ERS TASK FORCE

Bronchoalveolar lavage in children

ERS Task Force on bronchoalveolar lavage in children

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Bronchoalveolar lavage (BAL) is routinely performed in adults for sampling cellular and biochemical components. Many studies have been performed that have enabled the proposal of recommendations for BAL, methodology and processing, as well as reference values in different clinical settings. In contrast, despite a world-wide increase in the use of BAL in children, including neonates, there are, at the present time, no clear recommendations on the methodology, the clinical applications and the research areas for BAL. A Task Force on BAL in children has been approved by the European Respiratory Society (ERS) Task Force committee 1996. The objectives of this task force were: 1) to provide recommendations and guidelines on how to perform BAL and how to process the return fluid in children; 2) to collect and discuss available reference values; 3) to list indications and results in both immunocompetent and immunocompromised children; 4) to define areas for future research.

This task force included paediatric respiratory physicians involved in bronchoscopy and BAL and was run as a collaboration between the Paediatric Bronchology Group and the BAL scientific group of the ERS.

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On the basis of the available literature, this report provides recommendations about processing, "normal" values and indications for BAL. Members also discussed the main fields for further studies in order to standardize and improve the clinical value of BAL in children.

**Technical aspects**

**Instruments**

In children beyond the neonatal period, BAL is usually carried out via a paediatric flexible fibreoptic bronchoscope (FFB) with an external diameter of 3.5–3.7 mm. Patients >9 yrs of age usually tolerate the FFB with a diameter of 4.6–4.9 mm. In intubated patients BAL is performed after inserting the FFB through the endotracheal tube using a swivel Y connector that allows for further mechanical ventilation [1].

**Sedation**

While BAL can be performed under general anaesthesia, the majority of patients are studied under sedation and topical anaesthesia as it is the case for flexible bronchoscopy in children [2, 3].

In children the intravenous route is usually preferred for sedation [4]. Drugs most frequently used are meperidine associated with diazepam [5], meperidine with midazolam [6], or fentanyl with midazolam [7], or propofol [8, 9], or remifentanyl [10]. The aim is to achieve enough sedation, induction of anterograde amnesia and a short-lived action (table 1). The use of propofol and remifentanyl requires the supervision of the anaesthesiologist, because episodes of apnoea may occur during the induction of anaesthesia. This emphasizes the advantage of the availability of an intravenous access.

Many centres use also atropine [11, 12] as premedication because it minimizes vasovagally-induced bradycardia and decreases airway secretion. Adults pretreated with atropine [13] have a higher BAL return than untreated control subjects but this has not been formally assessed in children. Effective topical airway anaesthesia is achieved with 1–4% lignocaine in the upper airs and 0.5–2.0% lignocaine in the lower airs, the total dose of lignocaine being limited to a maximum of 7 mg·kg⁻¹ [14].

**Location of the BAL**

Usually BAL is carried out in the most-affected area (identified radiologically and/or endoscopically). In diffuse lung disease the right middle lobe is the preferred site because this area offers better fluid recovery [15]. In infants it is often easier to perform BAL in the right lower lobe. If BAL and transbronchial biopsies are planned in the same patient, BAL should be performed first.

**Volume of lavage solution**

BAL is carried out using normal sterile saline previously warmed to body temperature (37°C).

Limited information exists on the amount of fluid and the number of aliquots that should be used in order to obtain samples representative for the alveolar compartment in children of different ages and sizes. Various protocols for BAL have been applied. Some use two or four fractions of the same volume (10–20 mL) irrespective of the patient's body weight and age, as in adults [16, 17]. Others adjust BAL volume to the child's functional residual capacity (FRC) with 5–20 mL fractions depending on the patient's size [18]. Others [19] adjust BAL volume to body weight using 3 mL·kg⁻¹ of normal saline divided into three equal fractions in children weighing <20 kg and 3 mL·kg⁻¹ in 20 mL portions in children weighing >20 kg [20]. If the amount of instilled fluid is adjusted according to body weight in children aged 3–15 yrs, a constant proportion of the epithelial lining fluid (ELF) can be recovered [21].

The fractions are instilled using a syringe via the suction channel of the FFB, each instillation being followed by sufficient air to ensure that the channel's dead space is empty. The fluid may be recovered by hand suction using a syringe or by mechanical aspiration into a suction trap using pressures of 3.33–13.3 kPa (25–100 mmHg).

When three equal instilled fractions are used, the first aliquot collected, which is of more bronchial origin, is used for culture; the other two aliquots are pooled and submitted for cytological studies and analysis of BAL solutes, including proteins and inflammatory mediators.

Small volume BAL recovers material from more proximal airways whereas sequential lavages recover ELF from more distal sites [22]. In general, a BAL can be considered technically acceptable if the recovery is >40% and the lavage fluid (except for the first sample) contains few epithelial cells. Recovery may, however, be lower in children with obstructive lung disease.

**Safety**

Patients should be routinely monitored by continuous transcutaneous oximetry, using a well calibrated pulse oximeter [23], and electrocardiography with intermittent checks of blood pressure. Monitoring is continued until the patient has reached their presedation state of consciousness. FFB and BAL should be carried out in a well-equipped facility. Each child should have an intravenous access for the procedure. Immediate access to supplemental oxygen, resuscitation equipment [24] and antagonists for sedative agents (naloxone and flumazenil) should be readily available. An appropriate cleaning and disinfection procedure is mandatory to prevent cross contamination from one patient to another [25] or the distal propagation of infection [26], and any erroneous interpretation of laboratory results.

