (neutrophil elastase, myeloperoxidase), prostaglandins, cytokines, chemokines, reactive oxygen species, and components of surfactant can be detected, and their significance in various pathological conditions studied[7]. The results of the estimation of BAL acellular components of BAL via proteomic analysis are promising[8].

Cell pellet is suitable for cytological and immunocytochemical analyses, flow-cytometry and cultures of various microbes. Cytospin preparations are routinely stained according to May-Grenwalld-Giemsa method, and along the quality cell analysis, the quantity tests are performed as well. Examination of at least 600 nucleated white blood cells randomly, on a single slide is performed. The number of obtained cells is expressed as percentage. The presence of more than 5% squamous epithelial cells in BAL indicates cell contamination, and thus makes the sample inadequate for diagnosis of DLD. If the sample is adequate, the counting on 400–600 cells is repeated, but this time squamous epithelial cells are omitted. The numbers obtained represent lung lavage cell profile, ex-

TABLE 1
NORMAL VALUES OF CELLS IN BRONCHOALVEOLAR LAVAGE FLUID

	Non-smokers	Smokers
Alveolar macrophages	85–95%	90–95%
Lymphocytes	7.5–12.5%	3.5–7.5%
Neutrophils	<2%	<2.5%
Eosinophils	<1%	<1%
Mast cells	<1%	<1%
CD4/CD4 ratio	0.5–6.4 (median 2.6)	0.3–28.1 (median 6.5)

pressed as an absolute number out of 200 cells, or as a percent. Lung lavage cell profiles differ slightly between smokers and non-smokers, mostly in that the smokers generally have significantly increased total BAL cell count, as well as total macrophages and neutrophils *per* μL of BAL fluid. Table 1 shows normal values of the lung lavage cell profiles in smokers and non-smokers that are used at the University Hospital for lung Disease, Zagreb. Increased percentage of particular cell line, indicates the alveolitis and depending upon the cell line that is increased we recognize lymphocytic, neutrophil, eosinophil and mixed alveolitis. Elderly subjects appear to have increased percentages of lymphocytes and neutrophils in the differential cell counts, suggesting that advanced age may affect BAL cell differentials[9]. Additionally, the total volume of retrieved fluid declines with advanced age due to loss of elastic recoil and airway tethering that makes airways more likely to collapse with negative pressure.

Except the quantity analysis of inflammatory populations of lung lavage cells, it is of utmost importance to perform the quality analysis, to look for malignant cells, microorganisms and inorganic particles (Table 2). It is possible to do some additional testing, according to previous BAL findings or indications posed by the cytologist or clinician. To detect Pneumocystis jiroveci it is necessary to stain fresh smears with quick stain according to Papanicolaou and Kwik-Diff. Subsequently it is possible to stain the smears for pulmonary alveolar proteinosis (PAS – periodic acid-Schiff) and siderophages (Pearl's blue iron staining)[10]. The severity of the bleeding can be quantified using the Golde score which represents a numerical scale based on the blue intensity of macrophages on an iron stain from 0 to 4; the maximum score is 400 and scores less than 20 are considered normal[11]. The Golde score is not routinely used, instead the percentage of sideropages is counted. The percentage of siderophages equal or greater than 20% is sufficient to affirm the presence of diffuse alveolar hemorrhage[12]. An increase of BAL CD1a positive cells (Langerhans cells-LC) by immunocytochemical analyses, of more than 5% is detected al-

