The Significance of Bronchoalveolar Lavage Fluid Cytology in Diagnosing Lung Infiltrates in Children

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ABSTRACT
Aim: The aim of this research is to show why is it important in diagnosing children with lung infiltrates. Methods: Our study included 50 children with lung infiltrates during period 2005-2012, and was conducted on Pediatric Clinic of the University Clinical Center Sarajevo. We sent all cytological BAL analyses to the University Clinical Center Sarajevo. Cytology was performed by direct microscopy. BAL cytology was performed by the principle of sending samples for centrifuging, 12000 revolutions during a 10 min Shandon-cyto spin. Then the centrifuged sample is dried in the air during 1-2 hours, and is then dyed under the May-Grünwald-Giemsa staining, and analyzed under the Olympus BX41 microscope. Results: Nosocomial pneumonia has occurred in 32% children, acquired pneumonia in 38%, and 30% children had a lung infiltrates. 6 (12%) of children were younger than 1 year old, 23 (46%) children were between 1 to 5 years, 14 (28%) of children were between 5 to 10 ages, and 7 (14%) of children were between 10-15 ages. The most of the changes in observed children took place on the right lung, 34%, while 26% occurred on the left side, 22% were normal and 18% changes have affected both lungs, right and left. Percentage of cells in cytological smear in children with BAL were: cylindrical cells 28%, lung macrophage 26%, lymphocytes 17%, detritus 17% and phlegm 12%. Erythrocyte sedimentation rate (ESR) in children with BAL was up to 10-52%, to 50-30%, while ESR after first hour was above 50-18%. Conclusion: Clinical parameters and local inflammation of the affected lobe are associated with positive bronchoalveolar cytology lavage findings.

Key words: bronchoalveolar lavage, lung infiltrates, pneumonia, children.

1. INTRODUCTION
The majority of children’s diseases include respiratory diseases (75%-96%) at both preschool and school age. Of most respiratory diseases, the incidence of pneumonia in children of up to 5 years of age ranges from 3-4%, while in children beyond that age the incidence ranges from 0.7 to 1%. About 20% death outcomes are caused by pneumonia in children below 3 years of age (1). Introducing bronchoscopy at childhood age becomes a golden standard in pulmonary disease diagnosis. Pediatric flexible bronchoscopy has...
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been in routine use since the 1970's (2, 3, 4). Pneumonia occurs more commonly (15-25%) in mechanically ventilated patients, in which case we refer to ventilator-associated pneumonia (VAP). The risk of VAP is highest during the first days of mechanical ventilation and increases by 3% in the first 5 days, 2% from 5 to 10 days and 1% after 10 days. For intensive care patients the risk of hospital pneumonia is low in the first 5 days of hospitalization, but then increases rapidly by 5% every day, up to the day 14th, and then it starts to decrease by 1% on each day. One half of all VAPs occur during the first 4 days of mechanic ventilation (4, 5). In contemporary pulmonology, bronchoalveolar lavage (BAL) represents a diagnostic method that in an invasive way facilitates an insight into the state of cellular and humoral immunity of lower respiratory airways and the assessment of the function of inflammatory elements. The presence of alveolar macrophages with the cylindrical epithelial cells in BAL proves that the investigational samples were adequately taken. Neutrophils are also on the rise in bronchial glands, in parenchyma, indicating a crucial role in the development of hypersensitive mucus, as indicators of infection with the presence of bacteria. During the first saline withdrawal in bronchoalveolar lavage, less than 20% cells recover, while the next withdrawal of saline accounts for 40 to 70% of cell recovery (6). In the obtained BAL, by analyzing cells such as alveolar macrophages, lymphocytes and polymorph nuclear neutrophils we can determine the cell profile, so with the changes at the lungs and bronchi we can conclude about an etiology of the disease, and the diagnose and recovery of the lesion mucus. Hence the significance and the imperative of performing bronchoscopy with bronchoalveolar lavage in all respiratory diseases that remains unsolved using classical standard techniques (5, 6, 7).

