Translational Research in Pediatrics III: Bronchoalveolar Lavage
Dhenuka Radhakrishnan, Cory Yamashita, Carolina Gillio-Meina and Douglas D. Fraser
*Pediatrics* 2014;134;135; originally published online June 30, 2014;
DOI: 10.1542/peds.2013-1911

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://pediatrics.aappublications.org/content/134/1/135.full.html
Translational Research in Pediatrics III: Bronchoalveolar Lavage

abstract

The role of flexible bronchoscopy and bronchoalveolar lavage (BAL) for the care of children with airway and pulmonary diseases is well established, with collected BAL fluid most often used clinically for microbiologic pathogen identification and cellular analyses. More recently, powerful analytic research methods have been used to investigate BAL samples to better understand the pathophysiologic basis of pediatric respiratory disease. Investigations have focused on the cellular components contained in BAL fluid, such as macrophages, lymphocytes, neutrophils, eosinophils, and mast cells, as well as the noncellular components such as serum molecules, inflammatory proteins, and surfactant. Molecular techniques are frequently used to investigate BAL fluid for the presence of infectious pathologies and for cellular gene expression. Recent advances in proteomics allow identification of multiple protein expression patterns linked to specific respiratory diseases, whereas newer analytic techniques allow for investigations on surfactant quantification and function. These translational research studies on BAL fluid have aided our understanding of pulmonary inflammation and the injury/repair responses in children. We review the ethics and practices for the execution of BAL in children for translational research purposes, with an emphasis on the optimal handling and processing of BAL samples.

AUTHORS: Dhenuka Radhakrishnan, MD, MSc,a,b Cory Yamashita, MD,c,d,e Carolina Gillio-Meina, PhD,f and Douglas D. Fraser, MD, PhD,a,b,d,e,f

Departments of aPediatrics, cMedicine, dPhysiology and Pharmacology, and eClinical Neurologic Sciences, Western University, London, Ontario, Canada; fChildren’s Health Research Institute, London, Ontario, Canada; aCentre for Critical Illness Research, Western University, London, Ontario, Canada; and fTranslational Research Centre, London, Ontario, Canada

KEY WORDS translational research, pediatrics, repository, BAL, proteins, surfactant, DNA, RNA

ABBREVIATIONS

BAL—bronchoalveolar lavage
ELF—epithelial lining fluid
FB—flexible bronchoscopy

Drs Radhakrishnan, Yamashita, and Gillio-Meina contributed to drafting the review and revised the manuscript; Dr Fraser conceptualized and initiated the review, contributed to drafting the review, and revised the manuscript; and all authors approved the final manuscript as submitted.

doi:10.1542/peds.2013-1911

Accepted for publication Feb 7, 2014

Address correspondence to Douglas D. Fraser, MD, PhD, Paediatric Critical Care Medicine, Room C2-843, Children’s Hospital, London Health Sciences Centre, 800 Commissioners Rd East, London, ON, Canada, N6A 5W9. E-mail: douglas.fraser@lhsc.on.ca

PEDIATRICS (ISSN Numbers: Print, 0031-4005; Online, 1098-4275). Copyright © 2014 by the American Academy of Pediatrics

FINANCIAL DISCLOSURE: The authors have indicated they have no financial relationships relevant to this article to disclose.

FUNDING: Drs Radhakrishnan, Yamashita, Gillio-Meina, and Fraser are supported by the Children’s Health Foundation (http://www.childhealth.ca, London, ON, Canada) and the Lawson Health Research Institute (http://www.lawsonresearch.com, London, ON, Canada).

POTENTIAL CONFLICT OF INTEREST: The authors have indicated they have no potential conflicts of interest to disclose.
There is a lack of literature addressing the performance of bronchoalveolar lavage (BAL) in children for research purposes. Published reviews focus on adult populations or on the technical and procedural aspects of performing clinically indicated BAL. In this review, our third in a series on tissue sampling and procedural aspects of performing and biobanking for child health studies, we aim to expand on the 2000 European Respiratory Society Task Force for BAL in children, with a particular emphasis on BAL for pediatric translational research. We present the ethical considerations and methodologic issues for obtaining BAL samples for research purposes and review the processing and storage of BAL samples to allow for reliable and reproducible measurements. We also highlight studies that isolated specific cellular and noncellular components from BAL, including newer reports using sophisticated analytic techniques for investigating proteins and surfactants.

The procurement of BAL samples by using bronchoscopy (FB) is a skilled procedure that requires special training to achieve proficiency and ensure patient safety. FB can be performed with variable methods as outlined in Table 1. Pediatric FB and BAL are generally considered safe and well tolerated; however, they are technically invasive procedures and carry associated risks in <2% of patients, including bleeding, barotrauma, need for prolonged intubation, severe hypoxia, and/or bronchospasm. Minor desaturations and epistaxis can occur more frequently in up to 7% of patients, and an additional 19% can experience postbronchoscopy fever. Relative contraindications to FB or BAL include massive hemoptysis, bleeding diathesis, severe airway obstruction, foreign body removal, severe hypoxia, and unstable hemodynamic status.

### Ethics of Performing BAL for Research

Although complications from FB and BAL are relatively minor and rare, in some institutions the ethics of performing BAL for research purposes are being increasingly scrutinized, likely due to greater awareness of ethics guidelines regarding research studies in children, particularly those studies involving invasive procedures. Institutional ethics review and informed consent by the legal guardians of children undergoing BAL for research studies must be obtained before performing such studies. Assent may be required from adolescent patients. The consent process must ensure that children/families are not coerced into study participation for perceived clinical benefit.

Given the ethical considerations in obtaining control BAL fluid for research studies, sample procurement is generally limited to those subjects in whom FB is being performed for a specific clinical indication (Table 2) or in those whom sedation and intubation is clinically indicated for other reasons (e.g., elective abdominal surgery). In the former, control data consist of BAL samples in children without the respiratory disease under investigation. Although the use of these samples may not represent ideal controls, they may be an acceptable alternative for practical considerations in the absence of an acute infectious process and active inflammation on direct visualization of the airways. Even in healthy subjects, perioperative stress may influence levels of inflammatory cells, cytokines, and chemokines in lavage samples. Reference data for BAL cellular constituents in children have been published, but because of wide variability across studies, reference values for BAL cellular and noncellular