TABLE 2
LUNG LAVAGE CELLS PROFILES IN DIFFUSE LUNG DISEASE

Disease	Alveolar macrophages	Ly	N. gra	Eo gra	Other cells, spetial stains	CD4/CD8 ratio
Sarcoidosis		++	=/+	=/+		↓/=↑↑↑↑
Idiopathic pulmonary fibrosis		+	++	+		=
BOOP	Foamy	+	+	+		↓
Nonspecific interstitial pneumonia		+	+	+		↓
Acute interstitial pneumonia		=	++	=	Atypical pneumocytes type II, amorphous material –fragments of hyaline membranes	=
RB-ILD/DIP	Cigarette pigment	=	++	=		=
Connective tissue disease		+	+	=/+		↑↑/=/↓
Pneumoconioses	Coal pigment or asbestos bodies, silica…	+	+	–	Beryllium-sensitized BAL lymohocytes	↓
Hypersensitive pneumonitis	Foamy	++	+	=/+	Sporadically plasma cells and mastocytes	↓/=
Drug-induced pneumonitis	Foamy	++	+	+	Sporadically plasma cells and mastocytes	↓/=
Eosinophilic pneumonia		+	=	++ ≥25%	Sporadically plasma cells	↓
Diffuse alveolar hemorrhage	Siderophages ≥20%	=/+	+	=/+	Pearl's blue iron staining (Golde score)	=
Alveolar proteinosis	Foamy	+	=	=	PAS – periodic acid-Schiff	↓
Langerhans'scell histiocytosis		=	=	=/+	Langerhan's cells – CD1a and protein 100 positive	=
Carcinomatous lymphangiosis. Bronchioalveolar carcinoma					Malignant cells	
Pneumocystis jiroveci					Kwik-Diff	
Tuberculosis					Ziehl-Neelsen	
Fungal infection					Gomori methenamine silver (GMS)	

BOOP – bronchiolitis obliterans organizing pneumonia, RB – ILD/DIP – respiratory bronchiolitis associated interstitial lung disease

most exclusively in pulmonary LCH; the specificity of the test is high, but the sensitivity is low[13].

Cell subpopulations are readily detected by the aid of flow cytometer or immunocytochemichal method. The flow cytometry is a swift and simple method, but it can be performed only on the fresh samples, and connotes additional apparatuses and laboratory equipment. Immunocytochemical analysis is long-lasting and complicated, but it is very sensitive and perfectly depicts the cell morphology. It is performed on the non-stained smears which can be old as much as 7 days. And, above all, it is only necessary to have the light microscope and a panel of monoclonal antibodies which are used routinely (CD1a, CD2, CD19, CD4 and CD8). Taking into consideration all the advantages and all the limitations, it would be ideal to combine both methods. In our Institution we use the flow cytometry as the routine method, and immunocitochemical analysis only if indicated.

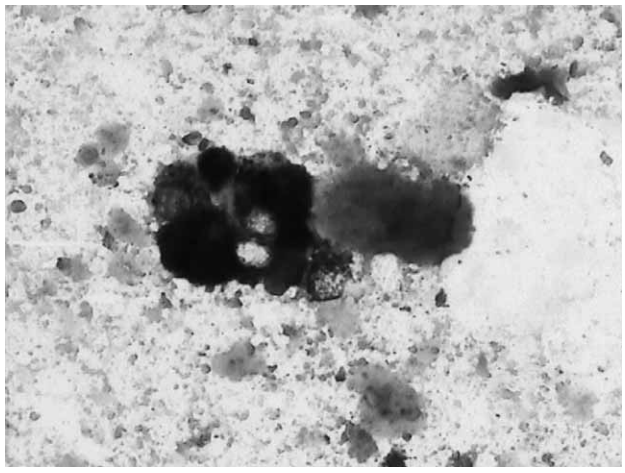

Fig. 2. PAS positive globules, background and macrophages in alveolar proteinosis, BALF, cytospin, original magnification 400x.

Diagnostic Value

Diffuse lung disease

Diffuse lung diseases[14,15] are a heterogeneous group with the common characteristic of diffuse infiltration of lung parenchyma with inflammatory or malignant cells, connective tissue or liquids, which disturb the passage of oxygen into the blood. The course of the disease may be acute but more often it is chronic. These diseases make about 15% of the pulmonary pathology. Most often they are classified into the group of known and a group of unknown causes. The significance of precise and thorough history of the diseases is of utmost importance, may be more important than in any other field of pulmonary pathology, because along with the systematic physical examination, it represents a key to the installation of working, sometimes final diagnosis and rational planning of the diagnostic procedures.

All DLDs, however, have certain common clinical, radiologic, and physiologic features that should be recognized.

The utilization of BAL for diagnostic purposes has significantly improved the diagnostic work-up of diffuse lung disease. BALF inflammatory cell populations in DLD usually differ from the findings in healthy parsons. There are only some conditions where BALF analysis results are diagnostic and provide conclusive evidence of the cause of diffuse pathological lung conditions[16,17].

