Translational Research in Pediatrics III: Bronchoalveolar Lavage

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KEY WORDS

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

ABBREVIATIONS

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

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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 pathophysiological 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. *Pediatrics* 2014;134:135–154

There is a lack of literature addressing the performance of bronchoalveolar lavage (BAL) in children for research purposes. Published reviews focus on adult populations[1,2] or on the technical and procedural aspects of performing clinically indicated BAL.[3–5] In this review, our third in a series on tissue sampling and biobanking for child health studies,[6,7] we aim to expand on the 2000 European Respiratory Society Task Force for BAL in children,[8] 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 flexible bronchoscopy (FB) is a skilled procedure that requires special training to achieve proficiency and ensure patient safety.[4] FB can be performed with variable methods as outlined in Table 1. Pediatric FB and BAL are generally considered safe and well tolerated[9–12]; 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.[3] Relative contraindications to FB or BAL include massive hemoptysis, bleeding diathesis, severe airway obstruction, foreign body removal,[12–14] severe hypoxia, and unstable hemodynamic status.[4]

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.[15] 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.[16]

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)[17–25] or in those whom sedation and intubation is clinically indicated for other reasons (eg, elective abdominal surgery).[26,27] 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.[28] Reference data for BAL cellular constituents in children have been published,[29–36] but because of wide variability across studies, reference values for BAL cellular and noncellular

TABLE 1 Pediatric FB Techniques

Point of Entry	Benefits	Pitfalls	Sedation/Anesthesia
Nose[4,105] (directly or via oxygen face mask with port for bronchoscope)	Allows best inspection of entire airway[39] Allows larger bronchoscope size[39] Allows best visualization of dynamic airway motion[39]	Lack of full control of airway[39]	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[39]
Mouth (laryngeal mask airway)[39,106]	Allows inspection of larynx and upper trachea[39] Allows largest bronchoscope size[39]	Lack of full control of airway[39] Less ideal for visualization of dynamic airway motion due to distortion of upper airway dynamics	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[39]
Mouth (ETT)[39]	Best airway protection[39]; may be required for transbronchial biopsy[39]	Size of bronchoscope limited by internal diameter of ETT[4] Visualization only of distal trachea and bronchial tree Difficult to maintain spontaneous ventilation to assess dynamic airways movement	GA required for deep sedation

ETT, endotracheal tube; GA, general anesthesia.

TABLE 2 Clinical Indications for Pediatric FB

Anatomic Evaluation	BAL	Airway Clearance	Biopsy	Other
Dynamic airway collapse (eg, bronchomalacia)	Microbiologic identification (eg, fungal/bacterial stains, culture, viral studies, PCR)	Foreign body assessment (removal should be performed by rigid bronchoscope)[13]	Endobronchial biopsy	pH of lower airways using pH probe[108]
External compression (eg, vascular ring, cardiac chambers)	Cell count and differential	Removal of mucus plugs	Transbronchial biopsy[39]	Ion transport properties of respiratory epithelium[109]
Tracheoesophageal fistula	Subpopulations of lymphocytes (eg, $CD4^+$:$CD8^+$ ratio)	Direct instillation of mucolytics (eg, dornase α, fibrinolytics)[107]	Bronchial brushings	
Endobronchial lesions (eg, tumor, hemangioma)	Noncellular components (eg, surfactant, lipid-laden macrophages, or hemosiderin)			
Bronchial webs/stenosis	Whole lung lavage			
Anatomic variants (eg, tracheal bronchus)				
Source of hemoptysis				

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

components should be established locally.[28]

BAL PROCEDURE

Performing BAL involves passing a flexible bronchoscope distally into an airway until the tip becomes wedged and cannot move any farther.[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.[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,[3,38,39] 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.[40] Distal sites may be better represented by a higher number of sequential aliquots taken from a particular wedge location.[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.[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.[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.[1] The concentrations of urea[18,44–46] and albumin[47,48] have been used to estimate the

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

Aliquot Size	Patient Size Adjustment
2–4 fractions of 10–20 mL[37,110]	N/A
5- to 20-mL fractions[111]	Adjusted by FRC
5 mL for infants[4]	N/A
10 mL for small child[4]	
15 mL for large child[4]	
3 mL/kg[3,30,112]	Divided into 3 aliquots for children <20 kg
	Divided into 20-mL aliquots for children >20 kg

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,[49] 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 number of specimens, the volume of each specimen, the percentage of BAL 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.[8] 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.[50–52] 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 4 Suggested Processing and Storage Practices for Samples From BAL Fluid