---

**TABLE 1** Pediatric FB Techniques

<table>
<thead>
<tr>
<th>Point of Entry</th>
<th>Benefits</th>
<th>Pitfalls</th>
<th>Sedation/Anesthesia</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nose105 (directly or via oxygen face mask with port for bronchoscope)</td>
<td>Allows best inspection of entire airway Allows larger bronchoscope size Allows best visualization of dynamic airway motion</td>
<td>Lack of full control of airway If conscious sedation is used, apply topical plain lidocaine (2% to 4%) at larynx and 0.5% to 1% at carina to maximum dose of 5–7 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Mouth (laryngeal mask airway)</td>
<td>Allows inspection of larynx and upper trachea Allows largest bronchoscope size</td>
<td>Lack of full control of airway If conscious sedation is used, apply topical plain lidocaine (2% to 4%) at larynx and 0.5% to 1% at carina to maximum dose of 5–7 mg/kg</td>
<td></td>
</tr>
<tr>
<td>Mouth (ETT)</td>
<td>Best airway protection, may be required for transbronchial biopsy Size of bronchoscope limited by internal diameter of ETT Visualization only of distal trachea and bronchial tree Difficult to maintain spontaneous ventilation to assess dynamic airways movement</td>
<td>GA required for deep sedation</td>
<td></td>
</tr>
</tbody>
</table>

ETT, endotracheal tube; GA, general anesthesia.
components should be established locally.\textsuperscript{28}

**BAL PROCEDURE**

Performing BAL involves passing a flexible bronchoscope distally into an airway until the tip becomes wedged and cannot move any farther.\textsuperscript{4} The location of BAL sampling is dependent upon the clinical indication, but in cases of diffuse lung disease or for samples acquired for research purposes sampling of the right middle lobe may be ideal from an operator standpoint.\textsuperscript{37} It is important to note that the outer diameter of the bronchoscope relative to the wedge position can influence epithelial lining fluid (ELF) recovery and composition; wedging a small bronchoscope into a more distal bronchus will sample a smaller lung volume than if a larger bronchoscope is used.

In certain instances only nonbronchoscopic, blind BAL can be performed, such as in clinically unstable patients and in very small infants for whom the endotracheal tube size precludes insertion of a bronchoscope smaller than 2.7-mm external diameter. Different methods for nonbronchoscopic BAL are described, including the blind insertion of an 8F catheter as far as possible down the endotracheal tube beyond the estimated site of the carina to instill and withdraw fluid in variously sized aliquots,\textsuperscript{3,28,39} or fixing a catheter to the external surface of a 2.2-mm flexible bronchoscope that does not contain an internal suction channel.

Different methods for determining BAL instillation volume have been reported (Table 3) and adjusting the amount of instilled fluid per the weight of the child (aged 3–15 years) was shown to improve the consistency of ELF sampling.\textsuperscript{40} Distal sites may be better represented by a higher number of sequential aliquots taken from a particular wedge location.\textsuperscript{41} It is not known whether the method of aliquot aspiration affects BAL composition. Two reported methods for aliquot aspiration include the following: mechanical aspiration using 25 to 200 mm Hg pressure (3.33–13.3 kPa) into a suction trap or hand suction using a syringe.

**FACTORS AFFECTING COMPOSITION OF BAL FLUID**

Many factors can influence the quality and composition of BAL samples, including the total volume of saline instilled and the length of the dwell time between saline instillation and withdrawal, because ELF can be diluted by fluid exchange occurring between alveolar, vascular, and interstitial compartments.\textsuperscript{42,43} The BAL sample should be considered adequate if there is >40% recovery of instilled fluid, <5% epithelial cells (unless an airway sample is desired), and minimal amounts of mucus after filtering.\textsuperscript{5,8,28} There is no reliable indicator to calculate the proportion of BAL fluid that represents ELF, which makes comparison between research studies difficult.\textsuperscript{1} The concentrations of urea\textsuperscript{18,44–46} and albumin\textsuperscript{47,48} have been used to estimate the

---

**TABLE 2 Clinical Indications for Pediatric FB**

<table>
<thead>
<tr>
<th>Anatomic Evaluation</th>
<th>BAL</th>
<th>Airway Clearance</th>
<th>Biopsy</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dynamic airway collapse (eg, bronchomalacia)</td>
<td>Microbiologic identification (eg, fungal/bacterial stains, culture, viral studies, PCR)</td>
<td>Foreign body assessment (removal should be performed by rigid bronchoscope)\textsuperscript{13}</td>
<td>Endobronchial biopsy</td>
<td>pH of lower airways using pH probe\textsuperscript{148}</td>
</tr>
<tr>
<td>External compression (eg, vascular ring, cardiac chambers)</td>
<td>Cell count and differential</td>
<td>Removal of mucus plugs</td>
<td>Transbronchial biopsy\textsuperscript{149}</td>
<td>Ion transport properties of respiratory epithelium\textsuperscript{190}</td>
</tr>
<tr>
<td>Tracheoesophageal fistula</td>
<td>Subpopulations of lymphocytes (eg, CD4+:CD8+ ratio)</td>
<td>Direct instillation of mucolytics (eg, dornase α, fibrinolytics)\textsuperscript{107}</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Endobronchial lesions (eg, tumor, hemangioma)</td>
<td>Noncellular components (eg, surfactant, lipid-laden macrophages, or hemosiderin)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bronchial webs/stenosis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Anatomic variants (eg, tracheal bronchus)</td>
<td>Whole lung lavage</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Source of hemoptysis</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

CD, cluster of differentiation; PCR, polymerase chain reaction.

---

**TABLE 3 Different Reported Methods for Determining BAL Instillation Volume in Children**

<table>
<thead>
<tr>
<th>Aliquot Size</th>
<th>Patient Size Adjustment</th>
</tr>
</thead>
<tbody>
<tr>
<td>2–4 fractions of 10–20 mL\textsuperscript{37,110}</td>
<td>N/A</td>
</tr>
<tr>
<td>5- to 20-mL fractions\textsuperscript{111}</td>
<td>Adjusted by FRC</td>
</tr>
<tr>
<td>5 mL for infants\textsuperscript{4}</td>
<td>N/A</td>
</tr>
<tr>
<td>10 mL for small child\textsuperscript{4}</td>
<td>Divided into 3 aliquots for children &lt;20 kg</td>
</tr>
<tr>
<td>15 mL for large child\textsuperscript{4}</td>
<td>Divided into 20-mL aliquots for children &gt;20 kg</td>
</tr>
<tr>
<td>3 mL/kg\textsuperscript{3,30,112}</td>
<td></td>
</tr>
</tbody>
</table>

FRC, functional residual capacity; N/A, not applicable.
ELF component, although each has its unique problems. Urea is present in ELF in equal concentrations to serum but diffuses into BAL fluid in a time-dependent manner, with higher concentrations observed in diseases with increased capillary permeability. Albumin diffuses only very slowly into BAL fluid, but its concentration is frequently altered by lung disease. Given the technical variations used for performing BAL sampling in children, the solute concentrations from BAL are best reported along with the following variables: total volume of normal saline instilled, the volume of fluid recovered (eg, number of recovered cells per milliliter of BAL fluid), as well as the site of BAL collection.