In general, evaluation of BALF data is more often diagnostic in patients with acute symptoms. In a number of diseases and/conditions such as diffuse alveolar hemorrhage, sarcoidosis, hypersensitivity pneumonitis, drug-induced pneumonitis, eosinophilic lung disease, Langerhans cell hystiocytosis (LCH) (Figure 1), alveolar proteinosis (Figure 2), occupationally-induced lung diseases (Figure 3), and infections and malignancies (Figure 4) which can mimic diffuse lung disease, lavage can be diagnostic (Table 3). But there are many diseases and condi-

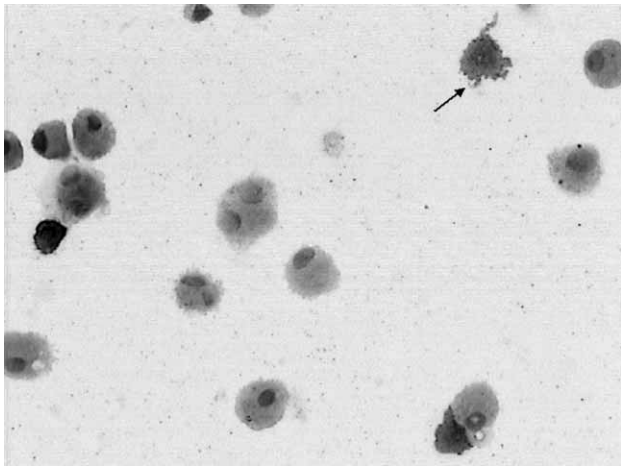

Fig. 1. CD1a positive cell (arrow) in Langerhans cell hystiocytosis (LCH), immunocytochemistry in BALF, cytospin, original magnification 400x.

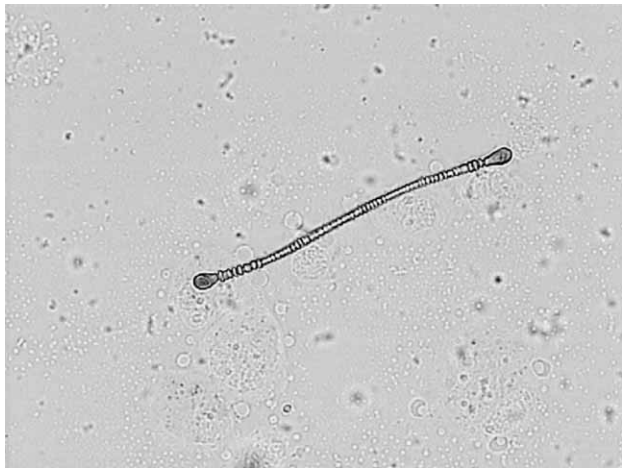

Fig. 3. Ferruginous body in unstained slide, BALF, cytospin, original magnification 400x.

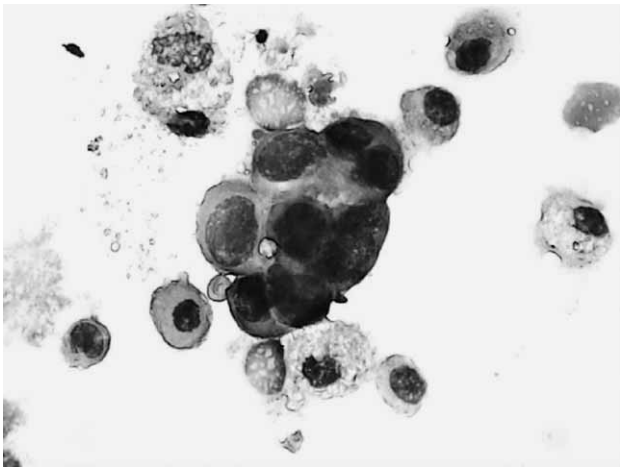

Fig. 4. Cluster of adenocarcinoma cells in BALF, cytospin, MGG staining, original magnification 400x.

tions where it is an accessory method, still helpful because it either narrow the diagnosis or focuses attention on prosecution of alternative diagnoses.

The significant increase of one particular inflammatory cell line in BALF narrows the differential diagnosis (Table 4). In combination with high resolution computed tomography (HRCT) and clinical features, it can be highly suggestive or even diagnostic of certain DLD. Also, the presence of certain cells that are normally not found in BALF is sometimes helpful. These cells are: plasma cells, pigmented macrophages, foamy macrophages, and reactive type II pneumocytes (typical and atypical).