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**Table 1. – Drugs most frequently used to perform flexible bronchoscopy and bronchoalveolar lavage**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose</th>
<th>Onset of effect min</th>
<th>Duration min</th>
</tr>
</thead>
<tbody>
<tr>
<td>Atropine</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ketamine</td>
<td>0.25–0.5</td>
<td>2–4</td>
<td>10–20</td>
</tr>
<tr>
<td>Diazepam</td>
<td>0.2</td>
<td>1–3</td>
<td>30–120</td>
</tr>
<tr>
<td>Midazolam</td>
<td>0.05–0.3</td>
<td>1–5</td>
<td>90</td>
</tr>
<tr>
<td>Meperidine</td>
<td>1–2</td>
<td>5–10</td>
<td>180–240</td>
</tr>
<tr>
<td>Propofol</td>
<td>1–3</td>
<td>0.5</td>
<td>30</td>
</tr>
<tr>
<td>Fentanyl</td>
<td>0.001–0.006</td>
<td>2–3</td>
<td>60</td>
</tr>
<tr>
<td>Remifentanyl</td>
<td>0.025–0.1</td>
<td>2-5</td>
<td>2–3</td>
</tr>
</tbody>
</table>

Dosage is given in milligrams per kilogram body weight, except for remifentanyl where it is given in micrograms per minute continuous infusion.
**Complications**

BAL increases the duration of the bronchoscopic procedure by 2–3 min, marginally increasing the risk of hypercapnia and/or hypoxia [27]. BAL can be performed safely in most critically-ill ventilated patients with stable haemodynamic and ventilatory parameters [28]. The risk of mucosal bleeding is theoretically higher in children with coagulopathies [29]. However, FFB and BAL has been performed without complications in patients with <20,000 platelets·mL\(^{-1}\) [30]. Some patients develop fever and transient pulmonary infiltrates [31] several hours after BAL. In rare cases, this may be related to infection spreading secondary to the BAL [26]. However, in most cases fever is transient and its origin remains obscure.

**Recommendations**

1) BAL is carried out with a paediatric FFB with an external diameter of 3.5–3.7 mm in children <9 yrs of age and with an external diameter of 4.6–4.9 mm in older children; 2) most BAL procedures in children can be performed under sedation in combination with local anaesthesia; 3) patients should be routinely monitored by continuous transcutaneous oximetry; 4) BAL is carried out in the most-affected area, the middle lobe or lingula are used in diffuse lung disease; 5) a BAL can be considered technically acceptable if the recovery is >40% and the lavage fluid (except for the first sample) contains few epithelial cells.

**Process of recovered BAL fluid: cellular and noncellular components**

**Process of the BAL fluid**

While it appears preferable to prewarm the BAL solution to body temperature in order to increase cell yield and recovery [15, 32], the recovered BAL samples should be kept at 4°C before analysis to optimize cell viability.

Before assessing cellular and noncellular components of BAL, the fluid should be filtered, using one layer of sterile gauze to remove mucous particles. Microbiological studies should be performed on unfiltered BAL fluid as organisms may be trapped in the mucous.

**Processing of the first sample**

There is no consensus as to whether the first BAL sample should be processed separately or whether it is acceptable to pool all specimens. The first BAL sample has a lower cellular yield and contains more neutrophils and less lymphocytes than the following samples in both healthy children and those with lung diseases [15, 33, 34]. Subsequent samples can be pooled for cellular and noncellular studies. The first sample may be of interest in healthy children and those with lung diseases [15, 33, 34].

**Epithelial lining fluid**

As mentioned above, there is currently no reliable indicator to calculate the amount of ELF recovered with BAL. The two substances which have been used in a number of studies as denominators for other BAL fluid constituents are urea and albumen [22, 40, 41]. Urea is thought to be present in equal concentrations in serum and ELF and would therefore potentially be a helpful reference substance for BAL fluid. However, urea diffuses into BAL fluid during the procedure in a time dependent manner [42–44]. In addition, higher concentrations are seen in diseases that alter capillary permeability [41, 42]. Albumen diffuses much more slowly into the BAL fluid but its concentration is almost always altered in lung diseases [43, 44]. BAL fluid concentrations of both urea and albumen are not different between healthy children and adults, whereas serum levels of both substances are lower in children. Therefore, a higher percentage of urea and albumen appears to be present in BAL fluid in children [21]. This may reflect an increased alveolar-capillary permeability in children or may be due to the more negative pressures used to overcome the higher resistance of the smaller suction channel of paediatric bronchoscopes. Both urea and albumen give similar measurements of ELF in children without lung disease although urea tends to overestimate ELF in larger volume lavages [21]. For a more detailed discussion of the problems in quantifying ELF see reference [45].
Reporting results

There is no consistency in reporting results of cellular BAL components in children. To improve comparison between centres the total volume instilled, the number of specimens, their volume and the percentage of recovery should be reported. Total cell counts are variable even in healthy children but should be included [19, 33, 34]. The percentage of individual cell population is the preferable method for data analysis. Individual cell populations can also be displayed in cells per millilitre of BAL fluid. Cells should not be "normalized" for measurements of the epithelial lining fluid (ELF) as there is no reliable method to calculate the latter.

Noncellular components

Despite the lack of a reliable reference substance that would enable the quantification of solubles in BAL fluid, a great deal of research has been performed in this area that has led to another recently published ERS Task Force report which also includes a chapter on special considerations in children [45]. Most of the measurements of noncellular BAL fluid components have been performed on supernatant obtained after centrifugation and separation of BAL cells. Studies of surfactant components need special consideration as large surfactant aggregates will be trapped in the cellular fraction if a conventional centrifugation speed (500 × g or higher) is used. Whether fresh or frozen BAL fluid specimens should be used for analysis has to be determined for every parameter individually. Most proteins appear to be stable if BAL fluid is stored at -80°C. Because of the low concentration of most noncellular BAL components, many bioassays have to be adjusted to a more sensitive range. It is advisable to use an assay on unconcentrated BAL fluid as concentration steps may affect the actual quantity of the component. In the absence of any reliable reference substance, measurements of noncellular constituents should be reported in concentrations per millilitre of recovered BAL fluid.

Recommendations

1) The recovered BAL fluid should be filtered through one layer of sterile gauze after separating material for microbiological studies; 2) while there is currently no consensus as to whether the first sample should be treated differently, the first sample is of more bronchial origin, has a smaller volume and contains more neutrophils and less lymphocytes than subsequent samples; 3) differential cell counts should be performed on at least 300 cells either by cytospins, smears or flow cytometry; 4) the presence of epithelial cells should be noted but not included in the differential cell count; 5) material for the determination of noncellular constituents is obtained from the supernatant after centrifugation. Concentration of BAL fluid should be avoided as it may affect the actual quantity of the component; 6) reports of BAL results should include the volume instilled, the volume recovered, total cell counts, percentage of cell types and for noncellular components, the concentration per millilitre of BAL fluid; 7) "normalization" for ELF should not routinely be performed due to a lack of a reliable method for its quantification.