**BAL FLUID HANDLING AND PROCESSING**

General recommendations on BAL in children have been published. Specific recommendations were proposed to optimize the handling and processing of samples to facilitate pathologic diagnosis, but less attention has been paid to the handling and processing of BAL fluid in the context of research practices. Because of the limited ability to perform pediatric FB strictly for research purposes, protocols by which samples are processed and handled will be dependent upon a number of factors and tailored on a “case-by-case” basis, including (1) the primary indication for the procedure, (2) immediate testing to be performed on samples to facilitate diagnosis, (3) local practices, and (4) availability of local resources. Furthermore, given the lack of control data, uniform handling, processing, and storage of samples should be observed to maximize the consistency and minimize variability in the results. Practices for the handling and processing of specimens that are designated for research purposes are summarized in Tables 4 and 5.

**INITIAL PROCUREMENT CONSIDERATIONS**

Upon retrieval of BAL fluid, the conditions for fluid transportation are primarily dependent on the anticipated duration of time from sample collection to laboratory analysis. Accordingly, the volume, location, quality of lavage, as well as underlying disease pathology may result in lavage fluid samples that vary considerably between individuals. For BAL samples in which the anticipated

<table>
<thead>
<tr>
<th>Suggested Processing and Storage Practices for Samples From BAL Fluid</th>
<th>Practice</th>
<th>BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport to the laboratory</td>
<td>BAL fluid samples must be fresh and should be transported on ice</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Sample pooling step</td>
<td>The first BAL sample should not be pooled with the next samples because it has a lower cellular yield with more neutrophils and less lymphocytes than subsequent samples; the first sample can be used for culture (microbiology)</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Filtration step</td>
<td>Before the evaluation of noncellular components, BAL fluid should be filtered</td>
<td>When total cell count is performed, filtration of pooled aliquots is important to prevent mixing of mucus with the cell pellet and to remove bronchial epithelial cells</td>
</tr>
<tr>
<td>Time to do total cell counting</td>
<td>Samples must be fresh and processed immediately after collection</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Time to centrifugation</td>
<td>The lavage sample is initially centrifuged at 250–500 g for 10 min at 4°C to separate the pellet (cellular components) from the supernatant (total surfactant or noncellular components)</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Centrifugation step</td>
<td>Immediately after centrifugation</td>
<td>Cells can remain viable in BAL fluid at 25°C for up to 4 h or at 4°C for up to 24 h</td>
</tr>
<tr>
<td>Time to freeze</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Storage</td>
<td>Pelleted cells can be resuspended in nutrient-supplemented media and stored at 4°C for up to 12 h</td>
<td>RT, room temperature (21°C).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>TABLE 4 Suggested Processing and Storage Practices for Samples From BAL Fluid</th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Culture, Noncellular Components</td>
<td>Cellular Components (Macrophages, Lymphocytes, Neutrophils, Eosinophils, etc)</td>
</tr>
<tr>
<td>Transport to the laboratory</td>
<td>BAL fluid samples must be fresh and should be transported on ice</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Sample pooling step</td>
<td>The first BAL sample should not be pooled with the next samples because it has a lower cellular yield with more neutrophils and less lymphocytes than subsequent samples; the first sample can be used for culture (microbiology)</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Filtration step</td>
<td>Before the evaluation of noncellular components, BAL fluid should be filtered</td>
<td>When total cell count is performed, filtration of pooled aliquots is important to prevent mixing of mucus with the cell pellet and to remove bronchial epithelial cells</td>
</tr>
<tr>
<td>Time to do total cell counting</td>
<td>Samples must be fresh and processed immediately after collection</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Time to centrifugation</td>
<td>The lavage sample is initially centrifuged at 250–500 g for 10 min at 4°C to separate the pellet (cellular components) from the supernatant (total surfactant or noncellular components)</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Centrifugation step</td>
<td>Immediately after centrifugation</td>
<td>Cells can remain viable in BAL fluid at 25°C for up to 4 h or at 4°C for up to 24 h</td>
</tr>
<tr>
<td>Time to freeze</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity</td>
<td>Same as noncellular components</td>
</tr>
<tr>
<td>Storage</td>
<td>Pelleted cells can be resuspended in nutrient-supplemented media and stored at 4°C for up to 12 h</td>
<td>RT, room temperature (21°C).</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Practice</th>
<th>BAL</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transport to the laboratory</td>
<td>BAL fluid samples must be fresh and should be transported on ice</td>
</tr>
<tr>
<td>Sample pooling step</td>
<td>The first BAL sample should not be pooled with the next samples because it has a lower cellular yield with more neutrophils and less lymphocytes than subsequent samples; the first sample can be used for culture (microbiology)</td>
</tr>
<tr>
<td>Filtration step</td>
<td>Before the evaluation of noncellular components, BAL fluid should be filtered</td>
</tr>
<tr>
<td>Time to do total cell counting</td>
<td>Samples must be fresh and processed immediately after collection</td>
</tr>
<tr>
<td>Time to centrifugation</td>
<td>The lavage sample is initially centrifuged at 250–500 g for 10 min at 4°C to separate the pellet (cellular components) from the supernatant (total surfactant or noncellular components)</td>
</tr>
<tr>
<td>Centrifugation step</td>
<td>Immediately after centrifugation</td>
</tr>
<tr>
<td>Time to freeze</td>
<td>Freeze-thaw should be limited to only 1 cycle to ensure sample integrity</td>
</tr>
<tr>
<td>Storage</td>
<td>Pelleted cells can be resuspended in nutrient-supplemented media and stored at 4°C for up to 12 h</td>
</tr>
</tbody>
</table>
time for processing is <60 minutes, samples can be transported “fresh” at room temperature (21°C).53 After 60 minutes, there is no formal consensus. In general, specimens should be transported on ice and may be stored at 4°C for up to 24 hours.54 If delays in cellular analysis are expected, samples should be centrifuged at 200 to 300 g × 10 minutes (to maintain cellular integrity), the cellular fraction should be resuspended in nutrient-supplemented media (eg, Minimum Essential Medium [MEM] supplemented with the pH buffering agent hydroxyethyl piperazineethanesulfonic acid [HEPES]), and the suspension can be stored at 4°C for up to 12 hours.54 Freeze/thaw cycles of samples should be avoided when possible.

Processing of cellular components and/or microbiologic agents should follow guidelines as previously described for freshly obtained clinical samples. When proteins and/or nucleic acids studies are required, BAL fluid supernatants can be stored from −20°C55 to −80°C56–58 to avoid degradation and then can be bulk analyzed at a later time.