Practice	BAL	
	Culture, Noncellular Components	Cellular Components (Macrophages, Lymphocytes, Neutrophils, Eosinophils, etc)
Transport to the laboratory	BAL fluid samples must be fresh and should be transported on ice[8,113,114] Samples can be transported at RT if processing will occur in <60 min[114,115]	Same as noncellular components
Sample pooling step	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[8]; the first sample can be used for culture (microbiology)[8]	Same as noncellular components
	After first lavage, subsequent lavages are higher in cell count, which remains consistent throughout subsequent lavages[59,60]; lavages should be pooled to increase yield of material	Same as noncellular components
Filtration step	Before the evaluation of noncellular components, BAL fluid should be filtered[8] Microbiologic studies (cultures) should be performed on unfiltered BAL fluid because organisms may be trapped in the mucus[8]	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[8,115]
Time to do total cell counting		Immediately after collection[113]
Time to centrifugation	Samples must be fresh and processed immediately after collection[62,113]	Same as noncellular components When delay in cellular analysis is expected, the BAL sample should be centrifuged at 200–300 *g* for 10 min, and pellet should be resuspended in nutrient-supplemented media and stored at 4°C for up 12 h[54]
Centrifugation step	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)[8]	Same as noncellular components
Time to freeze	Immediately after centrifugation[113]	Cells can remain viable in BAL fluid at 25°C for up to 4 h[114,115] or at 4°C for up to 24 h[54]
Storage	−70°C is recommended to maximize storage duration without changes in sample quality[8,113,116]	Pelleted cells can be resuspended in nutrient-supplemented media and stored at 4°C for up 12 h[54]
Freeze-thaw	Freeze-thaw should be limited to only 1 cycle to ensure sample integrity[8]	Same as noncellular components

RT, room temperature (21°C).

TABLE 5 Suggested Processing and Storage Practices for Surfactant Studies

Practice	Processing and/or Storage
Transport to the laboratory	BAL fluid should be transported on ice[113]
Time to centrifugation	Whether fresh or frozen, BAL supernatant from first centrifugation should be used for the isolation of surfactants[8]
	High-speed centrifugation immediately upon sample retrieval before freezing is recommended to maintain maximal consistency among large aggregate samples, particularly when functional activity is assessed
Centrifugation step	After initial centrifugation to pellet cells, functional large aggregate forms of surfactant should be collected through high-speed centrifugation of the previous supernatant (40 000–48 000 *g*, 4°C, 60 min)[117,118]
	Small aggregate forms should be collected from remaining supernatant after high-speed centrifugation[117,118]
Time to freeze	Immediately after high-speed centrifugation[119]
Storage	−70°C is recommended to maximize storage duration without changes in sample quality[8,113,116]
Freeze-thaw	Freeze-thaw should be limited to only 1 cycle to ensure sample integrity[8]

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°C[55] to −80°C[8,56–58] 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[8]; 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 cytospin preparations [Diff-Quick staining; Merz & Dade AG, Dudingin, Germany]) by using manual counts on simple smears or through automated counting techniques using a flow cytometer.[29,69,70] 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

Cell Type	Processing Tips, Detection Methods, and/or General Information
Macrophages	80% to 90% of the cells recovered from BAL from normal individuals are macrophages[68]
	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 pneumonitis, cytoplasmic inclusions associated with viral infection, ingested RBCs and RBC fragments and hemosiderin with DAH, ingested asbestos bodies, or other dust particles[68]
	Esterase staining distinguishes immature macrophages from lymphocytes[120]
	BAL macrophages may exhibit the same light scatter profile as lymphocytes, promoting errors in lymphocyte counts[121]
	Macrophages can be further characterized through flow cytometric techniques by using monoclonal antibodies[122]
	In DAH, alveolar macrophages will stain for iron (hemosiderin) if the onset of hemorrhage has preceded the time of BAL by 24–48 h[68,123]
	A high-lipid-laden macrophage index[124] may indicate chronic aspiration of oral or gastric contents[125–127]
	Immunostaining is used to assess for phagocytosis or apoptosis in asthma[18]
	KP-1 stains macrophages, which sometimes can be confused with epithelial cells[114]
Lymphocytes (eg, CD3, CD4, CD8)	5% to 15% of the cells recovered from BAL of normal individuals are lymphocytes[68]; the subsets of T lymphocytes in the normal adult lung are 75% of $CD3^+$, 45% of $CD4^+$, 25% of $CD8^+$, and <5% for B cells[121]; total T- and B-cell counts are similar in children and adults[128]
	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[128]
	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[129]
	A high percentage of lymphocytes (>50%) suggests HP or cellular NSIP, whereas a value >25% suggests granulomatous lung diseases (sarcoidosis, HP), NSIP, berylliosis, drug reaction, COP, LIP, or lymphoma[130]
	Although sarcoidosis involves predominantly $CD4^+$ T cells, HP involves typically lymphocytic alveolitis with a predominance of $CD8^+$ T cells[131]
	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[121]
	Lymphocytes can be assessed by using immunofluorescence-labeled monoclonal antibodies and flow cytometry for counting and assessment of polyclonality[20,115,132]
	Lymphocyte phenotype can be further characterized through flow cytometric techniques by using monoclonal antibodies[122]
	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[121]
	Cytocentrifugation 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[115,133]
	Cytocentrifugation (Cytospin) can underestimate the proportion of lymphocytes by ~45% compared with a smear of resuspended cells under a glass coverslip[8]
	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[121]
	Specific T-cell subset populations can be isolated by rosetting with neuraminidase-treated sheep erythrocytes followed by Ficoll-Hypaque gradient centrifugation[134]
Neutrophils	Less than 3% of the cells recovered from BAL from normal individuals are neutrophils[68]
	The percentage of neutrophils is higher in BAL fluid from children <12 mo than children aged 13–36 mo[31]
	A high percentage of neutrophils (>50%) strongly suggests pneumonia,[126] aspiration pneumonia, lung abscess, or acute lung injury[130]
	Increased neutrophils in BAL from patients with sarcoidosis has been associated with more progressive disease that is less likely to respond to immunosuppressive therapy[135]
	Increases in BAL neutrophils have been correlated with disease severity and prognosis for both HP[136,137] and IPF[138,139]
	ARDS is associated with lung neutrophil infiltration and elevated cytokines/chemokines[56]
	Elevated neutrophil levels are seen in CF,[140–142] asthma, PCD, PBB,[143] bronchiectasis, measles, and bronchiolitis obliterans[144] and in patients with tracheotomy[104]
	Neutrophil apopotosis has been studied in children with RDS/ECMO by using Giemsa staining of cytospin preparations[145]
	Filtration, as a method to obtain differential cell counts, should be avoided for neutrophils due to filter preparations that can underestimate cell number[115]
Eosinophils	Less than 1% of cells recovered by BAL from normal individuals are eosinophils[68]
	A high percentage of eosinophils (>25%) suggests eosinophilic lung disease,[130] especially EP if the presentation is acute[146]
	BAL eosinophilia has been linked to more severe disease and worse prognosis in IPF[147,148]
Granulocytes	Elevated during CF due to inflammatory reaction[142]
RBCs	RBC proportion is used to evaluate blood contamination in BAL fluid,[149] which is common
	If DAH is present, RBCs should be identifiable on the cytospin[68]
	RBC contamination can be removed by using lysis reagents including ammonium chloride, commercial lysing reagents, or mild hypotonic lysis solution[121]
	The use of lysing reagents to remove RBC contamination could lead to the release of cellular debris and interfere with lymphocyte gating purity[121]

