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Original article ■

Lymphocyte Subsets in Bronchoalveolar Lavage Fluid of Children with Lung Infiltrates

Amina Selimović[1], Senka Mesihović Dinarević[1], Tatjana Pejčić[2], Ermina Mujičić[3], Aida Hasanović [4], Siniša Ristić[5], Nataša Banjac[6], Željko Pavlović[7]

[1]*Pediatric Clinic of the Clinical Centre of the University of Sarajevo, Bosnia and Herzegovina*
[2]*University of Niš, Faculty of Medicine, Serbia*
[3]*Cardiosurgery Department and Clinic for Anesthesiology and Reanimatology, Clinical Centre of the University of Sarajevo, Bosnia and Herzegovina*
[4]*University of Sarajevo, Faculty of Medicine, Bosnia and Herzegovina*
[5]*University of East Sarajevo, Faculty of Medicine in Foča, Bosnia and Herzegovina*
[6]*Microbiology Department, Hospital Kasindo East Sarajevo, Bosnia and Herzegovina*
[7]*Emergency Centre, Hospital Kasindo East Sarajevo, Bosnia and Herzegovina*

SUMMARY

The analysis of the subpopulation of lymphocytes - CD4+, CD8+ lymphocytes in bronchoalveolar lavage (BAL) of paediatric patients can provide useful information related the lung parenchyma.

The aim of the paper was to analyze the results of bronchoscopy of patients presenting with persistent lung infiltrates and to find out of the diagnostic yield and complication rate of this procedure.

The study is a retrospective one. The data related to paediatric findings and BAL results of the bronchoscopies were retrieved from the hospital records. BAL was performed in tracheobronchial airways (middle lobe) by bronchoscope and sent to analysis of CD4+, CD8+ lymphocytes. Bronchoscopy was performed under general anesthesia (sedation, propofol, midazolam, morphium).

The records of seven patients were analyzed. All patients presented with persistent lung infiltrate (atelectasis and pneumonia). 71% of the patients with lung infiltrates in our study were below the age of 5. Our study results showed that CD4+, CD8+ lymphocytes in BAL in the studied group showed a small percentage of CD8+ lymphocytes as an immune response in 8-10% of patients, while the cellular response of CD4 +lymphocytes in the sample itself was present up to 14% in the entire group of the diseased children. There was no serious desaturation during bronchoscopy.

Bronchoscopy with BAL findings of lymphocyte populations is important in the early identification of inflammation and it helps in therapeutic strategies and monitoring of inflammatory response to the given therapy.

Key words: **flexible bronchoscopy with BAL, CD4+, CD8+ lymphocyte, lung infiltrate**

Corresponding author:

Amina Selimović •
phone: +38733 215577 •
e-mail: amina.selimovic@hotmail.com •

INTRODUCTION

Flexible bronchoscopy has been widely used as a tool for diagnostic research of paediatric patients with lung infiltrate (pneumonia and atelectasis) (1).

BAL is typically performed to diagnose the lung disease. The service of flexible bronchoscopy and BAL was introduced in our clinic for the first time in Bosnia and Herzegovina in 1998. We decided to review the results of bronchoscopy with bronchoalveolar (BAL) findings of CD4+, CD8+ lymphocytes of our pediatric patients who presented with lung infiltrate (atelectasis, pneumoniae, community-acquired pneumonia - CAP). CAP still remains a significant cause of childhood morbidity worldwide (2). BAL is a useful bronchoscopic technique (3).

The lung is continuously in contact with inhaled particles, some of which are of microbial origin. This requires adequate defense mechanisms in the form of immune reactions. Specific immune reactions depend on the interactions between lymphoid and accessory cells. Lymphocytes are found in the epithelium and lamina propria of the bronchi with different subset compositions. The vast majority of such lymphocytes express markers typical for "memory lymphocytes". The intrapulmonary migratory routes of lymphocytes and the integration of the lung are well-known. Lymphocytes present in the mucociliary epithelium of the trachea and bronchi are mainly the CD8+ T cells. In contrast to the epithelium, the bronchial lamina has more CD4+ than CD8+ T cells.

CD4+ lymphocytes are increased as inflamed cells in BAL of the children with lung infiltrates (4). An increase of T lymphocytes is noticed in the airways of patients with cystic fibrosis.

METHODS

We wanted to provide lymphocyte subsets in BAL in children with lung infiltrate (atelectasis in 4 patients and pneumonia in 13 patients). 17 children aged 6 months to 16 years underwent the diagnostic bronchoscopy and BAL (10 males and 7 females). Lymphocyte subsets were measured in the collected BAL fluid. Information about the patients who underwent bronchoscopy and BAL was collected, including demography, country, age and gender, as in other studies (5).