Lymphocytes

The increased BAL lymphocyte count is often present in a number of DLD like sarcoidosis, hypersensitivity pneumonitis, connective tissue disease, drug induced pneumonitis, interstitial pneumonias i.e. cellular non-specific interstitial pneumonitis (NSIP-cellular) and bronchiolitis obliterans organizing pneumonia (BOOP), inflammatory bowel disease, occupational lung disease (i.e. chronic beryllium disease, asbestosis), infection (mycobacteria, viral) and radiation pneumonitis. BALF lymphocytosis above 25% is quite probably caused by chronic granulomatous inflammation, sarcoidosis[18], hypersensitivity pneumonitis, cellular NSIP, chronic beryllium disease, viral infection or by drug toxicity. Extreme lymphocytosis, above 50% with supportive history of exposure, is highly suggestive of hypersensitivity pneumonitis[19].

According to the 2002 ATS/ERS Consensus Classification, a confident diagnosis of idiopathic pulmonary fibrosis (IPF) without surgical lung biopsy is made with consistent clinical/physiological findings and the typical features on high-resolution computed tomography (HRCT). Bronchoalveolar lavage (BAL) and/or transbronchial biopsy, one of four major criteria in the 2000 ATS/ERS IPF Statement, was no more essential in the diagnostic algorithm of 2002 ATS/ERS Consensus Classification. Recently, the group of researcher[20] reevaluated the additional utility of BAL for the diagnosis of IPF. The 74 patients met all the criteria recommended in the 2002 ATS/ERS Consensus Classification for making a diagnosis in the absence of surgical biopsy. The final diagnosis was made with further examinations, including pathological analysis, in patients who showed inconsistent findings for IPF on BAL. A cut-off level of 30% for lymphocytes in BAL demonstrated a favorable discriminative power for the diagnosis of IPF. Six of the 74 patients (8%) showed a lymphocytosis of 30% or greater in BAL. Their final diagnoses were idiopathic nonspecific interstitial pneumonia (n=3) and extrinsic allergic alveolitis (n=3). The change in perception of the diagnosis was validated by a surgical biopsy in two cases and by subsequent outcome in four cases. The conclusions were that the BAL lymphocytosis changed the diagnostic perception in six of 74 patients who would have been misdiagnosed as having IPF without BAL. This was a proof that certain diagnosis is readily suspected upon high lymphocytosis, and also that BAL should be performed in clinical settings suspicious of IPF.

CD4+:CD8+ T lymphocyte ratio in diseases with lymphocytic alveolitis shows different values (Table 5)[21]. High values increase the likelihood of sarcoidosis; the ratio higher than 3.5 is rather specific for sarcoidosis[22,23] but the sensitivity is quite low.

Neutrophils

Extreme values of BALF neutrophils, above 50% strongly support a diagnosis of suppurative lung process or diffuse lung damage. Neutrophil alveolitis, with values

TABLE 3
BROCHOALVEOLAR LAVAGE FLUID ANALYSIS HELPFUL IN ESTABLISHING THE DIAGNOSIS OF PARTICULAR DLD

	Disorder/disease
Diffuse lung diseases	Diffuse alveolar hemorrhage (DAH); Sarcoidosis; Hypersensitivity pneumonitis (HP); Drug induced lung disease; Eosinophilic lung disease; Langerhans cell histiocytosis (LCH); Pulmonary alveolar proteinosis (PAP)
Acute onset DLD	Adult respiratory distress syndrome (ARDS); Acute interstitial pneumonia (AIP)
Occupational lung disease	Chronic beryllium disease; Asbestosis; Silicosis
Pulmonary malignancies	Lymphangiitis carcinomatosis; Bronchoalveolar carcinoma (BAC); Lymphoma and other malignancies
Pulmonary infection	Immunocompromised host with infiltrates; Ventilator-associated pneumonia; Evaluation of persistent infiltrates/inadequate clinical response