Regardless of proven importance of BAL as a diagnostic and therapeutic method, there have been relatively few studies, evaluating its significance in childhood. The aim of the present study is to evaluate the importance of bronchoalveolar lavage cytology in diagnosing pulmonary disease in children.

2. PATIENTS AND METHODS

The subject of this retrospective study is a cohort of 50 children with lung infiltrates during the period 2005-2012. The children were admitted to the Pediatric Clinic of the University Clinical Center Sarajevo. A control group includes 50 children between the ages of 1 month to 15 years and observed group of children includes 50 children between the ages of 1 month to 15 years, who made the BAL which shows the presence of pulmonary infiltrates.

Children underwent to general anesthesia with sedation: Propofol (Diprivan) 1 mg/kg of body weight per dose repeated 4-6x during the procedure. Propofol has a short half-life, 2 to 3 minutes, and the effects from sedation to general anesthesia (8). Fentanyl with doses ranging from 4-6 mg/kg of body weight. Morphine: 1 mg/kg of body weight in 50 ml 0.9% NaCl or 5% glucose, 1 ml/kg of body weight, midazolam water solution, short-action benzodiazepine with rapid onset is one of the most popular sedatives in pediatric population i.v. 0.5 to 1 mg/kg of body weight initially. This procedure is performed in the lung segment that radiologically proved to be infiltrated, or from which purulent secretion oozes during bronchoscopy (central lobe or lingula), (9, 10).

We evaluated BAL samples in 50 of the children patients with lung infiltrates who were intubated. The main indications for flexible bronchoscopy and BAL were lung infiltrations, mostly on the right side of the lungs. Most optimal is the instillation of saline 0.9% NaCl, performed by 1 ml/kg of body weight 0.9% NaCl. We transported each bronchoalveolar lavage fluid sample to the laboratory within one hour, so that the cells were metabolically preserved. We sent all cytological BAL analyses to the University Clinical Center Sarajevo. Cytology was performed by direct microscopy. BAL cytology was performed by the principle of sending samples for centrifuging, 12000 revolutions during a 10 min Shandon-cyto spin. Then the centrifuged sample is dried in the air during 1-2 hours, and is then dyed under the May-Grünewald-Giemsa staining, and analyzed under the Olympus BX41 microscope. Cellularity is presented descriptively, qualitatively. Red blood cells sedimentation in peripheral blood was monitored in children who underwent bronchoscopy with BAL and children who did not undergo bronchoscopy.

3. STATISTICAL ANALYSIS

Statistical methods were applied in this case (mean value, standard deviation and percentage share), while the data should be analyzed through relevant tests. a) Z test of the hypothesis for proposition, and b) T test of the hypothesis for mean value. Z test is a statistical test used to determine whether two populations means are different when the variances are known and the sample size is large. The statistics is assumed to have a normal distribution, and parameters, such as standard deviation, should be known in order for an accurate Z test to be performed. Z–tests are closely related to T -tests, and are best performed when an experiment has a small sample size (XLSTAT software for statistical analysis in Ms Excel).

4. RESULTS

The bronchoalveolar lavage technique has an advantage in diagnosing because it covers a larger area of lower airways from which samples are taken. Nosocomial pneumonia in ventilated patients has occurred in 16 (32%) of children, 19 (38%) of children had community acquired pneumonia, 15 (30%) children had a lung infiltrates.

The most of the changes in observed children took place on the right lung, in 17 (34%) children, while 13 (26%) occurred on the left side, 11 (22%) were normal and 9 (18%) changes have affected both lungs, right and left.