Areas of future study

1) Comparative studies of different BAL protocols in children without lung disease to define which of the current protocols should be used in children; 2) comparative studies between bronchoscopic and nonbronchoscopic BAL procedures; 3) comparative studies between centrifuged and uncentrifuged smears of BAL cytology; 4) assessment of the influence of suction pressure on noncellular BAL constituents in children.

Processing of recovered BAL fluid: microbiological studies

BAL is well established in the diagnosis of pulmonary infections, especially in immunocompromised children [18, 46–54]. Molecular biology allows identification of pathogens from small samples [55, 56].

Processing of BAL specimens

Most authors sample the first aliquot separately for microbiological studies [19, 33, 34, 57]. The BAL fluid (BALF) for microbiological studies should not be put through any kind of mucus filter because of the risk of losing cells and attached diagnostic material such as Pneumocystis carinii, and should be processed as quickly as possible to avoid further contamination and loss of agents such as anaerobic bacteria [58]. For cultivation of anaerobes, BALF must not be exposed to air prior to inoculation on reduced culture medium; anaerobic transport media containing reducing agents may be used.

Techniques

Table 2 summarizes techniques for the detection of micro-organisms. As pointed out in the previous chapter, microbiological specimens should be obtained prior to filtration. Centrifugation is usually performed for detection of bacteria, fungi, protozoa, and viruses by direct light microscopy. Alternatively, smears can be prepared. Air-dried preparations allow the use of special stains (e.g. Gram, Papanicolaou, Gomori-Grocott, toluidine blue). For bacterial cultures uncentrifuged samples are used; cultures for fungi, protozoa, and viruses are performed after centrifugation.

Nonquantitated bacterial cultures of BALF are not reliable, as there is a potential for contamination of the suction channel of flexible bronchosopes. Quantitative cultures, bilateral BAL in patients with unilateral pneumonia, and protected BAL have been proposed as means to differentiate between bacterial infection and contamination or colonization [20, 59–61]. Recently, a semiquantitative dip slide method was reported to be reliable in measuring bacterial growth [62].

Many infectious agents are difficult to detect with traditional methods for various reasons, including slow growth in culture [55]. Thus, immunofluorescence and molecular methods are now increasingly used [18, 46, 47, 55, 56, 63–67]. The high sensitivity of polymerase chain reaction (PCR) may lead to the occurrence of false positive results and to the detection of organisms that are not causing infection or that are not viable [55, 56]. On the other hand, PCR may provide information on antibiotic sensitivities [68].
The diagnostic value of PCR for diagnosis of Legionella, respectively [71], and heteroduplex analysis to be able to classify zonation has been shown to be a sensitive method to detect Deoxyribonucleic acid (DNA) amplification and hybridization rapidly [69]. Generally, \( \geq 10^5 \) colony forming units per millilitre of BALF are considered to indicate established bacterial infection [70]. Growth of Legionella is best on buffered charcoal yeast extract agar.

For rapid culture confirmation, the hybridization techniques combine with monoclonal antibodies for early antigen detection with high sensitivity and specificity [55, 58].

For other viruses the shell vial technique allows detection of viruses within a day [58].

1) The recovery of a potentially pathogenic organism in the lavage fluid should be considered diagnostic only if colonization of the respiratory tract with the organism does not occur [58]; 2) results from BALF analysis have to be evaluated with regard to the underlying disease and the whole clinical picture [58]; 3) as pseudoinfections due to cross-contamination of bronchoscopically obtained specimens are relatively common, adherence to cleaning and disinfection guidelines is mandatory [75]; 4) routine microbiological checks of the instruments should be performed.

**Recommendations**

1) Assessment of the value of cytological studies for establishing the diagnosis of pulmonary infection (e.g. intracellular bacteria); 2) assessment of the value of different techniques for detection of viruses (especially CMV) and fungi; 3) comparison between BAL and protected brush samples for diagnosing bacterial pneumonia; 4) assessment of the value of molecular methods (e.g. PCR, in situ hybridization).

**Areas for further study**

1) The recovery of a potentially pathogenic organism in the lavage fluid should be considered diagnostic only if colonization of the respiratory tract with the organism does not occur [58]; 2) results from BALF analysis have to be evaluated with regard to the underlying disease and the whole clinical picture [58]; 3) as pseudoinfections due to cross-contamination of bronchoscopically obtained specimens are relatively common, adherence to cleaning and disinfection guidelines is mandatory [75]; 4) routine microbiological checks of the instruments should be performed.

**Normal values: cellular and noncellular components**

**Cellular components**

Since healthy children cannot be studied for ethical reasons, paediatric BAL reference values are difficult to obtain. Some investigators have approached the problem of recruiting "healthy children" by lavaging children during fibreoptic bronchoscopy performed for various clinical indications including stridor, chronic cough, evaluation of the stenosis of a main bronchus and follow-up of foreign body aspiration; children in whom upper and lower respiratory tract infections were excluded [19, 34, 76, 77]. Others have used children undergoing elective surgery under general anaesthesia for nonpulmonary illnesses [33]. BAL reference values have been reported using nonbronchoscopic lavage in children undergoing surgical procedures for a noninflammatory condition [78].