This study included children with lung infiltrates as indications for bronchoscopy performed between 2005 and 2010. The children were treated with conservative treatment (antibiotics, steroids, oxygene-O2) which yielded poor results. The patients were asked to sign a written consent form before having the bronchoscopy performed. All bronchoscopies were performed in the operative room for children or at the paediatric intensive care unit. During the bronchoscopy and BAL, the main vital signs (heart rate and oxygen saturation, respiration rate, blood pressure) were continuously monitored. The children were sedated with propofol, midazolam, morphium and intubated by an anesthesiologist.

Flexible bronchoscopy was performed with Olympus 3,5mm outside diameter. The exploration of the tracheobronchial wall or trunk was carefully inspected to search for abnormal motility of the airway wall and inflammatory change of the mucus, resolving the obstruction of the principal bronchus. BAL was performed by gently wedging the tip of the bronchoscope into the segmental or subsegmental bronchus of the right middle lobe or lingula or place with lung infiltrate detected by bronchoscopy and chest X ray or CT scan where pus was going out.

1 ml/kg of saline solution was instilled 2-3 times, which was followed by immediate aspirating the BAL fluid into sterile specimen container and sent to laboratory for citology analysis.

Studies on lymphocytes CD4+, CD8+ were performed in 17 patients with lung infiltrates (pneumonia, atelectasis). Identification of the CD4+, CD8+ lymphocytes in BAL was performed using a phenotyping technique with monoclonal antibodies. The centrifuging was carried out using a cytocentrifuge 1500 resolutions during the period of 20 min, colored by May-Grunwald-Giemsa. For the differentiation of the leukocytes, a conventional cytocentrifuge preparation or smears were air dried and stained with a hematological stain such as Wright-Giemsa or May-Grunwald-Giemsa. At least 200, more frequently 500 to 1000 cells were counted and classified as lymphocytes, neutrophils, eosinophils, macrophages, basophiles or epithelial cells. The final lymphocyte number was calculated by analyzing the presence or absence of lymphocytes using a microscope.

RESULTS

There were 11 patients up to 5 years of age, and 6 patients above the age of 5 (Figure 1).

71% of children were younger than 5 years of age.

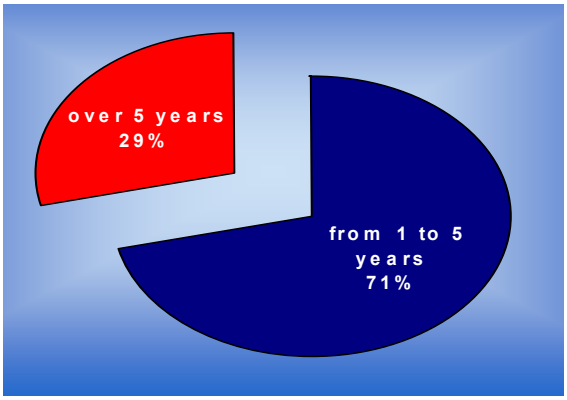

Figure 1. *Age of the patients with BAL*

The mean age of the study populations was up to 5 years.

This study provides BAL from children with lung infiltrates and could help in the early identification of lung parenchyma inflammation (Table 1).

Table 1. *The presence of lymphocyte subsets CD4+, CD8+ in BAL of children with lung infiltrates, during the period 2005-2010*

Date of hospitalization	Lung infiltrate	CD4+/CD8+ lymphocytes in BAL
2005	Pneumonia - (5 patients)	CD4+<10% CD8+<10%, CD4 + CD8 <5%, CD8 + CD4+ CD8 + negative
2006	Pneumonia - (2 patients)	CD4 + , CD8+ negative
	Atelectasis - (3 patients)	CD8 + CD4 + negative, CD8+ 5 %
2007	Pneumonia - 1 patient	CD4 + CD8 +
	Atelectasis - 1 patient	CD4 + negative, CD8 +
	Atelectasis - 1 patient	CD4+ negative, CD8 +
2008	Pneumonia - (2 patients)	CD4+ negative, CD8 +
2009	Pneumonia - (1 patient)	CD4 +negative, CD8 + negative
2010	Pneumonia (2 patients)	CD4+ negative, CD8+ negative
Total	**17**	

We analyzed the values of CD4+, CD8+ lymphocyte in BAL fluid of children with lung infiltrates (atelectasis and pneumonia). Figure 2 shows the percentage of CD4+, CD8+ lymphocytes in BAL in the studied group.