TABLE 4
INCREASED BALF INFLAMMATORY CELL POPULATIONS IN DLD

Balf cells	Diffuse lung disease
Lymphocytes ≥15% (Lymphocytic alveolitis)	Sarcoidosis; Hypersensitivity pneumonitis; Connective tissue disease; Drug induced pneumonitis; Interstitial pneumonias: NSIP*-cellular, BOOP**; Inflammatory bowel disease; Occupational lung dis. (e.g. chronic beryllium dis.); Infection (mycobacteria, viral); Radiation pneumonitis
Neutrophils ≥5% (Neutrophilic alveolitis)	Infection; Lung injury; Interstitial pneumonias: BOOP, DIP" IPF"", NSIP); Connective tissue diseases; Drug-induced pneumonitis; Hypersensitivity pneumonitis; Occupational lung disease; Aspiration pneumonia; Sarcoidosis
Eosinophils ≥3% (Eosinophilic alveolitis)	Eosinophilic pneumonia; Drug-induced pneumonitis; Churg-Strauss syndrome; Hypereosinophilic syndrome; Parasitic infection; Interstitial pneumonias. IPF, NSIP-fibrotic; Connective tissue diseases; Pneumocystis jiroveci pneumonia
Mast cells	Hypersensitivity pneumonitis; Drug-induced pneumonitis; IPF: Connective tissue diseases; BOOP; Eosinophilic pneumonia; Malignancy; Sarcoidosis
Plasma cells	Hypersensitivity pneumonitis; Drug-induced pneumonitis; Eosinophilic pneumonia; Malignancy; Infection (*Legionella, Pneumocystis*)
Pigmented macrophages	Pneumoconioses; Diffuse alveolar hemorrhage; RB-ILD
Foamy macrophages	Aspiration pneumonia; Drug-induced lung disease; Hypersensitivity pneumonitis; Pulmonary alveolar proteinosis; BOOP
Reactive type II pneumocytes	Systemic inflammatory response syndrome; Alveolar hemorrhage; Ventilator-associated pneumonia; *Pneumocystis jiroveci* pneumonia; Hypersensitivity pneumonitis; Drug-induced lung disease

*NSIP – non-specific interstitial pneumonia, **BOOP – bronchiolitis oblterans organizing pneumonia, " DIP-desquamative interstitial pneumonia, ""IPF – idiopathic pulmonary fibrosis, °RB-ILD – respiratory bronchiolitis interstitial lung disease

that are usually not increased at high rates, is also detected in number of other processes. For instance, the number of neutrophils in bronchoalveolar lavage fluid distinguishes between sarcoidosis patients who demonstrate remission and those having a more severe course of the disease; increased BALF neutrophils announce the severe course[24].

Eosinophils

Increase lavage eosinophils, especially extreme values are very helpful in narrowing the differential diagnosis of DLD. BALF eosinophils equal or above 25% are highly likely caused by eosinophilic lung disease, especially eosinophilic pneumonia, but also Churg-Strauss or hypereosinophilic syndrome[25,26].

Mast cells

Mast cells play an important role in tissue inflammation, fibrosis and remodeling. They are found in bronchoalveolar lavage fluid (BAL) of healthy persons only in small numbers. Increased number is associated with hypersensitivity pneumonitis, drug-induced lung disease, BOOP[27], connective tissue diseases, eosinophilic pneumonia, IPF, malignancies and sarcoidosis.

Plasma cells

Plasma cells are normally absent in bronchoalveolar lavage (BAL) fluid, but if detected they facilitate differentiation among diffuse pulmonary disorders. They are observed in hypersensitivity pneumonitis, drug-induced pneumonitis, eosinophilic pneumonia, malignancies and infection (*Legionella, Pneumocystis jiroveci*)[28].

Pigmented macrophages

The pigmented macrophages are alveolar macrophages whose cytoplasm contains divers materials which they have phagocytosed, such as carbon, cigarette pigment, hemosiderin, cell fragments, foreign bodies (asbestos bodies, pseudo-asbestos bodies) or debris. Their presence is helpful to diagnose various pneumoconioses, diffuse alveolar hemorrhage, and respiratory-bronchiolits interstitial lung disease (RB-ILD).

Foamy macrophages

Foamy macrophages are alveolar macrophages whose cytoplasm demonstrates complete vacuolisation. The pre sence of these foamy alveolar macrophages is usually

TABLE 5
CD4/CD8 RATIO IN DISEASES WITH LYMPHOCYTIC ALVEOLITIS

CD4:CD8 increased	CD4:CD8 normal	CD4:CD8 decreased
Sarcoidosis	Tuberculosis	Hypersensitivity pneumonitis
Beryllium disease	Lymphangitic carcinomatosis	BOOP*
Asbestos-induced alveolitis		Silicosis
Alveolar proteinosis		HIV infection
Crohn's disease		Drug-induced pneumonitis
Connective-tissue disorders		

*BOOP – bronchiolitis obliterans organizing pneumonia

non-specific, but they are frequently detected in BALF of patients with aspiration pneumonia, drug-induced lung disease, hypersensitivity pneumonitis, pulmonary alveolar proteinosis and BOOP.