The results of BAL cellular analysis in healthy children are reported in tables 3 and 4. The first report was published by Clement et al. [77] in 11 children. BAL was

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**Table 2. – Techniques for the detection of various microorganisms**

<table>
<thead>
<tr>
<th>Micro-organism</th>
<th>Techniques</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacteria</td>
<td>Gram, Quantitative culture</td>
</tr>
<tr>
<td>Chlamydia</td>
<td>Immunoassay (direct), Culture</td>
</tr>
<tr>
<td>Fungi</td>
<td>Molecular methods, Gomori-Grocott, Toluidine blue, Wright-Giemsa, Immunoassay (direct)</td>
</tr>
<tr>
<td>Legionella</td>
<td>Gomori-Grocott, Immunoassay (direct), Culture</td>
</tr>
<tr>
<td>Mycobacteria</td>
<td>Auramine-rhodamine, Ziehl-Neelsen, Culture</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>Immunoassay (early culture), Molecular methods</td>
</tr>
<tr>
<td>Viruses</td>
<td>Cytopathic effects, inclusion bodies, Culture</td>
</tr>
<tr>
<td>CMV, HSV</td>
<td>Immunoassay (direct, early culture), Electron microscopy</td>
</tr>
<tr>
<td>Other viruses</td>
<td>Immunoassay (direct, early culture), Culture</td>
</tr>
</tbody>
</table>

CMV: cytomegalovirus; HSV: herpes simplex virus.

**Micro-organisms**

**Bacteria.** Microscopy after Gram staining may indicate the presence of bacterial infection; especially identification of intracellular bacteria may offer diagnostic information rapidly [69]. Generally, \( \geq 10^5 \) colony forming units per millilitre of BALF are considered to indicate established bacterial infection [70]. Growth of Legionella is best on buffered charcoal yeast extract agar. Deoxyribonucleic acid (DNA) amplification and hybridization has been shown to be a sensitive method to detect [63], and heteroduplex analysis to be able to classify Legionellae, respectively [71].

**Fungi.** The diagnostic value of PCR for diagnosis of P. carinii infection is debated [65, 72]. Cultures for other fungi are usually performed on Sabouraud’s agar. Monoclonal antibodies may be used to increase the specificity [35].

**Mycobacteria.** For rapid culture confirmation, the hybridization of nucleic acid probes is now considered to be the gold standard [73]. Liquid culture methods may allow faster detection of mycobacteria than cultures on solid media; liquid and solid media should be used in combination routinely. DNA amplification is a method for the early diagnosis of tuberculosis [66], and molecular genetic tools allow rapid detection of multi-drug-resistant tuberculosis [68]. Molecular methods to determine drug susceptibility do not replace conventional growth-dependent methods [73].

**Cytomegalovirus and herpes simplex virus.** Sensitivity of viral cytopathic effects and intranuclear or intra-cytoplasmic inclusion bodies is low compared to direct antigen detection by immunofluorescence [35]. Cytological changes and immunofluorescent antibody staining lack sufficient negative predictive value for cytomegalovirus (CMV) infection; a rapid centrifugation culture combined with monoclonal antibodies for early antigen is highly sensitive and specific, and seems more useful [75]. PCR and in situ hybridization techniques combine early detection and high sensitivity [55, 58].

For other viruses the shell vial technique allows detection of viruses within a day [58].

1) The recovery of a potentially pathogenic organism in the lavage fluid should be considered diagnostic only if colonization of the respiratory tract with the organism does not occur [58]; 2) results from BALF analysis have to be evaluated with regard to the underlying disease and the whole clinical picture [58]; 3) as pseudoinfections due to cross-contamination of bronchoscopically obtained specimens are relatively common, adherence to cleaning and disinfection guidelines is mandatory [75]; 4) routine microbiological checks of the instruments should be performed.
performed under local anaesthesia if sarcoidosis was suspected (because of uveitis), or if endoscopy was used to evaluate stridor. This population had normal physical examination, chest radiographs, and arterial capillary blood gases. Only the last five aliquots were used for cellular analysis. RATJEN et al. [33] reported results from 48 children undergoing elective surgery for nonpulmonary illnesses. The study excluded children with chronic respiratory symptoms, an upper respiratory tract infection in the preceding 2 yrs, a history of hyperreactive airway disease or other atopic symptoms. The first sample was studied separately; subsequent samples were pooled for analysis. RIEDLER et al. [19] studied 18 children who fulfilled the following criteria: no history of lower respiratory tract symptoms, no upper respiratory tract infection in the previous 4 weeks, no systemic disorder, no medication at the time of the lavage, normal lung function tests, normal chest radiographs, normal physical examination of the chest, and normal bronchial mucosa on inspection. Patients were investigated for stridor. MIDULLA et al. [34] studied BAL in 16 children investigated for persistent stridor, left lobar emphysema associated with mild stenosis of the lingular bronchus, stenosis of the main left bronchus or 2 months after the removal of a foreign body. These children were considered free of par enchymal lung disease based on no visual evidence of bronchial inflammation, sterile BAL culture, normal white blood cell count, normal sedimentation rate and negative chest radiograph. TESSIER et al. [76] reported BAL cellular components in 16 children undergoing BAL during endoscopy to evaluate stridor or suspected inhalation of a foreign body. The last five aliquots of aspirated fluid were pooled and used for cellular studies. Whereas TESSIER et al. [76] reported that BAL cellular analysis followed a normal distribution, RATJEN et al. [33], RIEDLER et al. [19] and MIDULLA et al. [34] concluded that it did not. The latter findings are supported by the study of MERCHANT et al. [79] in healthy adult volunteers. Other discordances were observed between BAL studies in children, especially for the number of cells per millilitre, percentage of lymphocytes and neutrophils (tables 3 and 4). The differences could depend on children’s age. The patients in the paper by RIEDLER et al. [19] are in the same age range as those in the studies by TESSIER et al. [76] and CLEMENT et al. [77], but the absolute number of subjects studied in a given age range are small. Despite variations in differential cell counts between these studies, the differential cytology of BALF is fairly similar to that observed in healthy adults. Independent of the child’s age macrophages are the predominant cells in all studies followed by lymphocytes. The percentage of neutrophils appears to be higher in BALF from children <12 months than from children aged 13–36 months [34]. When expressed in cells per millilitre of recovered BALF, total cell counts tended to be higher in younger children [33]. The major difference between children and adults is in the CD4/CD8 ratio which has been found to be lower in children in two studies [19, 37].