We found a small percentage of CD8+ lymphocytes as an immune response in 8-10%, while the cellular response of CD4+ lymphocytes in the sample itself was present up to 14% in the entire group of the children with lung infiltrates. Data were analyzed statistically and were expressed in percentages (mean values).

There were no serious complications after and during bronchoscopy. Three patients had oxygen saturation within the range up to 90%. These cases of desaturation were transient and children responded to oxygen, while seven of them required ambu bagging. The size of the tracheobronchial tree in small kids under two years of age is three times smaller than in the adults, which is the main reason of oxygen desaturation. Bronchoalveolar lavage is a safe clinical procedure and helps resolving many diagnostic issues.

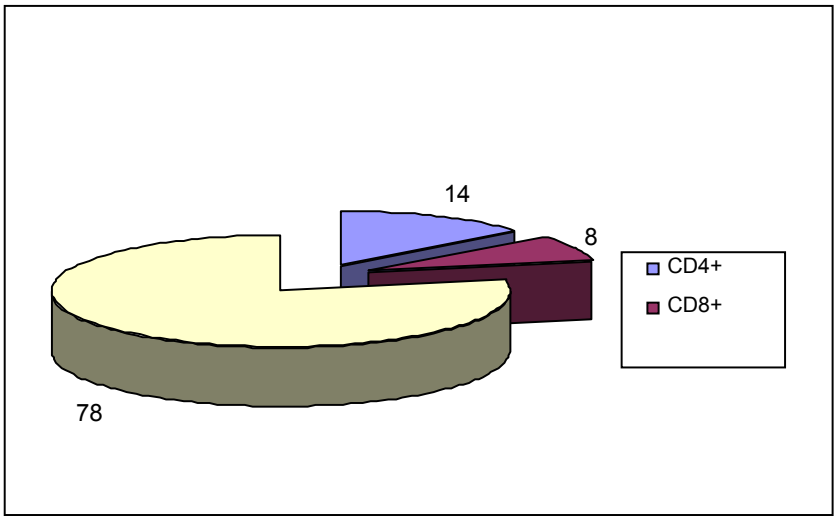

Figure 2. *Percentage of CD4+, CD8+ lymphocytes in BAL in the analyzed group*

DISCUSSION

This study was designed to analyze the BAL lymphocyte subsets. T lymphocytes have an essential role in many types of inflammatory responses (6-8). The most representative age group was up to 5 years. 71% of the patients with lung infiltrates in our study were below the age of 5, as other authors have reported (9, 10). In our study, the presentation of CD4+and CD8+ lymphocytes in BAL in the studied group shows a small percentage of CD8+ lymphocytes as an immune response in 8-10% of patients, while the cellular response of CD4 +lymphocytes in the sample itself was present up to 14% in the entire group of the diseased children.

The unresolved pneumoniae at this age group of up to 5 years lead to a large percentage of death outcomes. Lymphocytes in the bronchoalveolar space are the most easily accessible of the lymphocytes in the human lung. It has been estimated that the total number of these lymphocytes on the air side of the epithelium is between $2xl0^8$ and $4xl0^8$. This number represents about 5% of the total circulatory lymphocyte pool in humans or about 5% of the size of the interstitial lung pool.

Much of understanding of the role of pulmonary lymphocytes in host defense mechanisms and in disease comes from the study of lymphocytes recovered from the lung by bronchoalveolar lavage. BAL is the sampling of the lower respiratory tract by the instillation and subsequent aspiration of fluid. The technique recovers cells, soluble proteins, lipids and other chemical constituents from the epithelial surface of the lungs. Clinically, BAL has been helpful in the diagnosis and differentiation of various types of lung diseases including interstitial lung diseases, pulmonary infections and malignancies. It has also been used in defining the stages of disease, its progression and response to therapy (11).

This study provides the first data on BAL lymphocyte subsets findings of children with lungs infiltrates from this area of the East Europe. Before performing flexible bronchoscopy and BAL, it is necessary to have a systemic approach which includes a careful history and main laboratory findings including nose and throat swabs, cardiology findings, chest X-ray, physical examinations, CT scan of the chest.

T cells, as well as other inflammatory cell types such as neutrophils and macrophages, are probably essential in the initial inflammatory process leading to the breakdown of lung tissue, perhaps producing peptides. Once activated, T cells are present in the lung injury and the airways epithelium. CD4+ lymphocytes may occur in response to the recurrent persistent bacterial infections and development of lung damage.