TABLE 6 Continued

Cell Type	Processing Tips, Detection Methods, and/or General Information
Mast cells	Increased numbers of mast cells have been associated with HP, drug reactions, sarcoidosis, ILD associated with collagen vascular disease, IPF, COP, EP, and malignancy[68]
Squamous epithelial cells	Squamous epithelial cells suggest that the BAL fluid has been contaminated by oropharyngeal secretions, which may reflect operator inexperience in BAL or aspirated upper airway secretions[68]
Langerhans cells	Langerhans cells can be stained with S-100 protein and CD1a antibodies for the diagnosis of Langerhans cells histiocytosis; these 2 antibodies work well in formalin-fixed, paraffin-embedded sections[114]

ARDS, acute respiratory distress syndrome; CD, cluster of differentiation; CF, cystic fibrosis; COP, cryptogenic organizing pneumonia (distinctly adult disease); DAH, diffuse alveolar hemorrhage; ECMO, extracorporeal membrane oxygenation; EP, eosinophilic pneumonia; HP, hypersensitivity pneumonitis; ILD, interstitial lung disease; IPF, idiopathic pulmonary fibrosis (distinctly adult disease); LIP, lymphoid interstitial pneumonia; NSIP, nonspecific interstitial pneumonia (distinctly adult disease); PBB, persistent bacterial bronchitis; PCD, primary ciliary dyskinesia; RBC, red blood cell; RDS, respiratory distress syndrome; RPMI, Roswell Park Memorial Institute.

clinical or research purposes.[8] 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,[54] although neutrophil apoptosis with engulfment by alveolar macrophages can commence before 24 hours, and thus samples should be analyzed with minimum delay.[71,72]

NUCLEOTIDE ANALYSIS

Cellular gene expression studies from BAL samples in pediatric respiratory disease states have also been reported (ie, cytokine mRNA).[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,[73,74] mycobacteria,[75,76] fungi,[77–83] *Chlamydia*,[57] mycoplasma,[57] and viruses[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.[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[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.[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[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,[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,[96] or reverse-phase chromatography,[97] in addition to removal of ubiquitous proteins such as albumin.[98] Techniques such as affinity purification can be used to minimize the dynamic range and enrich the specific protein of interest.[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.[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