**INITIAL ALIQUOT**

As a general consideration, the initial BAL fluid aliquot should not be used for direct assessment of the alveolar environment.41 Although there is no specific consensus regarding the quality of the initial aliquot, previous studies performed in pediatric patients have established that this first sample has a lower cellular yield and may increase the likelihood of airway sampling rather than alveolar sampling.59 Thus, the initial aliquot may be of greater interest in the study of airway-related diseases. Subsequent BAL samples have higher cell counts and tend to remain consistent across multiple lavages.59,60

**MICROBIOLOGIC STUDIES**

Occult or suspected respiratory infection represents one of the most common clinical indications for FB and may include bacterial, fungal, and viral pathogens in both immunocompetent and immunocompromised patients.61 Accurate pathogen identification is also critical to investigate host response. Some advocate that microbiologic studies be performed on nonfiltered BAL samples to eliminate the possibility of inadvertently trapping organisms.8 Samples sent for microbiologic culture should be processed immediately to minimize the risk of contamination or degradation of anaerobic organisms, and the concurrent use of antibiotics should be noted, which may affect the interpretation of results.62 Cleaning and disinfection of all instruments used for the BAL procedure should be practiced to minimize the risk of false-positive results.12,63,64 Similarly, avoidance of suctioning while the bronchoscope is in the upper airway is critical to avoid contamination of lower airway samples.4 Specimens should be collected in leak-proof containers and transported in sealed plastic bags. If delays are anticipated in the processing of samples, refrigeration is preferable to storage at ambient temperatures; delays >48 hours are undesirable and results should be interpreted with caution.54

**CELLULAR ISOLATION**

Sequential aliquots of BAL fluid should be pooled and filtered through 1 layer of sterile gauze to remove excess mucoid debris; however, filtering of BAL fluid through gauze may result in a significant reduction in the volume of sample.65 Furthermore, filtering may result in lower cell counts, in particular adherent alveolar macrophages.66,67 Nevertheless, the total volume of retrieved BAL sample should be measured and cell viability should be initially assessed by using standard techniques such as trypan blue staining.68 Samples should then undergo centrifugation at 50 to 500 g for 10 to 15 minutes for cell subtype isolation and identification, and cell counting should be performed (ie, using cytocentrifuge preparations [Diff-Quick staining; Merz & Dade AG, Dudingen, Germany]) by using manual counts on simple smears or through automated counting techniques using a flow cytometer.59,69,70 Table 6 lists specific cellular components that can be isolated from BAL fluid and special considerations for processing.

A minimum of 300 to 350 cells should be counted to maximize accuracy, and multiple slides may be stored for...
### TABLE 6  Cellular Content Isolated From BAL and Processing Tips and Facts

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Processing Tips, Detection Methods, and/or General Information</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Macrophages</strong></td>
<td>80% to 90% of the cells recovered from BAL from normal individuals are macrophages. Morphologic changes can be seen in alveolar macrophages that include a foamy appearance in HP, markedly vacuolated cytoplasm with positive staining of vacuoles for fat in chronic aspiration pneumonia, cytoplasmic inclusions associated with viral infection, ingested RBCs and RBC fragments and hemosiderin with DAH, ingested asbestos bodies, or other dust particles. Esterase staining distinguishes immature macrophages from lymphocytes. BAL macrophages may exhibit the same light scatter profile as lymphocytes, promoting errors in lymphocyte counts. Macrophages can be further characterized through flow cytometric techniques by using monoclonal antibodies. In DAH, alveolar macrophages will stain for iron (hemosiderin) if the onset of hemorrhage has preceded the time of BAL by 24–48 h. A high-lipid-laden macrophage index may indicate chronic aspiration of oral or gastric contents. Immunostaining is used to assess for phagocytosis or apoptosis in asthma. KP-1 stains macrophages, which sometimes can be confused with epithelial cells.</td>
</tr>
<tr>
<td><strong>Lymphocytes (eg, CD3, CD4, CD8)</strong></td>
<td>5% to 15% of the cells recovered from BAL of normal individuals are lymphocytes; the subsets of T lymphocytes in the normal adult lung are 75% of CD3+, 45% of CD4+, 25% of CD8+, and &lt;5% for B cells; total T- and B-cell counts are similar in children and adults. In children, there is an increase in CD8+ subset of T cells in BAL that gives a lower CD4+CD8+ ratio than that in adults. Increased numbers of lymphocytes recovered in BAL fluid have been reported in diseases including hypersensitivity pneumonitis, sarcoidosis, berylliosis, tuberculosis, various drug-induced lung diseases, asbestosis, some collagen vascular diseases, and HIV infections. A high percentage of lymphocytes (&gt;50%) suggests HP or cellular NSIP; whereas a value &gt;25% suggests granulomatous lung diseases (sarcoidosis, HP, NSIP, berylliosis, drug reaction, CDP, LIP, or lymphoma). Although sarcoidosis involves predominantly CD4+ T cells, HP involves typically lymphocytic alveolitis with a predominance of CD8+ T cells. Immunoperoxidase reaction in immunocytochemistry is frequently used to enumerate lymphocyte populations in BAL fluid in patients with pulmonary diseases, but it is time-consuming and the accuracy and reliability of results depend on the number of cells counted and the experience of the observer. Lymphocytes can be assessed by using immunofluorescence-labeled monoclonal antibodies and flow cytometry for counting and assessment of polyclonality. Lymphocyte phenotype can be further characterized through flow cytometric techniques by using monoclonal antibodies. Flow cytometry rapidly counts large cell numbers compared with manual counting, but the heterogeneity of the cellular populations makes analysis difficult and can lead to the exclusion of cells of interest as well as the inclusion of unwanted cells. Cyto centrifugation is the best technique to avoid lymphocyte loss; differential counting of cells is performed on air-dried May-Grünwald-Giemsa– or Wright-Giemsa–stained preparations. Cyto centrifugation (Cytopsin) can underestimate the proportion of lymphocytes by ~45% compared with a smear of resuspended cells under a glass coverslip. Macrophages can be removed before lymphocyte immunophenotyping by adherence to plastic in media such as RPMI 1640 supplemented with serum for 30 min to 1 h, by the magnetic removal of ingested carbonyl iron, with complement-mediated lysis and anti-CD11c, or by passage through a nylon wool column. Specific T-cell subset populations can be isolated by rosetting with neuraminidase-treated sheep erythrocytes followed by Ficol-Hypaque gradient centrifugation. Morphologic changes can be seen in alveolar macrophages that include a foamy appearance in HP, markedly vacuolated cytoplasm with positive staining of vacuoles for fat in chronic aspiration pneumonia, cytoplasmic inclusions associated with viral infection, ingested RBCs and RBC fragments and hemosiderin with DAH, ingested asbestos bodies, or other dust particles. Esterase staining distinguishes immature macrophages from lymphocytes. BAL macrophages may exhibit the same light scatter profile as lymphocytes, promoting errors in lymphocyte counts. Macrophages can be further characterized through flow cytometric techniques by using monoclonal antibodies. In DAH, alveolar macrophages will stain for iron (hemosiderin) if the onset of hemorrhage has preceded the time of BAL by 24–48 h. A high-lipid-laden macrophage index may indicate chronic aspiration of oral or gastric contents. Immunostaining is used to assess for phagocytosis or apoptosis in asthma. KP-1 stains macrophages, which sometimes can be confused with epithelial cells.</td>
</tr>
<tr>
<td><strong>Neutrophils</strong></td>
<td>Less than 3% of the cells recovered from BAL from normal individuals are neutrophils. The percentage of neutrophils is higher in BAL fluid from children &lt;12 mo than children aged 13–36 mo. A high percentage of neutrophils (&gt;50%) strongly suggests pneumonia, aspiration pneumonia, lung abscess, or acute lung injury. Increased neutrophils in BAL from patients with sarcoidosis has been associated with more progressive disease that is less likely to respond to immunosuppressive therapy. Increases in BAL neutrophils have been correlated with disease severity and prognosis for both HP and IPF. ARDS is associated with lung neutrophil infiltration and elevated cytokines/chemokines. Elevated neutrophil levels are seen in CF, asthma, PCD, PBB, bronchiectasis, measles, and bronchiolitis obliterans. Neutrophil apoptosis has been studied in children with RDS/ECMO by using Giemsa staining of cytospin preparations. Filtration, as a method to obtain differential cell counts, should be avoided for neutrophils due to filter preparations that can underestimate cell number.</td>
</tr>
<tr>
<td><strong>Eosinophils</strong></td>
<td>Less than 1% of cells recovered by BAL from normal individuals are eosinophils. A high percentage of eosinophils (&gt;25%) suggests eosinophilic lung disease, especially EP if the presentation is acute. BAL eosinophilia has been linked to more severe disease and worse prognosis in IPF. BAL eosinophilia can be used to evaluate blood contamination in BAL fluid, which is common if DAH is present. RBCs should be identifiable on the cytospin. RBC contamination can be removed by using lysis reagents including ammonium chloride, commercial lysing reagents, or mild hypotonic lysis solution. The use of lysing reagents to remove RBC contamination could lead to the release of cellular debris and interfere with lymphocyte gating purity.</td>
</tr>
<tr>
<td><strong>Granulocytes</strong></td>
<td>RBC proportion is used to evaluate blood contamination in BAL fluid, which is common if DAH is present. RBCs should be identifiable on the cytospin. RBC contamination can be removed by using lysis reagents including ammonium chloride, commercial lysing reagents, or mild hypotonic lysis solution. The use of lysing reagents to remove RBC contamination could lead to the release of cellular debris and interfere with lymphocyte gating purity.</td>
</tr>
<tr>
<td><strong>RBCs</strong></td>
<td>RBC proportion is used to evaluate blood contamination in BAL fluid, which is common if DAH is present. RBCs should be identifiable on the cytospin. RBC contamination can be removed by using lysis reagents including ammonium chloride, commercial lysing reagents, or mild hypotonic lysis solution. The use of lysing reagents to remove RBC contamination could lead to the release of cellular debris and interfere with lymphocyte gating purity.</td>
</tr>
</tbody>
</table>
clinical or research purposes.\textsuperscript{9} If delays are anticipated for specific cellular analysis, cells can be stored at 4°C and analyzed up to 24 hours later without significant changes in the cellular composition or differential cell count,\textsuperscript{54} although neutrophil apoptosis with engulfment by alveolar macrophages can commence before 24 hours, and thus samples should be analyzed with minimum delay.\textsuperscript{71,72}

