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ERS TASK FORCE

Bronchoalveolar lavage in children

ERS Task Force on bronchoalveolar lavage in children

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CONTENTS

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

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

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

Technical aspects

Instruments

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

Sedation

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

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

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

Location of the BAL

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

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

Drug	Dose *i.v.*	Onset of effect min	Duration min
Atropine	0.01		10–20
Ketamine	0.25–0.5	2–4	10–20
Diazepam	0.2	1–3	30–120
Midazolam	0.05–0.3	1–5	90
Meperidine	1–2	5–10	180–240
Propofol	1–3	0.5	30
Fentanyl	0.001–0.006	2–3	60
Remifentanyl	0.025–0.1	2–5	2–3

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

Volume of lavage solution

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

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

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

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

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

Safety

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

Complications

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

Recommendations

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

Process of recovered BAL fluid: cellular and noncellular components

Process of the BAL fluid

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

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

Processing of the first sample

There is no consensus as to whether the first BAL sample should be processed separately or whether it is acceptable to pool all specimens. The first BAL sample has a lower cellular yield and contains more neutrophils and less lymphocytes than the following samples in both healthy children and those with lung diseases [15, 33, 34]. Subsequent samples can be pooled for cellular and noncellular studies. The first sample may be of interest in diseases with a primarily bronchial component such as asthma and bronchitis. In addition, all or part of the first sample is often used for microbiological cultures. The fluid recovery is lower in nonbronchoscopic lavage and comparative studies between formal BAL and nonbronchoscopic BAL are needed to define whether both techniques yield similar results for cellular and noncellular BAL constituents.

Cellular components

The processing of BAL fluid in children is not different to that used in adults [3]. The recovered volume should be measured and total cell counts performed. Cell viability should be assessed, *e.g.* using trypan exclusion. Differential cytology is usually performed on cell preparation after centrifugation. There is considerable variability between centres in centrifugation speed and time. In adults both factors affect the percentage of lymphocytes in cytospins prepared from BAL fluid [35, 36]. Most centres will use a centrifugation speed between 250–500 $\times g$ for 5–10 min. There are two approaches for obtaining differential cell counts. The one used more frequently is to perform cytospins of centrifuged BAL fluid after resuspension in small amounts of medium and to stain slides with May-Grünwald Giemsa, Diff-Quick staining (Merz & Dade A.G., Dudingen, Germany) or other stains used to identify microorganisms. Alternatively, simple smears of resuspended cells can be used instead of cytospins. The other possibility is to inject cells into a flow cytometre. The results of both technique yield similar results [19, 33, 37, 38, 39]. If cytospins are used, a minimum of 300–500 cells should be counted [35]. It is advisable to store one or two slides for clinical or research studies. Many laboratories consider red oil staining to detect lipid laden macrophages and periodic acid-Schiff (PAS) staining routine procedures. The presence of epithelial cells should be noted but not included in the differential cell count. The analysis of lymphocyte surface markers cannot be considered a routine procedure and is therefore not mentioned in this report.

Epithelial lining fluid

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

Reporting results

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

Noncellular components

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

Recommendations

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

Areas of future study

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

Processing of recovered BAL fluid: microbiological studies

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

Processing of BAL specimens

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

Techniques

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

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

Many infectious agents are difficult to detect with traditional methods for various reasons, including slow growth in culture [55]. Thus, immunofluorescence and molecular methods are now increasingly used [18, 46, 47, 55, 56, 63–67]. The high sensitivity of polymerase chain reaction (PCR) may lead to the occurrence of false positive results and to the detection of organisms that are not causing infection or that are not viable [55, 56]. On the other hand, PCR may provide information on antibiotic sensitivities [68].