TABLE 7 Nucleotides Isolated From BAL Fluid

Nucleotide	Detection Technique	Infectious Agent	Source of Nucleotide	Clinical Diseases
RNA	RT-PCR and Taqman primers for either serotype A or B[87]	RSV	BAL neutrophils	Severe bronchiolitis[87]
	RT (Superscript II; Invitrogen, Grand Island, NY) is performed with random hexamers[86]	HRV and HEV	BAL fluid, cell-free	Pneumonia and pericarditis[86]
DNA	PCR technique is used to amplify DNA[78,84,85]	*Aspergillus* species[78]	Second aliquot of BAL fluid[78] or BAL fluid,[85] cell-free	IPA[78]
	DNA amplification and hybridization is a sensitive method to detect bacteria[73]	HSV, CMV[85]	BAL fluid, cell-free	Lung transplantation[85]
	DNA is isolated with MagNa Pure LC DNA isolation kit II (Roche Diagnostics, Basel, Switzerland) and amplified by PCR using a bacterial broad-range 16S rDNA primer set[74]	HPV[84]	BAL fluid pellets after first centrifugation,[74] cellular	Asymptomatic immunocompetent children[84]
	Genomic DNA can be isolated by using a QIAMP DNA Blood mini kit (Qiagen, Venlo, The Netherlands) followed by PCR[57]	*Legionellae* species[73]	BAL fluid, first centrifugation, cell-free	Atypical pneumonia[73]
	LightCycler PCR detects virus DNA with high sensitivity[85]	Tropheryma whipplei[74]	BAL fluid, cell-free	Pneumonia[74]
	Total DNA content is measured with microtiter plates (Immulon 2 flat-bottom plates; Dynatech Laboratories, Chantilly, VA) and a fluorometer plate reader[150]	*Chlamydia* and *Mycoplasma*	From BAL fluid: supernatant (filtered)	Refractory asthma[57]
	One single and 2 nested PCR reactions are used to identify fungus; the 2 nested PCRs using primers that target ITS and mtLSU rRNA are more sensitive than a single assay[80]	CMV and HSV	BAL fluid, cell-free	Transplant recipients[85]
	A new single-round and nested PCR assay detects DNA with 2–3 orders of magnitude more than previous conventional PCR[79]	Neutrophil content	BAL fluid, cell-free	CF[150]
	Competitive PCR from BAL fluid is more sensitive for detecting low burdens of hyphae compared with routine culture[78,88,89]	PJP (previously classified as PCP)	Second aliquot of BAL fluid, first centrifugation,[78] cell-free	Immunocompromised patients[80]
	DNA amplification by PCR is used for early detection of mycobacteria in children[76]	PJP	BAL fluid, cell-free	HIV and non-HIV immunocompromised patients[79]
		Aspergillus species		IPA[78]
		Mycobacterium tuberculosis		Tuberculosis[76]
Purines (ATP, ADP, AMP, adenosine)[23]	Luminometry[23]	N/A	BAL fluid	CF

ADP, adenosine diphosphate; AMP, adenosine monophosphate; ATP, adenosine triphosphate; CF, cystic fibrosis; CLD, chronic lung disease; CMV, cytomegalovirus; HEV, enterovirus; HPV, human papillomavirus; HRV, human rhinoviruses; HSV, herpes simplex virus; IPA, invasive pulmonary aspergillosis; ITS, internal transcribed spacer region; mtLSU rRNA, mitochondrial large subunit ribosomal RNA locus; PCR, polymerase chain reaction; PCP, Pneumocystis pneumonia; PJP, *Pneumocystis* jirovecii pneumonia; RSV, respiratory syncytial virus; RT-PCR, reverse transcription polymerase chain reaction.

equilibrium buoyant density gradient centrifugation.[100] 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.[100] The evaluation of surfactant function can be performed through measurements of surface tension by using surfactometers or quantitative Brewster angle microscopy.[101–104] 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 8 Protein Isolation From BAL Fluid

Noncellular Constituents	Clinical Disease	Detection Technique	Isolation Method	General Information
Albumin (produced only outside the lungs)	Healthy Children[31] Fibrosing alveolitis[151,152] ILD[153] CF[46]	Nephelometric method Radial immunodiffusion in agar[151,152] Isotope counting[153] Immunoblotting[46]	Isolated from supernatant after first centrifugation Cell-free	At least 66% of the proteins in BAL fluid are serum derived[151] An increase in immunoglobulins and albumin is detected in these diseases[151–153] Specific antibodies in BAL can indicate infection
Immunoglobulins (synthesized both within and outside the lungs)	Fibrosing alveolitis, IPF, HP, pulmonary fibrosis[152] *Cryptococcus* infection[154] Fungal infection	Radial immunodiffusion in agar Radioimmunoabsorbent method ELISA Immunoblot assay		
Osteoporin (alveolar epithelium),[155] PEDF (fibrotic interstitium and epithelium)[156]	IPF	ELISA Microarray analysis IHC Western blot analysis	Isolated from cell pellet derived from BAL fluid after first centrifugation step Cellular	
Pepsin	GER CF	ELISA Proteolytic enzyme assay		BAL pepsin indicates GER and aspiration of contents[26,127,157]
Serpin B1	CF CLD of prematurity[158]	Immunoblot Western blot		Innate inhibitor of serine proteases such as elastase in CF airways[46]
RSV F, G, and N proteins[87]	Bronchiolitis	IF IHC	Proteins are isolated from the surface of neutrophils from BAL fluid[87] Cellular	
NE,[159,160] myeloperoxidase,[19,125,161] endotoxin[162]	CF Pulmonary infection CLD of prematurity GER Bronchiolitis[161]	NE-specific substrate assay,[159] nitroanilide colorimetric assay,[160] kinetic conversion assay[158] ELISA[26, 46,125] RIA kit[163] Immune-beads,[21] FACS[161] Limulus amebocyte lysate assay[162]	Isolated from BAL fluid supernatant after first centrifugation Cell-free Transport of frozen samples does not affect level of oxidized proteins[163]	Neutrophil elastase and myeloperoxidase in BAL are indicators of pulmonary inflammation in several disease processes
PDGF[164]	Bronchiolitis obliterans	Radio-ligand binding assay Western blot analysis, heparin affinity, gel filtration, IHC		
Mannose-binding lectin[160]	Pulmonary infections	ELISA		
BAFF APRIL[165]	RSV	ELISA		
GM-ab[166]	Pulmonary alveolar proteinosis	ELISA		
IL-6 receptor single nucleotide polymorphism[167]	Asthma	IHC	BAL cells air-dried, fixed, and stored at −20°C	
TGF-β[168]	Asthma	RT-PCR		
PPAR-γ, paraoxonase 2[169]	CF	qPCR	RNA extracted from cell pellets	
MCP-1, MCP-1 mRNA expression[170]	ILD	Multiplex, particle-based commercial assay qRT-PCR[170]	mRNA extracted from lysed BAL fluid cells	
MMP-8[171]	Preterm infants of mothers with chorioamnionitis	ELISA		Partial or complete courses of antenatal corticosteroids had no effect on MMP-8 concentrations
Lipoxin A, Clara cell protein 10[172]	CF	ELISA One-dimensional electrophoresis on 10% bis-Tris NuPAGE gel		
sICAM-1, MMP-9, TIMP-1[22,158] AAT[158]	Persistent wheeze CLD of prematurity[158]	RIA[22] Western blot ELISA[158]		Measurement of protease-antiprotease balance was reflected by molar ratio of TIMP-1 to MMP-9[22]
LTB_4, LTC_4, PGD_2, PGE_2, HETE, β-tryptase[173,174]	Wheezing Asthma[174]	EIA Sandwich EIA RIA[174]		