Reactive type II pneumocytes

The reactive type II pneumocytes in bronchoalveolar lavage (BAL) fluid samples are observed as large cells with a high nuclear to cytoplasmic ratio and deeply blue-stained, vacuolated cytoplasm (Figure 5). The highest prevalence of reactive type II pneumocytes is detected in patients with systemic inflammatory response syndrome – SIRS (SIRS is nonspecific and can be caused by ischemia, inflammation, trauma, infection, or a combination of several insults)[29] and alveolar hemorrhage. In addition, they occur in ventilator-associated pneumonia, *Pneumocystis jiroveci* pneumonia, hypersensitivity pneumonitis and drug-induced pulmonary disorders[30].

The group of authors[31] aimed to quantify how the likelihood for a given diagnosis changes with the knowledge of bronchoalveolar lavage cell differentials. As an initial estimate (a priori probability), frequencies of final diagnoses were taken. Using categorizations for cell differentials, a posteriori probabilities were then derived for each disease. The analysis was performed in three of five groups of diagnoses suspected prior to BAL: interstitial lung disease, inflammatory disease, or lung tumor mimicking DLD. Overall, out of 1 971 patients, 18.3% had sarcoidosis, 7.7% usual interstitial pneumonia (UIP), 4.4% hypersensitivity pneumonitis (HP) and 19.0% tumors. In the group with suspected DLD, the likelihood for sarcoidosis increased from 33.7 to 68.1% when lymphocyte numbers were 30–50% and granulocyte numbers were low; the likelihood for UIP increased from 15.8 to 33.3% when lymphocyte numbers were 30% with granulocytes elevated. CD4/CD8 was informative, especially in sarcoidosis and HP. Despite considerable increases, the likelihood of rare diseases rarely reached appreciable values. Similar results were obtained in the other two groups of suspected diagnoses. In conclusion, these data suggest that bronchoalveolar lavage cell counts *per se* provide substantial diagnostic information only in relatively frequent diseases, such as sarcoidosis and usual interstitial pneumonia, and are less helpful in infrequent diseases.

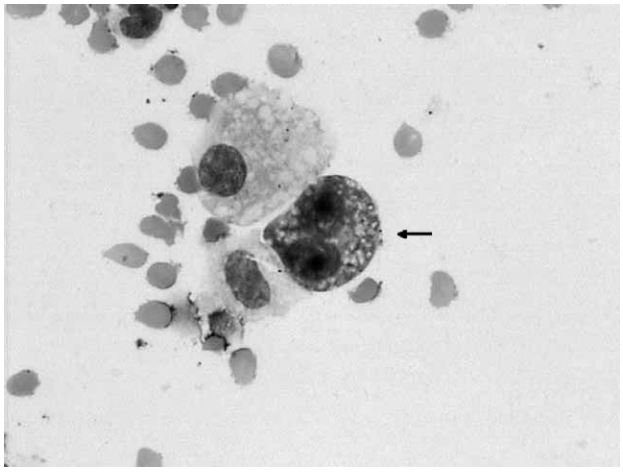

Fig. 5. Reactive pneumocyte (arrow) in BALF, cytospin, MGG staining, original magnification 400x.

Acute onset DLD

Acute onset DLD are the group of diseases characterized by duration of less than 4 weeks, dyspnoa, hypoxemia, and diffuse lung infiltrates upon radiological examination, not caused by know agent, including ARDS. Apart of the infection, acute onset DLDs include acute interstitial pneumonia (AIP), acute eosinophilic pneumonia (EP), acute BOOP, drug toxicity or acute exacerbation of unrecognized idiopathic pulmonary fibrosis (IPF). Bronchoalveolar lavage is helpful in diagnosing alveolar hemorrhage and infection, increased eosinophils point to eosinophilic pneumonia (EP) and lymphocytes indicate HP or drug toxicity, especially if mast cells or plasma cells are present.

Occupational lung disease

Occupational lung diseases presenting with DLD features include asbestosis, coal worker's pneumoconiosis, silicosis, beryllium disease, hard metal pneumoconiosis, aluminum associated lung disease, flavorings workers bronchiolitis obliterans (popcorn workers' lung) and hypersensitivity pneumonitis of various causes.