Table 2. – Techniques for the detection of various micro-organisms

Micro-organism	Techniques
Bacteria	Gram
	Quantitative culture
Chlamydia	Immunoassay (direct)
	Culture
	Molecular methods
Fungi	Gomori-Grocott
Pneumocystis carinii	Toluidine blue
	Wright-Giemsa
	Immunoassay (direct)
	Molecular methods
Other fungi	Gomori-Grocott
	Gram
	Immunoassay (direct)
	Culture
Legionella	Gomori-Golcott
	Immunoassay (direct)
	Culture
	Molecular methods
Mycobacteria	Auramine-rhodamine
	Ziehl-Neelsen
	Culture
	Molecular methods
Mycoplasma	Immunoassay (early culture)
	Culture
	Molecular methods
Viruses	Cytopathic effects, inclusion bodies
CMV, HSV	Immunoassay (direct, early culture)
	Culture
	Electron microscopy
	Molecular methods
Other viruses	Immunoassay (direct, early culture)
	Culture

CMV: cytomegalovirus; HSV: herpes simplex virus.

Micro-organisms

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

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

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

Cytomegalovirus and herpes simplex virus. Sensitivity of viral cytopathic effects and intranuclear or intra-cytoplasmic inclusion bodies is low compared to direct antigen detection by immunofluorescence [35]. Cytological changes and immunofluorescent antibody staining lack sufficient negative predictive value for cytomegalovirus (CMV) infection; a rapid centrifugation culture combined with monoclonal antibodies for early antigen is highly sensitive and specific, and seems more useful [74]. PCR and *in situ* hybridization techniques combine early detection and high sensitivity [55, 58].

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

Recommendations

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

Areas for further study

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

Normal values: cellular and noncellular components

Cellular components

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

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

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

	Clement *et al.* [78]	Ratjen *et al.* [33]	Riedler *et al.* [19]	Midulla *et al.* [34]	Tessier *et al.* [76]
n	11	48	18	16	11
Age range	1–15	3–5	1#–10	2#–3	4–16
Sedation	LA	GA	GA	LA	LA
No aliquots	6	3	3	2	6
Volume saline	10% FRC	3 mL·kg^{-1}	3 mL·kg^{-1}	20 mL	10% FRC
BAL fluid recovered %					
mean±SD	ND	58±15	ND	43.1±12.2	69.7±9.6
median	ND	ND	62.5	42.5	68
range	ND	ND	42.5–71.5*	20–65	52–87
$\times 10^4$ cells·mL^{-1}					
mean±SD	25.5±13.6	10.3±11.1	ND	59.9±32.9	35.1±18.4
median	24	7.3	15.5	51	30.5
range	7.0–50.0	0.5–57.1	7.5–25.8*	20–130	9–68
AM %					
mean±SD	89.7±5.2	81.2±12.7	ND	86±7.8	89.9±5.5
median	89	84	91	87	92.5
range	85–97	34.6–94	84.2–94*	71–98	77–98
Lym %					
mean±SD	8.7±4.6	16.2±12.4	ND	8.7±5.8	8.9±5.6
median	10	12.5	7.5	7	8
range	1–17	2–61	4.7–12.8*	2–22	2–22
Neu %					
mean±SD	1.3±0.9	1.9±2.9	ND	5.5±4.8	1.2±1.2
median	1	0.9	1.7	3.5	1
range	0–3	0–17	0.6–3.5*	0–17	0–3
Eos %					
mean±SD	ND	0.4±0.6	ND	0.2±0.3	0
median	ND	0.2	0.2	0	0
range	ND	0–3.6	0–0.3*	0–1	0

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

performed under local anaesthesia if sarcoidosis was suspected (because of uveitis), or if endoscopy was used to evaluate stridor. This population had normal physical examination, chest radiographs, and arterial capillary blood gases. Only the last five aliquots were used for cellular analysis. Ratjen *et al.* [33] reported results from 48 children undergoing elective surgery for nonpulmonary illnesses. The study excluded children with chronic respiratory symptoms, an upper respiratory tract infection in the preceding 2 yrs, a history of hyperreactive airway disease or other atopic symptoms. The first sample was studied separately; subsequent samples were pooled for analysis. Riedler *et al.* [19] studied 18 children who fulfilled the following criteria: no history of lower respiratory tract symptoms, no upper respiratory tract infection in the previous 4 weeks, no systemic disorder, no medication at the time of the lavage, normal lung function tests, normal chest radiographs, normal physical examination of the chest, and normal bronchial mucosa on inspection. Patients were investigated for stridor. Midulla *et al.* [34] studied BAL in 16 children investigated for persistent stridor, left lobar emphysema associated with mild stenosis of the lingular bronchus, stenosis of the main left bronchus or 2 months after the removal of a foreign body. These children were considered free of parenchymal lung disease based on no visual evidence of bronchial inflammation, sterile BAL culture, normal white blood cell count, normal sedimentation rate and negative chest radiograph. Tessier *et al.* [76] reported BAL cellular components in 16 children undergoing BAL during endoscopy to evaluate stridor or suspected inhalation of a foreign body. The last five aliquots of aspirated fluid were pooled and used for cellular studies.