TABLE 8 Continued

Noncellular Constituents	Clinical Disease	Detection Technique	Isolation Method	General Information
HA[175] TNF-α, IL-6[180]	Healthy adults and lung cancer Lung transplant	RIA	Isolated from BAL supernatant after first centrifugation Cell-free	ELISA is the most frequently used method for detecting various protein elements in BAL[31,176–178] In some cases, radiolabeled[175] and dye-based immunoassays are used[176,177]
Matrix components (elastin, collagen glycosaminoglycans)[176] and intracellular cytokines (TNF, IL-1β, IL-8, IL-6, IL-1ra)[177]	CF	Dye binding assay kits[176] IHC[177]		
Intracellular cytokines (IFN-γ, IL-2, IL-4, IL-5, IL-10)[181]	Asthma	Flow cytometry after stimulation with PMA and ionomycin	Anti-CD4 added to prevent PMA or ionomycin-caused reduction in surface expression of CD4	
IL-2, IFN-γ CCR2$^+$, CCR4$^+$, CCR3$^+$, CCR5$^+$, CXCR3[170]	ILD	Flow cytometry		
IP-10, ITAC, Mig, TARC, MDC, IL-5 IL-4[182]	Asthma, chronic cough	ELISA, particle-based multiplex array		
IL-8[22,144]	Measles bronchiolitis obliterans[144] Persistent wheeze[22]	ELISA		
TNF-α, IL-8, nitrite[183] nitrotyrosine[184]	CF, asthma	ELISA Griess colorimetric assay[185]		
hBD-2, hBD-3[186]	Healthy adults	ELISA		Salivary components and mucin can mask detection of hBD-2 and -3
β-defensins: hBD-1, hBD-2 Cathelicidin LL-37, hCAP-18[187]	CF	Immune dot blot assay		ELISA found to be more sensitive than semiquantitative Western blots
PAI-1, sTREM-1, RAGE[188,189]	VAP	ELISA		PAI-1 was a good biomarker for discriminating VAP in ventilated patients
Pulmonary lymphocyte expression of CXCR3$^+$, CCR5$^+$, CCR4$^+$, CCR3$^+$[182]	Asthma, chronic cough	Flow cytometry		
CXC chemokines (CXCL 10, CXCL8, CCL2, CCL3, CCL5, CCL1)[190]	Infants with severe RSV bronchiolitis	ELISA		
RANTES, MCP-3, MCP-4, eotaxin 1,2[179]	Asthma	ELISA		RANTES, MCP-3, MCP-4, eotaxins regulate eosinophil trafficking into the airways of asthmatic children in a coordinated manner[179]
Fibronectin[31]; TNF-α, IL-4, IL-5, IL-18, IL-8, eotaxin[28]	Healthy children	ELISA with/without Western blot		
92 Gelatinase, TIMP-1, IL-6[191]	ARDS			
IL-4, IL-5, IL-13 and IL-18,[178] TNF-α, IL-8, MMP-9, TIMP-1[192]; eicosanoid mediators (LTB_4, thromboxane)[193]	Asthma			Requires purification with Sep pak system[193]
IL-6, sIL-6r, sgp-130,[194] TNF-α, IL-1β, IL-8, IL-1Ra, IL-10 and TNF-sR[177]; IL-1[110]; A1AT and SLPI,[159] IL-18, IL-2,[195] complement receptors (CR1, CR3)[196]	CF			Upregulation of these molecules can indicate neutrophil activation[196]; CR3 is necessary for migration and phagocytosis[196]
Serum proteins (eg, albumin, β2-microglobulin and fibrinogen), IGFBP-3 and pulmonary proteins (eg, as SP-D and Clara cell protein)	ARDS[197]			ELISA used to confirm proteins identified by proteomics[197]
Carbonylated proteins[172]	GER	Immunoblot		Protein carbonyls used as a measure of oxidative stress
Bile acids		Commercial enzymatic assay		
IL-8		ELISA		
Carbonylated proteins, 8-isoprostane, catalase, glutathione peroxidase[198]	Bronchiolitis obliterans	DNPH-based procedure Exponential decay of H_2O_2 in K-PO_4 buffer ELISA		
Foxp3$^+$[17]	Lung transplant	PCR		Reduced FOX3p$^+$ cells found in immunosuppressed transplant patients
VEGF-C	RDS, BPD infants[199]	ELISA		