**NUCLEOTIDE ANALYSIS**

Cellular gene expression studies from BAL samples in pediatric respiratory disease states have also been reported (ie, cytokine mRNA).\textsuperscript{35} More commonly, nucleotide analysis has been useful to detect a variety of infectious pathogens localized in different cell types and/or in cell-free compartments of the respiratory tract and to detect increases in the number of specific cell-type populations (lymphocytes, macrophages, neutrophils, etc). Several molecular techniques with high sensitivity and specificity, such as polymerase chain reaction and hybridization, have been used to identify bacteria,\textsuperscript{73,74} mycobacteria,\textsuperscript{75,76} fungi,\textsuperscript{77–83} Chlamydia,\textsuperscript{57} mycoplasma,\textsuperscript{87} and viruses,\textsuperscript{84–87} whereas consensus has not been reached about the value of polymerase chain reaction for fungal detection due to positive results in patients who do not develop the associated disease.\textsuperscript{88–90} Examples of nucleotide analysis used for samples isolated from cell or cell-free compartments are shown in Table 7.

**NONSURFACTANT PROTEIN ANALYSIS**

Protein analyses have been used to assess the functional consequences of gene expression and to provide greater insight into protein expression and modification within complex disease states\textsuperscript{91} (Table 8). Recently, there have been significant advancements in proteomics, which analyzes large numbers of proteins in biological tissues with two-dimensional gel electrophoresis, multidimensional liquid chromatography, and/or mass spectrometry.\textsuperscript{92,93} Because of the abundance of high-molecular-weight proteins that predominate in the BAL fluid proteome in both diseased and nondiseased states, the detection of less abundant pathologic proteins may be more difficult\textsuperscript{94} and thus require special consideration when initially harvesting BAL fluid.

Because of the inherent nature of biological samples, several factors have the potential to interfere with proteomic analysis, including the presence of insoluble substances and biological salts,\textsuperscript{95} in addition to the dilute concentrations of proteins that are being measured. The initial centrifugation of samples before direct analysis or storage will initially remove insoluble factors present in the BAL. Subsequently, desalting of BAL samples has been described through a variety of techniques including dialysis, size-exclusion filtering, protein precipitation,\textsuperscript{96} or reverse-phase chromatography,\textsuperscript{97} in addition to removal of ubiquitous proteins such as albumin.\textsuperscript{98} Techniques such as affinity purification can be used to minimize the dynamic range and enrich the specific protein of interest.\textsuperscript{99}

Although proteomic analysis has been used across a spectrum of pediatric lung diseases, lack of uniformity exists across published studies and has likely contributed to proteomic variability.\textsuperscript{99} Currently, no standardized protocols exist for procedural aspects of sample retrieval and, furthermore, a standardized approach to optimizing samples for proteomic analysis has not been clearly defined. Thus, key information such as volume and protein concentration of the initial lavage, the number of freeze-thaw cycles, and methodology used in sample preparation should be carefully documented and reported.

**SURFACTANT ANALYSIS**

Analysis of the protein-phospholipid surfactant complexes remains an area of particular interest in pediatric respiratory research. Newer techniques allow in-depth analysis of the surfactant system, including quantification of the functional (large aggregate) and nonfunctional (small aggregate) forms and may offer insight into in vivo function (Table 9). Functional large aggregate forms of surfactant can only be retrieved via high-speed centrifugation (pellet), with nonfunctional small aggregate forms retrieved from the remaining supernatant. An alternative method of aggregate separation includes...
equilibrium buoyant density gradient centrifugation. Although both methods have been validated, high-speed centrifugation can be performed on multiple samples in a less labor-intensive manner compared with equilibrium buoyant density gradient centrifugation. The evaluation of surfactant function can be performed through measurements of surface tension by using surfactometers or quantitative Brewster angle microscopy. Standard practices for the handling, processing, and storage of specimens that are designated for surfactant isolation are listed in Table 5.