Table 1. shows that 6 (12%) of children were younger then 1 year old. 23 (46%) children were between 1 to 5
years. 14 (28%) of children were between 5 to 10 ages, and 7 (14%) of children were between 10-15 ages.

| Children’s age (years) | Number of children or patients | Percentage % |
|------------------------|--------------------------------|--------------|
| < 1                    | 6                              | 12%          |
| 1-5                    | 23                             | 46%          |
| 5-10                   | 14                             | 28%          |
| 10-15                  | 7                              | 14%          |
| Total                  | 50                             | 100%         |

Table 1. Age distribution of children with lung infiltrates

Table 2. Percentage of cells in cytological smear in children with BAL

| Cell type           | Number | Percentage % |
|---------------------|--------|--------------|
| Epithelial cells    | 1      | 1.2987       |
| Goblet cells        | 1      | 1.2987       |
| Lung macrophage     | 11     | 14.2857      |
| Bacteria            | 27     | 35.065       |
| Fungus              | 3      | 3.8961       |
| Lymphocytes         | 9      | 11.6883      |
| Red blood cells     | 2      | 2.5974       |
| Cylindric cells     | 12     | 15.5844      |
| Squamous cells      | 1      | 1.2987       |
| White blood cells   | 2      | 2.5974       |
| Phagocytes          | 1      | 1.2987       |
| Neutrophils         | 6      | 7.7922       |
| Eosinophils         | 1      | 1.2987       |
| **Total**           | **77** | **100.00**   |

Table 3. Cytological analysis of children’s bronchoalveolar lavage fluid

Table 4. Erythrocyte sedimentation rate (ESR) in children of the observed and control group

| Erythrocyte sedimentation rate (ESR) (mm) | Observed group | Control group |
|------------------------------------------|----------------|---------------|
| Up to 10                                 | 26             | 36            |
| 11-50                                    | 15             | 10            |
| > 50                                     | 9              | 4             |
| **Total**                                | **50**         | **50**        |

Table 4. Erythrocyte sedimentation rate (ESR) shown in children with and without BAL

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5. DISCUSSION

Out of total number patients in our sample, 58% of the sick children were under the age of five, as other authors have reported similar results in the previous studies recruiting children and adult (2). The issue of unresolved lung infiltrates in this age group up to five years may lead to a large percentage of death outcomes. Inflammation parameters and causative agents of disease should be monitored and measured in various phases of disease. Inflammation in distal regions of the lungs is often associated with a lesion in the alveolar structure. Flexible bronchoscopy with BAL gives importance to resolving the pneumonia problem. BAL samples were taken from the right middle lobe in 95% of the children, while 5% of samples were taken from the lingula, in line with the ESR guidelines. A cytological analysis of BAL is dominated by neutrophils (number of children (n) was 31, cylindrical cells 28%, lung macrophage 26%). The usefulness of the BAL analysis is the main factor in diagnosing lung diseases. BAL allows for a final diagnosis. In ventilator-associated pneumonias (VAP), an analysis of bronchoalveolar lavage fluid is a great help in establishing bacteriological pneumonia, the state of bronchi epithelium and the presence of inflammatory cellular elements, neutrophils, lymphocytes, macrophages. The macrophages and neutrophils carry surface receptors to recognize the most common ingredients on the surface of numerous bacteria and swallow them. Most of the authors compare the results of BAL with peripheral blood cells, as we did with the sedimentation of red blood cells from the peripheral blood that was larger than 50 during the first hour (n=9.8%). The advantage of flexible bronchoscopy with BAL in children is in an easy and good entrance into the trachea bronchial tree of the intubated child, which facilitates a direct access to the lungs and allow for a BAL examination of cells, mucus removal, infection colonization and addressing a chronic inflammation of respiratory airways (11). A cytological analysis
can confirm the presence of dysplastic changes in epithelial cells, and therefore, bronchoscopy and BAL allow the preservation of the bronchial wall, especially in children on mechanical ventilation.

6. CONCLUSION
Clinical parameters and local inflammation of the affected lobe are associated with positive bronchoalveolar cytology lavage findings. A cytological analysis of the BAL is dominated by neutrophils. Flexible bronchoscopy with BAL facilitated diagnosing by collecting cells from the location of the diseased lungs, that is, distal parts of lungs, as well as the therapeutic effect of instilled medicines at the site of changed bronchi mucus. Bronchoscopy with BAL cytology evaluation is the golden standard for alveolar inflammatory process.

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- Conflict of interest: none declared.

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