Worldwide studies show that CD8+ cytotoxic T lymphocytes play a crucial role in virus eradication from the respiratory tract, as shown in our study (Table 1). A smaller percentage of the CD4+ lymphocytes share in children with BAL with tuberculosis is explained by a weak cellular response in children (Table 1) (12).

CD4+, T-helper cells are primarily responsible for helping the immune cells directly or by excreting cytokines after recognizing virus peptides that bind with the main histocompatible MHC class 2 complex. CD4+ lymphocyte cell molecules develop airway inflammation and hypersensitive response and lead to alveolitis. Reducing airway dimensions is associated with an increase of macrophages and T lymphocytes, as is the case with bacterial pneumoniae analyzed in Table 1 (2005-2006).

Children who have been subjected to mechanical ventilation or children that had the so-called acquired pneumonia had a weak cellular CD4+ response as well as cytotoxic CD8+, which was especially the case

with the children who had been exposed to mechanical ventilation for weeks, as presented in Table 1 (2009-2010).

It can be seen from the foregoing analysis that BAL enables the recovery of cellular and acellular components in the distal sections of airways, because if alveolitis is not resolved it grows into pneumonial fibrosis through the FAS ligand protein located on lymphocytes, which infiltrates the bronchial wall and alveolar epithelial wall and causes bronchial damage.

CONCLUSION

Bronchoscopy with BAL findings of lymphocyte populations is important in the early identification of inflammation and it helps in therapeutic strategies and monitoring of inflammatory response to the given therapy.

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SUBPOPULACIJA LIMFOCITA U BRONHOALVEOLARNOJ LAVAŽI KOD PEDIJATRIJSKIH BOLESNIKA SA PLUĆNIM INFILTRATOM

Amina Selimović[1], Senka Mesihović Dinarević[1], Tatjana Pejčić[2], Ermina Mujičić[3], Aida Hasanović [4], Siniša Ristić[5], Nataša Banjac[6], Željko Pavlović[7]

[1]Pedijatrijska klinika Kliničkog centra Univerziteta u Sarajevu, Sarajevo, Bosna i Hercegovina
[2]Univerzitet u Nišu, Medicinski fakultet, Niš
[3]Odeljenje za kardiohirurgiju i Klinika za anesteziologiju u reanimaciju, Klinički centar Univerziteta u Sarajevu, Bosna i Hercegovina
[4]Univerzitet u Sarajevu, Medicinski fakultet, Bosna i Hercegovina
[5]Univerzitet u Istočnom Sarajevu, Medicinski fakultet u Foči, Bosna i Hercegovina
[6]Odeljenje za mikrobiologiju, Bolnica Kasindo, Istočno Sarajevo, Bosna i Hercegovina
[7]Urgentni centar, Bolnica Kasindo, Istočno Sarajevo, Bosna i Hercegovina

Sažetak

Analiza subpopulacija limfocita u bronhoalveolarnoj lavaži (BAL) kod pedijatrijskih bolesnika može nam pružiti korisne informacije o dešavanju u parenhimu pluća.

Cilj rada bio je analiza rezultata bronhoskopije i BAL-a kod bolesnika sa perzistentnim plućnim infiltratima i stope komplikacija kod ove procedure.

Ovo je retrospektivna studija. Podaci o pedijatrijskim nalazima i rezultatima BAL bronhoskopija uzeti su iz bolničke evidencije. BAL je izvršena bronhoskopom unutar traheobronhalnog stabla (srednji lobus) i upućena na CD4+, CD8+ limfocita. Za vreme bronhoskopije nije zabeležena ozbiljnija desaturacija. Bronhoskopija je urađena u opštoj anesteziji (propofol, midazolam, morfijum).

Analizirano je ukupno 17 istorija bolesti. Svi bolesnici imali su perzistentne plućne infiltrate (atelektazu i upalu pluća). 71% bolesnika sa plućnim infiltratima bili su mlađi od 5 god. Rezultati naše studije pokazali su da CD4+, CD8+ limfociti u BAL-u na datoj grupi pokazuju mali procenat CD8+ limfocita kao imunu reakciju kod 8-10% bolesnika, dok je ćelijska reakcija CD4+ limfocita u samom uzorku bila zabeležena kod 5%, odnosno 14% bolesnika u čitavoj grupi obolele dece.

Bronhoskopija sa BAL-om je značajna metoda u identifikaciji imunog odgovora u plućima i primeni adekvatne terapije.

Ključne reči: **fleksibilna bronhoskopija sa BAL-om, CD4+, CD8 +limfociti, plućni infiltrati**