TABLE 8 Continued

Noncellular Constituents	Clinical Disease	Detection Technique	Isolation Method	General Information
VEGF receptor-3,[199] VEGF[192]	Asthma	LPS assay[192]		In situ pulmonary concentration of VEGF-C estimated by using secretory IgA in tracheal aspirate fluid versus human colostrum reference standard[199]
Gene expression for TLR-2, TLR-3, TLR-4, CCR3, CCR5, CXCR1, neutrophins, TAC1, TAC3, CGRP, NGF substance P[126] TLR-2, TLR-4, TLR-7, TLR-8, TLR-9, CD11b[87]	Healthy children (with bacterial colonization), bronchiolitis[87]	Duplex real-time PCR[126]	Commercial multiple tissue RNA preparation[126] TLR-2, TLR-4, and LPS-stimulated neutrophils used as calibration control sample	
Eosinophil cationic protein[22]	Persistent wheeze, asthma	ELISA Fluoro-immunoassay[174]		
Serine protease Grb[200]	RSV	Immdunoassay		
Glutathione, glutathione disulfide, MDA (lipid/DNA oxidation)[18]	Asthma	HPLC Colormetric assays Immunostaining		Glutathione is an antioxidant found in airway cells. Glutathione supplementation inhibited apoptosis and rescued phagocytosis of airway cells[18]
Chitinase activity,YKL-40[154]	Asthma	Incubation of BAL fluid with 4-methylumbelliferyl-D-N, N′-diacetylchitobioside ELISA		Chitinases break down chitin within cell walls of fungi; there may be a role for chitinases in asthma pathogenesis[154]
Carbonylated proteins[172]	CF	Dot blot colorimetric assay	Protease inhibitors added to cell-free supernatant 1 or 2 thaw/refreeze cycles did not impact protein carbonylation	A marker of oxidation of pulmonary ELF proteins
Carbonylated proteins (ie, albumin, IgG heavy chain, transferrin, hemopexin, complement C3, superoxide dismutase, transthyretin, IgA s-chain, IgA heavy-chain, ceruloplasmin and haptoglobin)[201]	Sarcoidosis, IPF, and SSc	2-DE PAGE, Shotgun proteomics, SELDI-TOF, LC-MS/MS analysis, DIGE, cation exchange chromatography Immunoblotting ELISA	Isolated from BAL fluid supernatant after first centrifugation Cell-free	To further identify carbonylated proteins, high-power 2-DE combined with Western blot technique should be used[201] Changes in pulmonary oxidant/antioxidant balance can be detected with proteomics techniques[201] 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[201] Ceruloplasmin and haptoglobin are 2 glycoproteins with antioxidant function and which are only carbonylated in patients with IPF[201]
ApoA1, and S100 calcium binding protein A8 and A9[56]	Bronchial lung endotoxin instillation and ARDS			SELDI-TOF and 2-DE PAGE techniques are useful to detect unique/differential expression patterns of biomarker in acute lung inflammation[56] Low protein content, high salt concentration, elevated albumin and immunoglubulins can affect results[56]
Calgranulin A[92]	CF		SELDI-TOF spectrometry Identification of proteins by peptide mass finger printing of trypsin digested fragments	Chromatographic chips used to avoid unintended "preselection" of proteins Before protein identification, samples purified and submitted to PAGE
IgG and IgA, plasma proteins, calgranulin, antioxidant peroxysomal enzyme, thioredoxin peroxidase 2, and proteins with low molecular masses (<35 kDa) and acidic isolelectric points (4< pI < 7) such as cyclophilin A, calgranulin B, TCTP, and MIF	IPF, sarcoidosis, HP, and SSc[55,202–205]			2-DE analysis of BAL fluid showed quantitative differences between IPF, sarcoidosis, and HP[55,202–204], and among sarcoidosis, IPF, and SSc[205] Plasma proteins increased in sarcoidosis and SSc, and low-molecular-weight proteins