Table 3. – Bronchoalveolar lavage (BAL) differential cell counts from different studies of “normal” children

<table>
<thead>
<tr>
<th>Study</th>
<th>n</th>
<th>Age range</th>
<th>Sedation</th>
<th>No aliquots</th>
<th>Volume saline</th>
<th>BAL fluid recovered</th>
<th>AM</th>
<th>Lym</th>
<th>Neu</th>
<th>Eos</th>
</tr>
</thead>
<tbody>
<tr>
<td>CLEMENT et al.</td>
<td>11</td>
<td>1–15</td>
<td>GA</td>
<td>6</td>
<td>10% FRC</td>
<td>58±13.6</td>
<td>89.7±5.2</td>
<td>1.3±0.9</td>
<td>0.4±0.6</td>
<td>ND</td>
</tr>
<tr>
<td>RATJEN et al.</td>
<td>48</td>
<td>3–5</td>
<td>LA</td>
<td>3</td>
<td>3 mL·kg⁻¹</td>
<td>81.2±12.7</td>
<td>81.2±12.7</td>
<td>1.9±2.9</td>
<td>0.4±0.6</td>
<td>0.2±0.3</td>
</tr>
<tr>
<td>RIEDLER et al.</td>
<td>18</td>
<td>1–10</td>
<td>GA</td>
<td>3</td>
<td>3 mL·kg⁻¹</td>
<td>62.5</td>
<td>91</td>
<td>2.9±5.8</td>
<td>0.2±0.3</td>
<td>0.2±0.3</td>
</tr>
<tr>
<td>MIDULLA et al.</td>
<td>16</td>
<td>2–3</td>
<td>LA</td>
<td>2</td>
<td>20 mL</td>
<td>42.5–71.5*</td>
<td>84.2–94*</td>
<td>1.7±3.5</td>
<td>0.6–3.5*</td>
<td>0.0–3.6</td>
</tr>
<tr>
<td>TESSIER et al.</td>
<td>11</td>
<td>4–16</td>
<td>LA</td>
<td>6</td>
<td>10% FRC</td>
<td>7.3</td>
<td>7.5±5.8</td>
<td>1.7±3.5</td>
<td>0.6–3.5*</td>
<td>0.0–3.6</td>
</tr>
</tbody>
</table>

Age range is given in years, except where indicated by # where they are given in months. *: First interquartile to third interquartile. LA: local anaesthesia; GA: general anaesthesia; FRC: functional residual capacity; ND: not done; AM: alveolar macrophage; Lym: lymphocyte; Neu: neutrophil; Eos: eosinophil.
Table 4. – Lymphocyte subsets in children

<table>
<thead>
<tr>
<th>CD3 %</th>
<th>CD3/HLA-DR %</th>
<th>CD4 %</th>
<th>CD4/CD8 %</th>
<th>CD8 %</th>
<th>CD25 %</th>
</tr>
</thead>
<tbody>
<tr>
<td>mean</td>
<td>median</td>
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<td>85.8</td>
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<td>72–92</td>
<td>35.1</td>
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<td>ND</td>
<td>7.8</td>
<td>5.0</td>
<td>1.6</td>
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Age range is given in yrs. *: first interquartile to third interquartile. ND: not done; HLA-DR: human leukocyte antigen-D.

Noncellular BAL components

Obtaining paediatric reference values for noncellular components is complex. Apart from the criteria of patient selection, technical details related to the procedure will influence the final results [38]. None of the markers for ELF (external, methyl blue or internal, urea and albumin) proposed for BAL dilution have proved reliable.

The concentration of serum derived proteins is higher in children than in adults, whereas locally produced mediators do not differ [80–82]. Concentration of BAL fibronectin and hyaluronic acid in BALF are not different in children and adults [34, 80, 81]. Surfactant phospholipid concentrations are higher in children 3–8 yrs of age than in older children, whereas protein concentrations are independent of the child’s age [83].

The differences between children and adults can reflect the growing children’s lung, the maturation process of the immune system, or they can simply be a technical problem such as higher suction pressures bringing in more proteins from the circulation.

Recommendations

1) Few reference data are currently available for BAL constituents in children and results of these studies vary due to differences in patient selection, BAL protocols and in the age group studied; 2) results of differential cell cytology as well as noncellular BAL constituents in children with lung disease should be compared to reference data obtained with a similar BAL protocol in the same age group.

Indications and results in immunocompetent children

The main indications of BAL in immunocompetent children are:

Diagnosis of infection. When infection is suspected and other techniques of collecting airway secretions are not possible and/or efficient, BAL can provide specimens for microbial cultures as well as cytological examination. The development of molecular techniques allows for identification of a number of micro-organisms. However, the usefulness of BAL for the detection of infection in the alveolar compartment can be limited by contamination. Therefore, results need to be interpreted based on qualitative cultures with the use of a diagnostic threshold and/or identification of intracellular bacteria on direct examination of the sample [60].

Therapeutic removal of airways materials. In lipid pneumonia, BAL can help removing material present in the airways [84, 85]. These pneumonias result from the presence of lipoid material in alveolar structures, either from external or internal sources. Endogenous lipid pneumonia includes various disorders such as cholesterol pneumonia, disseminated lipogranulomatosis and alveolar proteinosis. Although controlled studies are lacking, several reports on proteinosis have shown that whole lung lavage is an effective treatment as it can remove the material from the alveolar space and consequently improves gas exchange and decreases the risk of infection. Application of this technique can be limited by the tolerance of the child. However, the limitation can be overcome by performing pulmonary lavage of a single lobe under endoscopy in association with ventilation of the other lung [86].

Diagnosis of noninfectious lung diseases. The role of BAL depends on the pathological conditions studied (table 5). BAL can be diagnostic in alveolar proteinosis, alveolar haemorrhage, and pulmonary histiocytosis. In alveolar proteinosis, BALF has a milky appearance [85–87]. On cytocentrifugation preparations stained with May-Grünwald Giemsa, basophil extracellular material mixed with enlarged foamy alveolar macrophages is observed. This material and the cytoplasmic content of the macrophages display a pink periodic-acid-Schiff staining. New biological investigations include evaluation of surfactant component accumulation in the BAL fluid, such as surfactant apoproteins. Idiopathic and secondary forms of alveolar proteinosis can be differentiated.