### CONCLUSIONS

This review summarizes pertinent issues regarding BAL in children for research purposes, including ethical and methodologic considerations for obtaining BAL fluid, and the cellular and noncellular elements that can be obtained by FB. The
<table>
<thead>
<tr>
<th>Noncellular Constituents</th>
<th>Clinical Disease</th>
<th>Detection Technique</th>
<th>Isolation Method</th>
<th>General Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>Albumin (produced only outside the lungs)</td>
<td>Healthy Children</td>
<td>Nephelometric method</td>
<td>Isolated from supernatant after first centrifugation</td>
<td>At least 69% of the proteins in BAL fluid are serum derived. An increase in immunoglobulins and albumin is detected in these diseases. Specific antibodies in BAL can indicate infection.</td>
</tr>
<tr>
<td>Immunoglobulins (synthesized both within and outside the lungs)</td>
<td>Fibrosing alveolitis, IPF, HP, pulmonary fibrosis</td>
<td>Radial immunodiffusion in agar</td>
<td>Isolated from cell pellet derived from BAL fluid after first centrifugation step</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Cryptococcus infection</td>
<td>Radioimmunoabsorbent method</td>
<td>Isolated from BAL fraction</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Fungal infection</td>
<td>Immunoblot assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>IPF</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>RSV F, G, and N proteins</td>
<td>Western blot analysis</td>
<td>Isolated from BAL fluid supernatant after first centrifugation</td>
<td></td>
</tr>
<tr>
<td></td>
<td>NE, myeloperoxidase, endotoxin</td>
<td>NE-specific substrate assay</td>
<td>Transport of frozen samples does not affect level of oxidized proteins</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Pulmonary infection</td>
<td>ELISA</td>
<td>Neutrophil elastase and myeloperoxidase in BAL are indicators of pulmonary inflammation in several disease processes</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Acidic fibroblast growth factor</td>
<td>Multiplex, particle-based commercial assay</td>
<td>Western blot analysis, heparin affinity, gel filtration, IHC</td>
<td></td>
</tr>
<tr>
<td>Manose-binding lectin</td>
<td>Pulmonary infections</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>BAFF APRIL</td>
<td>Pulmonary alveolar proteinosis</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6 receptor single nucleotide polymorphism</td>
<td>Asthma</td>
<td>Immune-beads</td>
<td>Cells are isolated from the surface of neutrophils and other cells in BAL fluid</td>
<td></td>
</tr>
<tr>
<td>TGFB1</td>
<td>Asthma</td>
<td>RT-PCR</td>
<td>Neutrophil elastase and myeloperoxidase in BAL are indicators of pulmonary inflammation in several disease processes</td>
<td></td>
</tr>
<tr>
<td>PPAR-y, paraxanthase</td>
<td>CF, ILD</td>
<td>qPCR</td>
<td></td>
<td></td>
</tr>
<tr>
<td>MCP-1, MCP-1 mRNA expression</td>
<td>Preterm infants of mothers with chorioamnionitis</td>
<td>qRT-PCR</td>
<td>mRNA extracted from lysed BAL fluid cells</td>
<td></td>
</tr>
<tr>
<td>MMP-8</td>
<td>CF</td>
<td>ELISA</td>
<td>Isolated from BAL fluid supernatant after first centrifugation</td>
<td></td>
</tr>
<tr>
<td>Lipoxin A, Clara cell protein</td>
<td>CF</td>
<td>ELISA</td>
<td>Neutrophil elastase and myeloperoxidase in BAL are indicators of pulmonary inflammation in several disease processes</td>
<td></td>
</tr>
<tr>
<td>sILAM-1, MMP-9, TIMP-1</td>
<td>Persistent wheeze</td>
<td>ELISA</td>
<td>Neutrophil elastase and myeloperoxidase in BAL are indicators of pulmonary inflammation in several disease processes</td>
<td></td>
</tr>
<tr>
<td>AAT</td>
<td>ILD</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>uTAC, uTCA, PGE2, HETE, β-tryptase</td>
<td>Wheezing</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Noncellular Constituents</td>
<td>Clinical Disease</td>
<td>Detection Technique</td>
<td>Isolation Method</td>
<td>General Information</td>
</tr>
<tr>
<td>--------------------------</td>
<td>------------------</td>
<td>---------------------</td>
<td>------------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>HA</td>
<td>Healthy adults and lung cancer</td>
<td>RIA</td>
<td>Isolated from BAL supernatant after first centrifugation</td>
<td>ELISA is the most frequently used method for detecting various protein elements in BAL, in some cases, radiolabeled and dye-based immunoassays are used</td>
</tr>
<tr>
<td>TNF-α, IL-6</td>
<td>Lung transplant</td>
<td>Flow cytometry after stimulation with PMA and ionomycin</td>
<td>Anti-CD4 added to prevent PMA or ionomycin caused reduction in surface expression of CD4</td>
<td></td>
</tr>
<tr>
<td>Matrix components (elastin, collagen glycosaminoglycans) and intracellular cytokines (TNF, IL-1β, IL-8, IL-6, IL-1ra)</td>
<td>CF</td>
<td>Dye binding assay kits</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intracellular cytokines (INF-γ, IL-2, IL-4, IL-5, IL-10)</td>
<td>Asthma</td>
<td>Flow cytometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-2, IFN-γ, CD28, CD45, CD40, CD80, CD86, CXCR3</td>
<td>ILD</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10, ITAC, MIG, TARC, MDG, IL-5, IL-18</td>
<td>Asthma, chronic cough</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL8, IL16</td>
<td>Measles bronchiolitis obliterans</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α, IL-8, nitrite</td>
<td>Persistent wheeze</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>hBD-2, hBD-3</td>
<td>Healthy adults</td>
<td>Griess colorimetric assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>β-defensins: hBD-1, hBD-2</td>
<td>CF</td>
<td>Immune dot blot assay</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cathelicidin LL-37, hCAP-18</td>
<td>VAP</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PA-1, STREM-1, RAGE</td>
<td>Asthma, chronic cough</td>
<td>Flow cytometry</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pulmonary lymphocyte expression of CXCR3, CXCR4, CCR4, CCR5</td>