TABLE 8 Continued

Noncellular Constituents	Clinical Disease	Detection Technique	Isolation Method	General Information
				were detected in IPF, suggesting different pathogenesis of these diseases[205]
IgJ chain, α1-acid glycoprotein[206]	Healthy adults			2-DE–based proteomics method is used to show differences in proteins from BAL fluid[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[206] 2-DE cannot detect low-abundance proteins and hydrophobic proteins; there is a large intersubject variability in relative protein intensity[206]
Membranes, nuclear, cytosolic, extracellular, and secreted proteins, as well as protein from cytoskeleton, serum, and intracellular compartments (ie, fibrinogen α chain, α2-HS-glycoprotein, cerulopasmin, α1 chymotrypsin, antitrypsin inhibitor, C3a, leukotrienes, collagenases A and B, IGFBP-3, and proteases)	ARDS[197]			Shotgun proteomics can obtain a set of BAL fluid protein profiles of markers of lung injury[197] Shotgun proteomics can detect proteins that are limited by 2-DE[197] LC-MS/MS analysis is an excellent screening tool to characterize sample containing unknown protein composition[197] Extra steps including isolation of subpopulations of proteins should be done to increase the yield of proteins using LC-MS/MS proteomic screen[197] Cation exchange chromatography of trypsin-digested samples followed by MS/MS analysis is limited by overlap in the proteins, but it has the advantage of high throughput[197]
Opsonins, antioxidants, basement membrane proteins, coagulation proteins, and serum acute-phase reactants (ie, S100A8, S100A9, C3, C4, C9, cystatin S, transthyretin, hemoglobin, PRDX2, FGA, FTL, annexin 1, SAA1, etc[207]	ARDS[207]			DIGE allows profiling of protein expression in BAL fluid samples at the onset and during the course of acute lung injury 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[207]

A1AT, α1 antitrypsin; AAT, Alpha-1 antitrypsin; ApoA1, apolipoprotein A1; APRIL, A proliferation including ligand; ARDS, acute respiratory distress syndrome; BAFF, B-cell activating factor; BPD, bronchopulmonary dysplasia; C3, complement C3 precursor; C4, complement C4 precursor; C9, complement C9 precursor; CCL2, monocyte chemotactic protein 1; CCL3, macrophage inflammatory protein 1α; CCR5, chemokine (C-C motif) receptor type 5; CD11b, cluster of differentiation molecule 11B; CF, cystic fibrosis; CGRP, calcitonin gene–related peptide; CLD, chronic lung disease; CR1, C3b receptor; CR3, iC3b receptor; CXCL 10, interferon inducible protein 10; CXCL8, interleukin-8; CXCR3, chemokine (C-X-C motif) receptor 3; DIGE, difference gel electrophoresis; DNPH, 2,4-dinitrophenylhydrazin; EIA, competitive enzyme immunoassay; ELISA, enzyme-linked immunosorbent assay; FACS, ; FGA, fibrinogen a chain; FTL, ferritin light chain; GER, gastroesophageal reflux; GM-ab, immunoglobulin G–granulocyte-macrophage colony-stimulating factor antibody; Grb, granzyme B; HA, hyaluronic acid; hBD, human b defensin; human cathelicidin antimicrobial peptide (hCAP) and LL-37, human cathelicidin; HETE, 15-hydroxyeicosatetraenoic acid; HP, hypersensitivity pneumonitis; HPLC, high-performance liquid chromatography; IF, immunofluorescence; Ig, immunoglobulin; HS, Homo sapiens; IGFBP-3, insulin-like growth factor binding protein-3; IHC, immunohistochemistry; IL, interleukin; IL-1ra, interleukin-1 receptor antagonist; ILD, interstitial lung disease; IP-10, interferon-γ inducible 10 kDa protein; IPF, idiopathic pulmonary fibrosis (distinctly adult lung disease); ITAC, interferon-γ inducible cell chemoattractant; LC-MS/MS, liquid chromatography–tandem mass spectrometry; LPS, lipopolysaccharide; LTB_4, leukotriene B_4; LTC_4, leukotriene C_4; MCP-1, monocyte chemotactic protein 1; MDA, malondialdehyde; MDC, macrophage-derived chemokine; MIF, migration inhibitory factor; Mig, monokine induced by interferon-γ; MMP, matrix metalloprotein; MS/MS, tandem mass spectrometry; NE, neutrophil elastase; NGF substance P, nerve growth factor substance P; PAGE, polyacrylamide gel electrophoresis; PAI-1, plasminogen activation inhibitor; PCR, polymerase chain reaction; PDGF, platelet-derived growth factor; PEDF, pigment epithelium–derived factor; PGD_2, prostaglandin D_2; PGE_2, prostaglandin E_2; pI, isoelectrical point; PMA, phorbol 12-myristate 13-acetate; PPAR-γ, peroxisome proliferator activated receptor-γ; PRDX2, peroxiredoxin 2; qPCR, quantitative polymerase chain reaction; qRT-PCR, quantitative reverse transcription polymerase chain reaction; RAGE, receptor for advanced glycation end-products; RANTES, regulated on activation, normal T-cell expressed and secreted; RDS, respiratory distress syndrome; RIA, radioimmunoassay; RSV, respiratory syncytial virus; RT-PCR, reverse transcription polymerase chain reaction; S100A8, calgranulin A; S100A9, calgranulin B; SAA1, serum amyloid protein A; SELDI-TOF, surface-enhanced laser desorption ionization time-of-flight; sgp-130, human soluble glycoprotein of 130 kDa; sICAM-1, soluble intercellular adhesion molecule-1; sIL-6r, soluble interleukin-6 receptor; SLPI, secretory leukocyte protease inhibitor; SP-D, surfactant protein-D; SSc, pulmonary fibrosis associated with systemic sclerosis; sTREM, soluble triggering receptor expressed on myeloid cells; TAC-1/3, tachykinin precursor 1/3; TARC, thymus- and activation- regulated chemokine; TCTP, translationally controlled tumor protein; TGF-β, transcription growth factor-β; TIMP-1, tissue inhibitor of metalloproteinase 1; TLR, Toll-like receptor; TNF-sR, tumor necrosis factor–soluble receptor; TNF-α, tumor necrosis factor-α; VAP, ventilator-associated pneumonia; VEGF, vascular endothelial growth factor; YKL-40, chitinase-3-like protein 1 (CHI3L1); 2-DE, two-dimensional gel electrophoresis.