The diagnosis of alveolar haemorrhage is made easy when the BALF has either a bloody or orange pink colour and when the triad of haemoptysis, infiltrates on chest radiograph and anaemia is present. However, active pulmonary haemorrhage can occur without these elements, and the gross appearance of the BAL may be normal. Light microscopy examination can show the presence of free red blood cells, red blood cells in alveolar macrophages, or haemosiderin laden alveolar macrophages [88].
The diagnosis of pulmonary histiocytosis can be rapidly documented with the use of the monoclonal antibodies revealing the presence of CD1a positive cells. A presence of ≥5% of CD1a positive cells is required to confirm the diagnosis, as ≤3% of these cells can be present in healthy patients [89, 90]. In other pathological situations, BAL is not considered to be diagnostic, but may be a useful tool in the differential diagnosis. In many instances the various patterns of BAL cell profiles will help to orientate further investigations. (table 5).


Alveolar proteinosis
Primary, idiopathic
Secondary
haematological malignancies
exposure to inhaled chemicals and minerals
lipid pneumonia
Alveolar haemorrhage
associated with vascularitis and collagen vascular diseases
associated with glomerulonephritis
associated with drugs
secondary to cardiac diseases, pulmonary vascular lesions or malformations
associated with haematological malignancies or coagulopathy
idiopathic
Pulmonary histiocytosis
class I: Langerhans cell histiocytosis
class II: haemophagocytic syndromes
class III: malignant proliferation of cells (lymphoid origin or monocyte-macrophage lineage)
BAL: helpful for diagnosis
Increase in BAL T-lymphocytes
prevalence of CD4+ cells
sarcoidosis
Crohn’s disease
prevalence of CD8+ cells
hypersensitivity pneumonitis
histiocytosis X
drug-induced pneumonitis
interstitial lung disease associated with collagen vascularitis
bronchiolitis obliterans organizing pneumonia
Increase in BAL eosinophils
cosinophilic lung diseases
simple pulmonary eosinophilia
chronic eosinophilic pneumonia
asthma
Churg-Strauss syndrome
drug reactions
allergic bronchopulmonary aspergillosis
idiopathic hypereosinophilic syndrome
pulmonary diseases sometimes associated with eosinophils
interstitial lung diseases
Increase in BAL neutrophils
interstitial lung diseases
chronic bronchitis
asthma

Table 5. – Bronchoalveolar lavage (BAL) in non-infectious lung diseases in immunocompetent children

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<th>BAL: essential for diagnosis</th>
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of interstitial lung diseases and the role of BAL in paediatric interstitial lung disease remains to be defined. BAL provides information on the cellular and noncellular components present in the alveolar space in an attempt to evaluate the activity of the disease and therefore to adapt the therapeutic strategy. In active disease, patients may present with clinical signs of activity, and/or biological/immunological markers of active alveolitis, and/or active progression to fibrosis. The major problem is to interpret the role of markers of alveolitis as there is no general consensus on their meaning [91–93].

Recommendations

1) BAL should be performed in every child presenting with interstitial lung disease; 2) if lipid pneumonia or pulmonary haemorrhage are suspected, BAL represents an essential step for the diagnosis; 3) in pulmonary disorders with involvement of the alveolar structures, BAL provides information on the profiles of cell population and consequently helps to orientate the investigations. BAL is therefore helpful for evaluation of alveolar inflammatory processes.

Areas for further study

The goals for the next few years need to focus on the definition of biological markers in order to: 1) improve the management of lung diseases; 2) provide information on the pathogenesis of various types of lung diseases; 3) help predict the outcome as well as to predict the development of lung disease in at-risk patients; 4) it is important to highlight that results reported in adult populations cannot apply to paediatric populations without further evaluation.

Indications and results in immunocompromised children

BAL has become an important tool in the evaluation of diffuse or localized pulmonary infiltrates in immunocompromised children (IC).

Interpretation of microbiological results

Microbiological studies must be interpreted with care [70]. The identification of primary pathogens not usually found in the lung in BAL effluent is diagnostic. Such pathogens include P. carinii, Mycobacterium tuberculosis, Legionella pneumophila, Nocardia, Histoplasma, Blastomyces, Mycoplasma, Influenza virus, and Respiratory Syncytial Virus. Conversely, the identification of Herpes simplex virus (HSV), CMV, Aspergillus, atypical mycobacteria, bacteria, and Candida in BAL from IC does not necessarily establish a diagnosis of infection, since these organisms may be present as airway contaminants or commensals.

BAL in human immunodeficiency virus positive children

As survival time has increased in patients with human immunodeficiency virus (HIV), the incidence of acute pneumonia and recurrent bronchopneumonia has risen [94]. In several prospective studies of BAL in HIV-positive patients with acute interstitial pneumonitis (IP) or lower respiratory tract disease, one or more infectious
agent was identified in 55–84% of cases, with P. carinii being most frequently identified [47, 48, 95–99]. The sensitivity of BAL for the identification of P. carinii in acute IP is felt to be high. CMV is also frequently identified in BAL effluent, often in combination with a second pathogen such as P. carinii, but as in adults, the diagnosis of CMV pneumonia by BAL remains difficult. The clinical relevance of Staphylococcus aureus, Escherichia coli, Klebsiella, and Pseudomonas in BAL effluent from this patient population, however, is questionable as these organisms may be airway contaminants.

In children with pulmonary lymphoid hyperplasia-lymphocytic interstitial pneumonitis (PLH-LIP), BAL cytology reveals lymphocytosis with a low percentage of neutrophils and no infectious opportunistic agents [47].

**Other conditions**

Children with primary immune deficiencies or immunosuppression secondary to chemotherapy for malignancy, bone marrow transplantation (BMT) or nonlung organ transplantation [100, 101] may develop pneumonitis. In studies of BAL in such patients, the rate of establishing a microbiological diagnosis ranged 28–86%, and P. carinii and CMV were the infectious organisms most frequently isolated [18, 46, 48, 50, 52, 102–106]. Other pathogens isolated by BAL included other viruses or other organisms such as Mycoplasma hominis, and Legionella pneumophila [55]. The wide variation in diagnostic rates reported may be due to heterogeneity of patient selection, delay between onset of lung involvement and BAL, BAL technique used, and techniques used for detection of organisms.