Whereas Tessier *et al.* [76] reported that BAL cellular analysis followed a normal distribution, Ratjen *et al.* [33], Riedler *et al.* [19] and Midulla *et al.* [34] concluded that it did not. The latter findings are supported by the study of Merchant *et al.* [79] in healthy adult volunteers.

Other discordances were observed between BAL studies in children, especially for the number of cells per millilitre, percentage of lymphocytes and neutrophils (tables 3 and 4). The differences could depend on children's age. The patients in the paper by Riedler *et al.* [19] are in the same age range as those in the studies by Tessier *et al.* [76] and Clement *et al.* [77], but the absolute number of subjects studied in a given age are small.

Despite variations in differential cell counts between these studies, the differential cytology of BALF is fairly similar to that observed in healthy adults. Independent of the child's age macrophages are the predominant cells in all studies followed by lymphocytes. The percentage of neutrophils appears to be higher in BALF from children <12 months than from children aged 13–36 months [34]. When expressed in cells per millilitre of recovered BALF, total cell counts tended to be higher in younger children [33]. The major difference between children and adults is in the CD4/CD8 ratio which has been found to be lower in children in two studies [19, 37].

Table 4. – Lymphocyte subsets in children

	RATJEN *et al.* [32]	RIEDLER *et al.* [30]
n	28	10
Age range	3–14	3–10
CD3 %		
mean	85.8	ND
median	87	81
range	72–92	75.5–88*
CD4 %		
mean	35.1	ND
median	34.5	27
range	10–57	22–32*
CD8 %		
mean	56.8	ND
median	57	45
range	30–84	33.8–57*
CD4/CD8 %		
mean	0.7	ND
median	0.6	0.6
range	0.1–1.9	0.4–1*
CD19 %		
mean	0.9	ND
median	0.5	5
range	0–7	4–9.5*
CD25 %		
mean	1.9	ND
median	2	2
range	0–4	0–3*
CD3/HLA-DR %		
mean	1.4	ND
median	1	ND
range	0–7	ND
CD56 %		
mean	7.8	ND
median	5	4

Age range is given in yrs. *: first interquartile to third interquartile. ND: not done; HLA-DR: human leukocyte anitigen-D.

Noncellular BAL components

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

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

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

Recommendations

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

Indications and results in immunocompetent children

The main indications of BAL in immunocompetent children are:

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

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

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

The diagnosis of alveolar haemorrhage is made easy when the BALF has either a bloody or orange pink colour and when the triad of haemoptysis, infiltrates on chest radiograph and anaemia is present. However, active pulmonary haemorrhage can occur without these elements, and the gross appearance of the BAL may be normal. Light microscopy examination can show the presence of free red blood cells, red blood cells in alveolar macrophages, or haemosiderin laden alveolar macrophages [88].

The diagnosis of pulmonary histiocytosis can be rapidly documented with the use of the monoclonal antibodies revealing the presence of CD1a positive cells. A presence of ≥5% of CD1a positive cells is required to confirm the diagnosis, as ≤3% of these cells can be present in healthy patients [89, 90].

In other pathological situations, BAL is not considered to be diagnostic, but may be a useful tool in the differential diagnosis. In many instances the various patterns of BAL cell profiles will help to orientate further investigations. (table 5).