TABLE 9 SP-Phospholipid Complexes Isolated From BAL Fluid

	Clinical Disease	Isolation/Detection Method	General Information
Total phospholipid[208]	Healthy children[208]	Cell-free BAL fluid, chloroform/methanol followed by HPLC	Surfactant phospholipid concentrations are higher in children between 3 and 8 y than in older children[208]
SP-A[24,27,101,116,118,141,142,197,201,202,206–215] SP-B[24,25,101,116,118,142,197,210,211,214,215,217] SP-C[24,25,101,118,215,217,219] SP-D[116,118,142,188,209–211,215,216,218]	Healthy children[206,208] PAP[24,216] Acute inflammatory airway disease[25] Chronic bronchitis[27] Mechanical ventilation[101] ALI/ARDS[116,118,197,207,210,214] CF[27,141,142,212,213,215] CLD[217] GER[209] Acute bacterial pneumonia[214] IPF[201,202] RDS[211] VAP[188,218] Sarcoidosis[201,202,219]	Isolated from BAL supernatant after first centrifugation Immunoblot[24,25] Chemiluminescence assay[217] Agglutination assay[27] Gel chromatography[27,212] Mass spectrometry[210] ELISA[101,116,118,141,142,188,210,211,214,215,218] Western blot[24,209,210,216,217,219] Commercial protein assay kit[217]	SP-A concentrations are independent of the child's age[208] CF, pneumonia, and ARDS shift the composition of phospholipids and reduce the amount of SP-A but not SP-B[142,214] Deglycosylation of reacting proteins using recombinant N-glycosidase F[217]
Phosphatidylcholine/ phosphatidylglycerol[116,142,214,220,221] Phosphatidylinositol[116,142,214,220,221] Sphingomyelin[116,142,214,220,221] Phosphatidylserine[116,142,220] Phosphatidyl-ethanolamine[142,221]	CF[142] Acute bacterial pneumonia[214] ARDS[116,220] Thromboembolic disease[221]	Isolated from BAL supernatant after first centrifugation; a high-speed centrifugation isolation is conducted for further isolation of lipid-protein complex (from cell-free supernatant or pellet)	Phospholipid content can be determined by phosphorus assay of lipid extract of surfactant pellet after high-speed centrifugation[142] For further separation and analysis of different classes of phospholipids, HPTLC can be used[142,214] Extensive alterations in the biochemical and biophysical properties of surfactant have been described in CF, pneumonia, and ARDS[116,142,214] The lipid-protein complexes from lung lavage are not surface-active in ARDS[116,220] Lipids can also be extracted from BAL fluid by using chloroform/methanol[222]
Lecithin[116,220]	ARDS[116,220]	Isolated from BAL supernatant after first centrifugation Supernatant is used for analysis of lipids and enzymatic activities High-speed centrifugation is conducted on the first supernatant for the measurement of surface activity of lipid-protein complex	Disaturated lecithin is low, but sphingomyelin and phosphatidylserine are elevated in ARDS[116,220] Additional phospholipids present in the airways that could dilute surfactant and nonsurfactant lipids can originate from outside or within the lung (type II pneumocytes)[220]
Napsin[24]	PAP[24]	Activity assay	
Cathepsin H[24]	PAP[24]	Proteolysis by elastase, cathepsin G, or proteinase 3, then gold or silver staining plus Western blot[24,213]	
Cathepsin G[213]	CF[213]	Coupled spectrophotometric reaction[213]	
Surfactant function[104]	Chronic airway inflammation (chronic bronchitis and tracheostomy patients)	Capillary surfactometer after separation of BAL fluid to large surfactant aggregates (LA) and supernatant with inhibitory constituents	Function of LA-supernatant recombinations is poor because of protein influx during lavage procedure

ALI, acute lung injury; ARDS, acute respiratory distress syndrome; CF, cystic fibrosis; CLD, chronic lung disease; ELISA, enzyme-linked immunosorbent assay; GER, gastroesophageal reflux; HPLC, high-performance liquid chromatography; HPTLC, high-performance thin layer chromatography; IPF, idiopathic pulmonary fibrosis (distinctly adult lung disease); LA, large aggregates; PAP, pulmonary alveolar proteinosis; RDS, respiratory distress syndrome; SP, surfactant protein; VAP, ventilator-associated pneumonia.

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.

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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

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