Clinical and research applications of bronchoalveolar lavage

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Bronchoalveolar lavage

- Technical aspects
- Normal reference values
- Indications
Bronchoalveolar lavage in children

ERS TASK FORCE

Bronchoalveolar lavage in children

ERS Task Force on bronchoalveolar lavage in children

Members of the Task Force: J. de Blic and F. Midulla (co-chairmen), A. Barbato, A. Clement, I. Dab, E. Eber, C. Green, J. Grigg, S. Kotecha, G. Kurland, P. Pohunek, F. Ratjen and G. Rossi.
Bronchoalveolar lavage

“Definition”

It is a technique that allows the recovery of cellular and non-cellular components from the epithelial surface of the lower respiratory tract.
Bronchoalveolar lavage
“Technical aspects”

• Injecting pre-warmed sterile saline through the working channel of the bronchoscope (1.2 mm).

  • 1-3 ml/kg for aliquot
  • Fixed aliquots of 10 - 20 ml
  • 10% FRC

• 2 - 6 aliquots

• Cytospin preparation from whole BAL fluid (> 3-4 slides)
Bronchoalveolar lavage

“Where?”

• **Localized lung diseases:** Involved lobe.

• **Diffuse lung diseases:** Middle lobe or lingula.
Bronchoalveolar lavage

“Can be influenced”

- Site of lavage
- pH, temperature, volume of saline
- Number of aliquots
- Size of the bronchoscope
- Dwell time
- Aspiration pressure (100-150 mmHg)
Bronchoalveolar lavage

- Technical aspects
- Normal reference values
- Indications
Bronchoalveolar lavage
“Parameters studied”

- Percent of BAL Fluid Recovered (n.v. >40%)
- Cellular Components
  - n° cells/ml of BAL fluid recovered
  - Differential cell counts
  - Lymphocytes subsets
  - Morphological aspects
  - Specific inclusions
  - Proliferation assay
- Non Cellular Components
- Microbiological Studies
### Total and Differential Cell Counts in Broncoalveolar Lavage Fluid from Control Children

<table>
<thead>
<tr>
<th>First author</th>
<th>[Ref.]</th>
<th>Subjects n</th>
<th>Age range</th>
<th>Total count $\times 10^3$ cells mL$^{-1}$</th>
<th>AM %</th>
<th>Lym %</th>
<th>Neu %</th>
<th>Eos %</th>
<th>Bas %</th>
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</thead>
<tbody>
<tr>
<td>Clement</td>
<td>[29]</td>
<td>11</td>
<td>1–15 yrs</td>
<td>240</td>
<td>89.0</td>
<td>10.0</td>
<td>1.0</td>
<td>-</td>
<td>-</td>
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<tr>
<td>Modulla</td>
<td>[26]</td>
<td>16</td>
<td>2–32 months</td>
<td>510</td>
<td>87.0</td>
<td>7.0</td>
<td>3.5</td>
<td>0</td>
<td>-</td>
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<tr>
<td>Ratjen</td>
<td>[31]</td>
<td>48</td>
<td>3–16 yrs</td>
<td>73</td>
<td>84.0</td>
<td>12.5</td>
<td>0.9</td>
<td>0.2</td>
<td>0.1</td>
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<tr>
<td>Redler</td>
<td>[33]</td>
<td>18</td>
<td>3 months–10 yrs</td>
<td>155</td>
<td>91.0</td>
<td>7.5</td>
<td>1.7</td>
<td>0.2</td>
<td>-</td>
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<tr>
<td>Tessier</td>
<td>[30]</td>
<td>16</td>
<td>2 months–8 yrs</td>
<td>305</td>
<td>89.5</td>
<td>8.0</td>
<td>1.0</td>
<td>0</td>
<td>-</td>
</tr>
</tbody>
</table>

Values are presented as medians, with those of Clement et al. [29] and Tessier et al. [30] being calculated from their published raw data. AM: alveolar macrophages; Lym: lymphocytes; Neu: neutrophils; Eos: eosinophils; Bas: basophils.
LYMPHOCYTE SUBSETS IN BRONCHOALVEOLAR LAVAGE FLUID FROM CONTROL CHILDREN