Identification of a specific noninfectious disease by BAL is rare in this patient population, ranging 0–20% [18, 102, 103, 106, 107]. Noninfectious diseases are a relatively uncommon cause of pulmonary infiltrates in immunocompromised children, but have been documented by open lung biopsy (OLB) or autopsy [48, 52, 104, 108, 109]. Their true frequency is unknown.

**BAL in paediatric lung transplant recipients**

BAL and transbronchial biopsy (TBB) are frequently performed in children after lung transplantation, either routinely or as a result of clinical and/or radiological pulmonary deterioration [110]. Although TBB is currently required to establish the diagnosis of acute rejection, it is also important for microbiological studies [50]. Studies in adults and experimental animals have failed to identify molecular markers in BAL effluent which can accurately distinguish infection from rejection [111, 112], and no equivalent studies have been carried out in paediatric lung recipients.

**Recommendations**

The indications for its use are: 1) acute onset of tachypnoea, dyspnoea, and hypoxaemia with radiographic findings of new diffuse interstitial pulmonary infiltrates. In this circumstance, BAL should be performed before antibiotic therapy is commenced. If BAL is not performed and antibiotic therapy has been started, BAL should be performed in those children who do not improve on antibiotic therapy. BAL should be repeated in children with a positive BAL diagnosis but who deteriorate clinically in spite of appropriate treatment; 2) acute focal infiltrates which do not respond to standard broad spectrum antibiotic therapy within 48 h; 3) chronic interstitial pneumonitis, especially in HIV infected children. In non-HIV infected children BAL should be used in conjunction with either OLB or TBB; 4) chronic recurrent bronchopneumonia in HIV infected children if organisms are not found by less invasive techniques; 5) in association with TBB in lung transplant recipients as part of a routine surveillance programme and/or for diagnosis of suspected lung pathology.

**Areas for further study**

Although BAL is widely accepted as an important tool in the management of respiratory disease in immunocompromised children, further study of BAL in this setting is required. Suggested areas for research include studies to determine the following: 1) the microbiological diagnostic yield, sensitivity and specificity of BAL; 2) the incidence of noninfectious pulmonary infiltrates; 3) the noncellular component in noninfectious involvement; 4) a comparison of quantitative and semi-quantitative bacterial cultures of BAL with protected brush samples to determine the sensitivity and specificity of each technique; 5) the sensitivity and specificity of diagnostic tools for CMV, Pneumocystis carinii pneumonia (PCP), and Aspergillus; 6) the effect of potential pulmonary toxins on BAL cellular and noncellular components.

**Special consideration in neonates**

Since the smallest bronchoscopes with an external diameter of 2 mm have an inadequate suction channel, nonbronchoscopic bronchoalveolar lavage (NB-BAL) of intubated newborn infants is used to obtain BALF [113, 114, 115]. Only intubated infants can be lavaged, thus data from "normal" ventilated infants is lacking. NB-BAL is performed at the time of routine toileting of the endotracheal tube with simple modifications. Serial NB-BAL permits the study of the evolution of neonatal respiratory disorders. However, bias may be introduced as extubated infants can no longer be studied. In addition, mechanical ventilation itself is a risk factor for respiratory disease so "normal" data in newborn infants especially preterm infants is lacking and difficult to obtain. Many rely on infants ventilated for nonrespiratory reasons, e.g. surgery or muscular disorders [116, 117]. Thus, the reference group may vary according to the aims of the study but true reference data for term and preterm infants is unlikely to be available.

**Methods and site of sampling**

There are many variations of NB-BAL with few groups attempting to standardize the technique. Many do not pay any regard to positioning whilst others report a supine child with the head turned to the left to aid insertion of the catheter into the right lower lobe. It is unknown whether repeated insertion of the catheter samples the same area. Although most investigators use normal saline, at either room temperature or warmed to 37°C, the volume instilled is either based on weight [21, 114] or is fixed empirically [118]. The number of aliquots is usually one or two with the first aliquot probably reflecting bronchial milieu and the second more bronchoalveolar regions [114]. A further
variation lies with catheter size and the positioning of the
distal holes being either end-hole or two side-holes. End-
hole catheters are more likely to sample the areas of inter-
est. The suction pressure used to retrieve the fluid is often
not quoted, many use mechanical suction whilst others
prefer to use syringes. Higher pressures will collapse the
airways whereas lower pressures will reduce recovery.

Transient bradycardia due to a vasovagal response to
catheter insertion and a need to increase the inspired
oxygen by 5–10% to maintain oxygen saturation at >90%
are the most commonly encountered side effects [114].
Repeated NB-BAL of the same site over several weeks
has a theoretical risk of washing out surfactant but in the
authors’ experience regular examination of the chest
radiographs has not demonstrated any irregularities in the
right lower lobe [119].

Processing

Processing of BALF is discussed in detail elsewhere. In
neonatology, one or two aliquots of fluid are usually
instilled and the recovered fluid pooled mainly due to the
small volumes that are returned. Some workers filter the
fluid but in the authors’ experience this results in lower cell
counts than in unfiltered fluid [114]. In addition, filtering
is likely to result in loss of adherent cells such as alveolar
macrophages and the gauze is likely to soak up BALF,
thus it may affect the concentration of the constituents.
The BALF should be placed on ice after collection,
processed within 30 min of collection and the supernatant
stored at -70°C. Analysis of the supernatant and cells will
depend on the aims of the study but the methodology
should be optimized for the particular questions being
asked. In particular, the supernatant should be analysed
within 6 months as the effect of prolonged storage even at
-70°C has been poorly studied.

Estimation of epithelial lining fluid

ELF estimation remains a controversial area [43, 120].
External markers such as inulin or methylene blue are not
used in neonates. Internal markers include urea [116,
117], albumen [120], total protein and secretory com-
ponent of immunoglobulin (Ig)A (scIgA) [120]. Because
of capillary leak in many respiratory disorders, albumen
and protein, are unsuitable to estimate ELF. Urea is easy
to measure. However, it may diffuse across the epithelium
during the procedure, which may influence the results
[44, 121]. GRIGG et al. [114] did not show any differences
for ELF between the first and second aliquots thus
suggesting that significant diffusion of urea does not
occur. The use of scIgA has some merits [120]. scIgA is
secreted by the epithelium, is thought to not be affected
by gestation, age, lung injury or permeability and does
not require a serum sample. Whether scIgA production is
affected by lung injury, where epithelial cell damage may
be extensive, is unknown. Direct comparisons between
urea and scIgA have not been performed to estimate ELF.