The influence of nanoparticles on lung health has recently been raised. Nanoparticles, classified as anthropogenic and natural particles, and fibers of diameters less than 100 nm, have unrestricted access to most areas of the lung due to their size. Size relates to the deposition efficiency of the particle, with particles in the nano-range having the highest efficiencies. The deposition of nanoparticles in the lung can lead to chronic inflammation, epithelial injury, and further to pulmonary fibrosis. Although studies of nanoparticle toxicity have focused on lung disease the molecular link between nanoparticle exposure and lung injury remained unclear. It has recently been show that cationic Starburst polyamidoamine dendrimer (PAMAM), a class of nanomaterials that are being widely developed for clinical applications can induce acute lung injury *in vivo*. PAMAM triggers autophagic cell death by deregulating the Akt-TSC2-mTOR signaling pathway[32].

The contribution of BALF analysis in the evaluation of various occupational lung diseases until now has not been marked, except for diagnosing the hypersensitivity pneumonitis. It has already been mentioned that high lymphocyte counts, above 50% and low CD4/CD8 ratio are helpful in these respect. Also, the detection of silica particles and asbestos bodies in BALF[33] is usually performed if pneumoconiosis is suspected, but it is not possible to distinguish between sheer exposure and dust-induced disease.

Malignancies mimicking DLD

Bronchoalveolar lavage is a useful diagnostic tool in diffuse lung malignancies which mimic DLD, and do not involve the bronchial structures visible by bronchoscope. Adenocarcinoma and tumors with lymphangitic growth patterns are easily diagnosed by bronchoalveolar lavage; in these cases the diagnostic yield reported is higher than 80%. In hematologic malignancies the diagnostic yield is quite good in secondary diffuse indolent B-cell lymphomas and in primary B-cell lymphomas of mucosa-associated lymphoid tissue (MALTQ3) type but low in Hodgkin disease[34]. Application of immunophenotypic and gene rearrangement analysis[35] and clonality investigations is useful to identify pulmonary lymphomas[36].

Disorders in which bronchioloalveolar cell hyperplasia/dysplasia is a significant morphological component may have cytological features in bronchoalveolar lavage fluid that mimic lung neoplasms like acute respiratory distress syndrome (ARDS), acute interstitial pneumonitis (AIP), and acute exacerbation of idiopathic pulmonary fibrosis.

Conclusion

Lung lavage cell profile analyses are helpful in diagnosing of various diffuse lung disease, but it applies only to a small number of diseases and/conditions such as diffuse alveolar hemorrhage, sarcoidosis, hypersensitivity pneumonitis, drug-induced pneumonitis, eosinophilic lung disease, Langerhans cell hystiocytosis (LCH), alveolar proteinosis, and to some extent to occupationally-induced lung diseases. Also lavage can be helpful to diagnose infections and malignancies which can mimic diffuse lung disease.

There are numerous, other DLDs where it is an accessory method, but nevertheless helpful because it either narrow the diagnosis or focuses attention on prosecution of alternative diagnoses. Except the quantity analysis of inflammatory populations of lung lavage cells, it is of utmost importance to perform the quality analysis, to look for malignant cells, microorganisms and inorganic particles.

New techniques, for instance gene microarray technology or proteomic analysis may allow BAL to assume a more prominent role in diagnosis and management of lung disease in the near future.

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STANIČNI SASTAV BRONHOALVEOLARNOG LAVATA U DIFUZNIM BOLESTIMA PLUĆA

S A Ž E T A K

Standardni armamentarium testova kojima se koriste pulmolozi su laboratorijski testovi, testovi plućne funkcije, različte slikovne tehnike (konvencionalni rendgenološki pregled, kompjutorizirana tomografija i druge) i patohistološka analiza bioptata. Minimalno invazivna tehnika bronhoalveolarne lavaže, uz ranije spomenute tehnike, važan je dijagnostički instrument koji olakšava dijagnostiku različitih difuznih plućnih bolesti. Analiziraju se upalne stanice bronhoalveolarnog lavata, traže se maligne, i u određenim okolnostima vrše se dodatna bojenja kako bi se detektirale i neke druge stanice. Dodatno, analiza lavata je vrlo značajna u dijagnostici infekcija respiratornog trakta. Sve ove analize mogu se izvesti u umjereno ekipiranom citološkom laboratoriju.