<table>
<thead>
<tr>
<th>First author</th>
<th>[Ref.]</th>
<th>Subjects</th>
<th>Lymphocyte subset</th>
<th>% of total lymphocytes</th>
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<tr>
<td></td>
<td></td>
<td>n</td>
<td>CD3</td>
<td>CD4</td>
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<tr>
<td>Clement</td>
<td>[29]</td>
<td>11</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Ratjen</td>
<td>[80]</td>
<td>28</td>
<td>87</td>
<td>35</td>
</tr>
<tr>
<td>Riedler</td>
<td>[33]</td>
<td>10</td>
<td>81</td>
<td>27</td>
</tr>
</tbody>
</table>

Values are presented as medians, with the exception of the CD4/CD8 ratio reported by Clement et al. [29] reported as mean.
Neutrophilic Alveolitis
Eosinophilic Alveolitis
Lymphocytic Alveolitis
Bronchoalveolar lavage

• Technical aspects

• Normal reference values

• Indications
Bronchoalveolar lavage

• DIAGNOSTIC
  - Microbiology studies (first aliquot)
  - Non specific radiological findings
  - Radiological findings of ILD
  - Non specific chronic respiratory symptoms

• THERAPEUTIC
  Atelectasis
  - Total lung lavage (Alveolar Proteinosis, acute lipoid pneumonia)

• RESEARCH
Clinical indications of bronchoalveolar lavage

“Infectious Diseases”

• Immunocompromised children
  Lung Transplant
  HIV Infected
  Chemotherapy

• Immunocompetent children
  Chronic pneumonia
  Tuberculosis
  Cystic Fibrosis
Microbiological studies

- **Centrifuged is performed:**
  - For direct detection of bacteria, virus and fungi.
  - To culture fungi, protozoa and virus

- **Centrifuged in NOT performed:**
  - To culture bacteria ($10^4$cfu/ml)
Child with Common Variable Immunodeficiency, cough and fever
Foamy alveolar casts, modified Diff-Qik, x500
Silver staining illustrating cyst details, x 1250
Patient with Cystic Fibrosis and Lungs Transplantation
Aspergillus showing 45° angle branching. Gomori methenamine silver stain x 720
• P.S. Age 5 months

• At 3 months fever and dyspnea

• Retractions. RR 80/m. SaO₂ 95% (FiO₂ 30%).

• PFR: Restrictive.

• WBC 6600 (Neu 30%, Lym 66%, E 4%), CRP neg, SR 8.

• IgG ed IgM for CMV positive
Chronic pulmonary aspiration
Chronic pulmonary aspiration

Is the repeated passage of food material, gastric refluxate or saliva into the subglottic airways causing chronic or recurrent respiratory symptoms

Bronchoalveolar lavage

“Diagnostic test”

- Lipid laden macrophage index
- Immunocytochemical staining for alfa-lactoalbumin and beta-lactoglobulin
- Gastric pepsin assay
Lipid laden macrophage index

- Lipids present in aspirated food are phagocytosed by alveolar macrophages.

- A quantitative index of lipid-laden macrophages in BAL samples has repeatedly been evaluated as a test for CPA of various types, but conflicting results have been reported.

- Theoretically, an increase prevalence of L-L macrophages in the lower airways suggests aspiration of food intake directly or following reflux in the stomach.
Bronchoalveolar lavage and lipid index

- Amount of lipid per cell graded from 0 to 4
- 100 consecutive AM were observed
- The graded for the 100 cells were summed

Lipid laden macrophage index

“Limitations”

- A lipid stain cannot differentiate between exogenous and endogenous lipids.
- It is a sensitive tool, but its usefulness is still controversial because it often yields positive values in children with lung diseases not related to aspiration, or even in healthy children.
- Lack of reproducibility.
Lipid laden macrophage index

“advantages”

- Easy to perform during endoscopic evaluation of the airways.
- A specific cut-off increase the specificity of the test.
- Remains elevated for several days after aspiration.
- It remains a useful diagnostic tool in PZ with a history and clinical symptoms suggestive of aspiration who should undergone diagnostic FB
- R.A. 20 days

- BW 3.300 Kg. Apgar 8,10.