<td>Infants with severe RSV bronchiolitis</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CX3 chemokines (CXCL 10, CXCL8, CCL2, CCL3, CCL5)</td>
<td>Asthma</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RANTES, MCP-3, MCP-4, eotaxin</td>
<td>Healthy children</td>
<td>ELISA with/without Western blot</td>
<td>Requires purification with Sep pak system</td>
<td></td>
</tr>
<tr>
<td>Fibronecin, TNF-α, IL-4, IL-5, IL-18, IL-8, IL-12</td>
<td>ARDS</td>
<td>ARDS</td>
<td>Upregulation of these molecules can indicate neutrophil activation</td>
<td></td>
</tr>
<tr>
<td>92 Gelatinase, TIMP-1, IL-6</td>
<td>Asthma</td>
<td>ARDS</td>
<td>CR3 is necessary for migration and phagocytosis</td>
<td></td>
</tr>
<tr>
<td>IL-4, IL-5, IL-15 and IL-18, IL-17</td>
<td>IL-10 and TNF-α</td>
<td>ELISA</td>
<td>EUSA used to confirm proteins identified by proteomics</td>
<td></td>
</tr>
<tr>
<td>MMP-9, TIMP-1, eicosanoid mediators (LTB4, thromboxane)</td>
<td>IL-8, IL-12</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-6, sIL-6r, sgp-130</td>
<td>Protein carbonyl used as a measure of oxidative stress</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>TNF-α, IL-1β, IL-8, IL-1Ra</td>
<td>IL-1B</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-10 and TNF-α</td>
<td>A1AT and SLPI</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>IL-18, IL-2</td>
<td>CR3, CR5</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Serum proteins (eg, albumin, β2-microglobulin and fibrinogen), IGFBP-3 and pulmonary proteins (eg, as SP-D and Clara cell protein)</td>
<td>ARDS</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbonated proteins</td>
<td>GER</td>
<td>Immuno blot</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bile acids</td>
<td>Commercial enzymatic assay</td>
<td>ELISA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Carbonated proteins, 8-isoprostan, catalse, glutathione peroxidase</td>
<td>Bronchiolitis obliterans</td>
<td>DNPW-based procedure</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foxp3+</td>
<td>Lung transplant</td>
<td>Exponential decay of H2O2 in KPO4 buffer</td>
<td></td>
<td></td>
</tr>
<tr>
<td>VEGF-C</td>
<td>RDS, BPD infants</td>
<td>ELISA</td>
<td>Reduced Foxp3+ cells found in immunosuppressed transplant patients</td>
<td></td>
</tr>
<tr>
<td>Noncellular Constituents</td>
<td>Clinical Disease</td>
<td>Detection Technique</td>
<td>Isolation Method</td>
<td>General Information</td>
</tr>
<tr>
<td>-------------------------</td>
<td>-----------------</td>
<td>---------------------</td>
<td>-----------------</td>
<td>---------------------</td>
</tr>
<tr>
<td>VEGF receptor-3, VEGF</td>
<td>Asthma</td>
<td>LPS assay</td>
<td>Commercial multiple tissue RNA preparation</td>
<td>In situ pulmonary concentration of VEGF-C estimated by using secretory IgA in tracheal aspirate fluid versus human colostrum reference standard</td>
</tr>
<tr>
<td>Gene expression for TLR-2, TLR-3, TLR-4, CCR5, CCR6, OCK-1, neuropeptides, TAC1, TAC3, GPR57, NGF substance P, TLR-2, TLR-4, TLR-7, TLR-8, TLR-9, CD11c</td>
<td>Healthy children (with bacterial colonization), bronchiolitis</td>
<td>Duplex real-time PCR</td>
<td>TLR-2, TLR-4, and LPS-stimulated neutrophils used as calibration control sample</td>
<td></td>
</tr>
<tr>
<td>Eosinophil cationic protein</td>
<td>Persistent wheeze, asthma</td>
<td>ELISA</td>
<td>Fluoro-immunoassay</td>
<td>Glutathione is an antioxidant found in airway cells</td>
</tr>
<tr>
<td>Serine protease Grb2</td>
<td>RSV, Asthma</td>
<td>Immunoassay</td>
<td>HPLC</td>
<td>Glutathione supplementation inhibited apoptosis and rescued phagocytosis of airway cells</td>
</tr>
<tr>
<td>Glutathione, glutathione disulfide, MDA (lipid/DNA oxidation)</td>
<td>Asthma</td>
<td>Colormetric assays</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chitinase activity, YKL-40</td>
<td>Asthma</td>
<td>Immunostaining</td>
<td>Incubation of BAL fluid with 4-methylumbelliferyl-D-N,N-9-diacetylchitobioside</td>
<td>Chitinases break down chitin within cell walls of fungi; there may be a role for chitinases in asthma pathogenesis</td>
</tr>
<tr>
<td>Carbonylated proteins</td>
<td>CF</td>
<td>Dot blot colorimetric assay</td>
<td>Protease inhibitors added to cell-free supernatant</td>
<td>A markeroxidation of pulmonary ELF proteins</td>
</tr>
<tr>
<td>Carbonylated proteins (ie, albumin, IgG heavy chain, transferrin, hemopexin, complement C5, superoxide dismutase, transthyretin, IgA s-chain, IgA heavy chain, ceruloplasmin and haptoglobin)</td>
<td>Sarcoidosis, IPF, and SSc</td>
<td>2-DE PAGE, Shotgun proteomics, SELDI-TOF, LC-MS/MS analysis, DIGE, cation exchange chromatography</td>
<td>Isolated from BAL fluid supernatant after first centrifugation</td>
<td>To further identify carbonylated proteins, high-power 2-DE combined with Western blot technique should be used</td>
</tr>
<tr>
<td>ApoA1, and S100 calcium binding protein A8 and A9</td>
<td>Bronchial lung endotoxin instillation and ARDS</td>
<td>SELDI-TOF spectrometry</td>
<td>Identification of proteins by peptide mass fingerprinting of trypsin-digested fragments</td>
<td>Chromatographic chips used to avoid unintended &quot;preselection&quot; of proteins</td>
</tr>
<tr>
<td>Calgranulin A</td>
<td>CF</td>
<td>ELISA</td>
<td>2-DE analysis and immunoblot analysis of BAL protein composition revealed different profiles in these diseases and that patients with IPF had a greater number of protein targets of oxidation in BAL compared with patients with sarcoidosis or SSc and controls</td>
<td></td>
</tr>
<tr>
<td>IgG and IgA, plasma proteins, calgranulin, antioxidant peroxysomal enzyme, thioredoxin peroxidase 2, and proteins with low molecular masses (&lt;35 kDa) and acidic isoelectric points (4 &lt; pI &lt; 7) such as cyclophilin A, calgranulin B, TCTP, and MIF</td>
<td>IPE sarcoidosis, HP, and SSc</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
TABLE 8 Continued