Recommendations

1) NB-BAL should be performed in a standardized
manner. For generalized lung disease, the infant should be
supine with the head turned to the left and inspiratory
oxygen fraction (F1O2) increased to maintain oxygen
saturation >90%; 2) two aliquots 1 mL·kg⁻¹ each of nor-
mal-saline at room temperature should be instilled with an
endhole catheter of size FG 5–8 which is wedged in the
right lower lobes. Fluid should be recovered with a con-
stant suction pressure of 5–10 kPa; 3) the returned aliquots
can be pooled unless central or peripheral airways are
specifically being investigated; 4) the volume of fluid
instilled and recovered should be recorded and reported.
While filtration through gauze is considered a standard
procedure in older children, it is generally unnecessary in
neonates; 5) until the most appropriate method for accurately
estimating ELF is determined, results should
be reported as a concentration per millilitre.

Areas for further study

1) the reproducibility of the recovery of BALF and its
constituents from the same site or from different sites is not
known; 2) it is unknown whether repeated sampling of the
same site over a period of several weeks results in any local
clinical, pathological or radiographic changes; 3) reference
values for healthy term and preterm infants are unavail-
able; 4) comparison between the use of urea and scIgA to
estimate ELF is urgently required; 5) the effects of using
gauze or mucolytics on mucousy samples are unknown.

Research studies

The main objectives of BAL research studies in children
are to improve the management of lung diseases, to pro-
gress in the understanding of lung disease pathogenesis,
and to allow identification of patients at risk for the develop-
ment of respiratory diseases and prediction of outcome.

Management of lung diseases in children

Future directions need to focus on the characterization
of markers of acute inflammation as well as of markers of
repair processes.

Markers of acute inflammation. Efforts should be made to
integrate the concept of balance between pro-inflammatory
and anti-inflammatory molecules, and to co-ordinate
measurements of these factors. This involves pro-
and anti-inflammatory cytokines [122, 123]. Other molecules
such as oxygen radicals, platelet activating factor and
metabolites of arachidonic acid, proteases, coagulation
cascade and complement play an important role in the
inflammatory response [124, 125]. These factors can be
produced by the various cells of the alveolar structure.
Future works need to address the role of these various
components in the development of acute pulmonary inflam-
mmatory processes in paediatric populations [126].

Markers of repair processes. The processes of repair have
been poorly investigated in children, although they are a
dominant element of the prognosis [127]. Persistence of
lung dysfunction following acute lung injury is likely to
result from an inadequately regulated healing re-
ponse. Inflammation of the distal part of the lung has
been shown to be associated with an alteration of the
alveolar structures. The cellular components directly
in contact with the inflammatory cells present in the alve-
olar space are the epithelial cells. Following injury, the
alveolar epithelium needs to be rapidly and properly
repaired. Any delay in the re-epithelialization process may impair the healing response by allowing proliferation of fibroblasts in the interstitium and consequently the excessive accumulation of fibrotic tissue in the air spaces [128]. At the present time, little is known on the factors involved in the repair of the alveolar structure in patients with lung diseases [129].

Markers of acute inflammation and repair processes should be measured in BALF at various stages of disease progression. In the future, they will be of interest for the follow-up of patients and may be helpful in optimizing therapeutic strategies [130].

Understanding lung disease pathogenesis in children

Future directions should provide information on the cellular and noncellular components involved in the development of lung diseases [131]. Characterization of the functional modifications of the alveolar cells needs to include studies on freshly isolated cells in view of the rapid changes induced by culture conditions. Various techniques can be performed to provide information at the level of messenger ribonucleic acid (mRNA) and protein using cellular component extraction and/or in situ hybridization and immunohistochemistry on fixed cells. In some specific situations, these studies should be associated with analysis of lung tissue obtained by biopsy. For the noncellular components, the list of factors which could be analysed is increasing rapidly. Investigations can now evaluate a large number of functions of the various cells of the alveolar structure. For cells present in the alveolar space (macrophages, lymphocytes, and neutrophils), functional studies can be performed directly on isolated cells. For the other cells, evaluation of cell function through BAL materials can only be indirect by measurement of secreted products in the BAL supernatant. This approach may explain the difficulty in data analysis. As an example, alterations in surfactant components in BAL fluid can be explained by impairment of lung alveolar type 2 epithelial cell function. However, despite the fact that these cells are the producers of surfactant, abnormalities in intra-alveolar surfactant may also be the result of altered macrophage functions [132].

Information on cellular and noncellular BAL components should be integrated in the specific context of the lung disease studied. A fascinating example is cystic fibrosis (CF). Recent studies suggest that inflammation in CF is present early in the course of lung disease before colonization and infection of the lungs with potentially pathogenic bacteria [133]. However, it remains difficult at the present time to conclude that inflammation precedes infection. Further studies using BAL are required to provide more information on the defective homeostasis in the lung of children with CF and on the mechanisms involved.

Identification of at-risk patients to develop respiratory diseases and prediction of outcome

Characterization of factors specifically involved in the development and progression of lung diseases should help in the follow-up of children. One of the best examples is asthma which is the most common chronic illness in childhood. From the current understanding of the disease, it is now well recognized that an optimal management implies that the underlying inflammatory process is controlled [134]. This raises several essential questions such as when does asthma start and can patterns of progression towards persistent disease be individualized? To answer these questions, BAL is likely to represent a useful tool in wheezing infants by documenting the patterns of inflammatory marker expression at various stages of the disease [135, 136].

Conclusion

In the next few years it can be expected that bronchoalveolar lavage will be considered as an essential procedure in specific pathological situations. However, one major limitation for the interpretation of the data will remain the lack of control subjects and therefore the lack of reference values. The only way to overcome this problem relies on the development of collaborative studies among the various groups involved in paediatric pulmonary medicine. The present Task Force is a good illustration that these collaborative studies can be successful.

References