- Since the age of 8 days tachypnea (RR 72/m), retractions. SaO₂ 91% (air).

- WBC 13700 (Neu 50%, Lym 37%, Mon 13%). SR 10, CRP 0.13

- PFR: Obstructive.
- PH study: pos for GER
After a single aspiration of human milk a large number of AM displayed positive staining for alpha LA and Beta LG

None of the negative controls display immunoreactivity for A1A or BLG

Immunocytochemical Detection of Milk Proteins in Tracheal Aspirates of ventilated Infants: A pilot Study

- 12% of infants who had never been fed (true negative control) have positive AM for Alpha LA and Beta LG

- 22% of infants from positive control have negative AM staining

Miller et al. Pediatric Pulmonol 2002; 34: 369-374
Gastric pepsin in BAL

- Proteolytic enzyme secreted by the gastric chief cells and mucus neck cells as inactive pepsinogen.
- It is cleaved at pH 5 to form active pepsin.
- The detection of pepsin in BAL would theoretically be highly specific for GER related aspiration.
Gastric pepsine in BAL

“limitations”

- Pepsin is present only in the stomach and for this reason it is a marker only of gastric reflux related aspiration.

- The pepsin assay is a “home made” assay and not available in all the lab.

- Results should be analyzed qualitatively or quantitatively?

- None of the studies have of a real gold standard.
BAL in Chronic Parenchymal Lung Diseases

Chronic parenchymal lung diseases are a group of disorders characterized by typical radiological findings, restrictive lung physiology and inflammation of the pulmonary interstitium.
BAL in Chronic Parenchymal Lung Diseases

"Indications"

- Characterized the alveolitis
- Diagnosis: specific and differential
- Follow-up: clinical and therapeutic
Diagnostic value of bronchoalveolar lavage in immunocompetent children with chronic diffuse pulmonary infiltrates


• 29 CHILDREN with ILD (radiological and clinical diagnosis)
  positive results 20/29
  negative results 9/20

• POSITIVE RESULTS:
  diagnostic of a primary disorders 5 (17%)
  diagnostic of a secondary disorders 8 (40%)
  consistent with a diagnosis 15 (75%)
BAL in Chronic Diffuse Perenchymal Lung Diseases.

“Diagnostic”

- Alveolar proteinosis
- Pulmonary haemorrhage
- Pulmonary histiocytosis
- Chronic lipoid pneumonia
- Pulmonary microlitiasis
• S.A.  Age 5 months

• Since the age of 15 days respiratory distress

• Dyspnea and retractions

• Enlarge liver and spleen.

• SaO$_2$ 94% with FiO$_2$ 41%.
• G.M. 4 years
• Adopted child
• Chronic constipation.
• RX performed before a minor operation
• Physical: negative
4 years later......
BAL in Chronic Diffuse Parenchymal Lung Diseases

“BAL as a useful tool for the diagnosis”

- BAL T-lymphocytes
  - Prevalence CD4
    - Sarcoidosis
    - m. di Crohn
  - Prevalence CD8
    - Allergic alveolitis
    - Istitocitosis X
    - ILD associated to collagen disease
    - BOOP
BAL in Chronic Diffuse Parenchymal Lung Disease

“BAL a useful tool for diagnosis”

- Neutrophilic alveolitis
- Idiopathic pulmonary fibrosis
- BOOP
- Eosinophilic alveolitis
- Eosinophil Pneumonia
- ILD
Bronchoalveolar lavage in children

“Therapeutic”

- Total lungs lavage in: Alveolar Proteinosis, Acute lipoid pneumonia

- Atelectasis, mucus plug (DNase)