<table>
<thead>
<tr>
<th>Noncellular Constituents</th>
<th>Clinical Disease</th>
<th>Detection Technique</th>
<th>Isolation Method</th>
<th>General Information</th>
</tr>
</thead>
<tbody>
<tr>
<td>lgJ, chain, α-1-acid glycoprotein[^205]</td>
<td>Healthy adults</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Membranes, nuclear, cytosolic, extracellular, and secreted proteins, as well as protein from cytoskeleton, serum, and intracellular compartments (ie, fibrinogen α chain, α2-HS-glycoprotein, ceruloplasmin, α1-antitrypsin, antithrombin, collagenogenes A and B, IGFBP-3, and proteases)</td>
<td>ARDS[^207]</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Opsonins, antioxidants, basement membrane proteins, coagulation proteins, and serum acute-phase reactants (ie, S100A8, S100A9, C3, C4, C5, cryatatin S, transthyratin, hemoglobin, PRDX2, FGA, FTL, annexin 1, SM1, etc[^207])</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

[^205]: were detected in IPF, suggesting different pathogenesis of these diseases
[^206]: A limitation to the 2-DE proteomics approach is that it is not easy to quantify all changes in relative protein expression among a large number of samples.
[^207]: Shotgun proteomics can detect proteins that are limited by 2-DE. 2-DE cannot detect low-abundance proteins and hydrophobic proteins; there is a large intersubject variability in relative protein intensity.
[^208]: LC-MS/MS analysis is an excellent screening tool to characterize sample containing unknown protein composition. Extra steps including isolation of subpopulations of proteins should be done to increase the yield of proteins using LC-MS/MS proteomic screen.
[^209]: Gilon exchange chromatography of tryptic-digested samples followed by MS/MS analysis is limited by overlap in the proteins, but it has the advantage of high throughput.
[^210]: DIGE allows profiling of protein expression in BAL fluid samples at the onset and during the course of acute lung injury.
[^211]: Computational analysis and bioinformatics can be applied to map complex protein interactions during the course of ARDS, in the study, several proteins found in low concentrations in BAL fluid (eg, cytokines, intracellular signaling proteins, and transcription factors) were added.

[^205]: were detected in IPF, suggesting different pathogenesis of these diseases.
[^206]: A limitation to the 2-DE proteomics approach is that it is not easy to quantify all changes in relative protein expression among a large number of samples.
[^207]: Shotgun proteomics can detect proteins that are limited by 2-DE.
[^208]: LC-MS/MS analysis is an excellent screening tool to characterize sample containing unknown protein composition. Extra steps including isolation of subpopulations of proteins should be done to increase the yield of proteins using LC-MS/MS proteomic screen.
[^209]: Gilon exchange chromatography of tryptic-digested samples followed by MS/MS analysis is limited by overlap in the proteins, but it has the advantage of high throughput.
[^210]: DIGE allows profiling of protein expression in BAL fluid samples at the onset and during the course of acute lung injury.
[^211]: Computational analysis and bioinformatics can be applied to map complex protein interactions during the course of ARDS, in the study, several proteins found in low concentrations in BAL fluid (eg, cytokines, intracellular signaling proteins, and transcription factors) were added.

[^205]: were detected in IPF, suggesting different pathogenesis of these diseases.
[^206]: A limitation to the 2-DE proteomics approach is that it is not easy to quantify all changes in relative protein expression among a large number of samples.
[^207]: Shotgun proteomics can detect proteins that are limited by 2-DE.
[^208]: LC-MS/MS analysis is an excellent screening tool to characterize sample containing unknown protein composition. Extra steps including isolation of subpopulations of proteins should be done to increase the yield of proteins using LC-MS/MS proteomic screen.
[^209]: Gilon exchange chromatography of tryptic-digested samples followed by MS/MS analysis is limited by overlap in the proteins, but it has the advantage of high throughput.
[^210]: DIGE allows profiling of protein expression in BAL fluid samples at the onset and during the course of acute lung injury.
[^211]: Computational analysis and bioinformatics can be applied to map complex protein interactions during the course of ARDS, in the study, several proteins found in low concentrations in BAL fluid (eg, cytokines, intracellular signaling proteins, and transcription factors) were added.
study of procured BAL fluid continues to be a fertile ground for pediatric translational research. Indeed, newer quantitative analytic techniques have been used to investigate cellular and noncellular components, thereby improving our ability to identify early markers of susceptibility to respiratory disease, monitor and predict disease progression, and understand pulmonary disease pathogenesis and outcomes. Although gaps still exist in BAL practices, the development of disease- or technique-specific guidelines would significantly enhance homogeneity and allow more accurate comparisons across different studies.

ACKNOWLEDGMENT

Dr Fraser is Director of the Translational Research Centre (http://www.translational-research.ca; London, ON, Canada).
REFERENCES

47. de Torre C, Ying SX, Munson PJ, Meduri GU, Sufredini AF. Proteomic analysis of inflammatory biomarkers in bronchoalveolar lavage. Proteomics. 2006;6(13):3849–3857
64. Prakash UB. Does the bronchoscope propagate infection? Chest. 1995;104(2):552–559
111. de Blic J, McKelvie P, Le Bourgeois M, Blanche S, Benoist MR, Scheinmann P. Value of bronchoalveolar lavage in the...


114. Travis WD, Colby TV, Koss MN, Rosado-de-Christenson ML, Muller NL, King TEJ. Handling and analysis of bronchoalveolar lavage and lung biopsy specimens with approach to patterns of lung injury. ARP Atlases. 2007:117–47


145. Kotecha S, Mildner RJ, Prince LR, et al. The role of neutrophil apoptosis in the


188. Yerkovich ST, Chang AB, Carroll ML, Petsky HL, Srinivasa S, Upham JW. Soluble receptor for advanced glycation end products (sRAGE) is present at high concentrations in the lungs of children and varies with age and the pattern of lung inflammation. *Respirology.* 2012;17(5):841–846


AN UNTIMELY DEATH: I was recently in Washington, DC, and stayed in a hotel close to the White House. I enjoyed walking around the National Mall, visiting the museums, and viewing the monumental architecture. The area around the White House was packed with stores, restaurants, and office buildings. It was a bit odd to realize, however, that I was walking on the remains of what was once an old sewage dump. Human waste was actually dumped into an area north of the White House where it often formed a marsh. In fact, the drinking water supply of the White House was only a few blocks from this dumping ground. Not many would care about that, but as an infectious disease physician I have enormous respect for the infectious diseases that may arise from improper disposal of human waste.

As reported in The New York Times (Science: March 31, 2014), President William Henry Harrison may have died of Salmonella acquired from these very sewage fields. President Harrison is best known for being the shortest-serving President, dying after only one month in office. The most commonly accepted reason for his death is that he died of pneumonia acquired while giving an interminable inaugural address in freezing weather. That theory is, however, under attack. For one, President Harrison had few signs and symptoms of pneumonia. An alternative theory is that he died of typhoid fever. Presidents Harrison, Polk, and Taylor all developed severe gastrointestinal disease while living in the White House, but Harrison may have been prone to severe disease as he took alkali for dyspepsia — which increases the risk of gastrointestinal infection. Moreover, his physician repeatedly treated his illness with enemas — which in the setting of invasive Salmonella infection would increase the risk of perforation and sepsis. Before his death, President Harrison had a thready pulse and cold extremities, both of which are features of sepsis. I am not entirely sure why President Harrison died, but I do know that the sewage system of Washington, DC, is just as important as the heroic above-ground architecture.

Noted by WVR, MD