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

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

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

Recommendations

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

Areas for further study

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

Indications and results in immunocompromised children

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

Interpretation of microbiological results

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

BAL in human immunodeficiency virus positive children

As survival time has increased in patients with human immunodeficiency virus (HIV), the incidence of acute pneumonia and recurrent bronchopneumonia has risen [94]. In several prospective studies of BAL in HIV-positive patients with acute interstitial pneumonitis (IP) or lower respiratory tract disease, one or more infectious

agent was identified in 55–84% of cases, with *P. carinii* being most frequently identified [47, 48, 95–99]. The sensitivity of BAL for the identification of *P. carinii* in acute IP is felt to be high. CMV is also frequently identified in BAL effluent, often in combination with a second pathogen such as *P. carinii*, but as in adults, the diagnosis of CMV pneumonia by BAL remains difficult. The clinical relevance of *Staphylococcus aureus*, *Escherichia coli*, Klebsiella, and Pseudomonas in BAL effluent from this patient population, however, is questionable as these organisms may be airway contaminants.

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

Other conditions

Children with primary immune deficiencies or immunosuppression secondary to chemotherapy for malignancy, bone marrow transplantation (BMT) or nonlung organ transplantation [100, 101] may develop pneumonitis. In studies of BAL in such patients, the rate of establishing a microbiological diagnosis ranged 28–86%, and *P. carinii* and CMV were the infectious organisms most frequently isolated [18, 46, 48, 50, 52, 102–106]. Other pathogens isolated by BAL included other viruses or other organisms such as *Mycoplasma hominis*, and *Legionella pneumophila* [58]. The wide variation in diagnostic rates reported may be due to heterogeneity of patient selection, delay between onset of lung involvement and BAL, BAL technique used, and techniques used for detection of organisms.

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

BAL in paediatric lung transplant recipients

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

Recommendations

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

Areas for further study

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

Special consideration in neonates

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

Methods and site of sampling

There are many variations of NB-BAL with few groups attempting to standardize the technique. Many do not pay any regard to positioning whilst others report a supine child with the head turned to the left to aid insertion of the catheter into the right lower lobe. It is unknown whether repeated insertion of the catheter samples the same area. Although most investigators use normal saline, at either room temperature or warmed to 37°C, the volume instilled is either based on weight [21, 114] or is fixed empirically [118]. The number of aliquots is usually one or two with the first aliquot probably reflecting bronchial milieu and the second more bronchoalveolar regions [114]. A further

variation lies with catheter size and the positioning of the distal holes being either end-hole or two side-holes. End-hole catheters are more likely to sample the areas of interest. The suction pressure used to retrieve the fluid is often not quoted, many use mechanical suction whilst others prefer to use syringes. Higher pressures will collapse the airways whereas lower pressures will reduce recovery.

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

Processing

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

Estimation of epithelial lining fluid

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

Recommendations

1) NB-BAL should be performed in a standardized manner. For generalized lung disease, the infant should be supine with the head turned to the left and inspiratory oxygen fraction (F_{I,O_2}) increased to maintain oxygen saturation >90%; 2) two aliquots 1 $mL \cdot kg^{-1}$ each of normal-saline at room temperature should be instilled with an endhole catheter of size FG 5–8 which is wedged in the right lower lobes. Fluid should be recovered with a constant suction pressure of 5–10 kPa; 3) the returned aliquots can be pooled unless central or peripheral airways are specifically being investigated; 4) the volume of fluid instilled and recovered should be recorded and reported. While filtration through gauze is considered a standard procedure in older children, it is generally unnecessary in neonates; 5) until the most appropriate method for accurately estimating ELF is determined, results should be reported as a concentration per millilitre.

Areas for further study

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

Research studies

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

Management of lung diseases in children

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

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

Markers of repair processes. The processes of repair have been poorly investigated in children, although they are a dominant element of the prognosis [127]. Persistence of lung dysfunction following acute lung injury is likely to result from an inadequately regulated healing response. Inflammation of the distal part of the lung has been shown to be associated with an alteration of the alveolar structures. The cellular components directly in contact with the inflammatory cells present in the alveolar space are the epithelial cells. Following injury, the alveolar epithelium needs to be rapidly and properly

repaired. Any delay in the re-epithelialization process may impair the healing response by allowing proliferation of fibroblasts in the interstitium and consequently the excessive accumulation of fibrotic tissue in the air spaces [128]. At the present time, little is known on the factors involved in the repair of the alveolar structure in patients with lung diseases [129].

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

Understanding lung disease pathogenesis in children

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

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

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

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

Conclusion

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

References

1. Novello A Jr, Barbato A. The use of bronchoscopy in the neonatal intensive care unit. *In*: Barbato A, Landau L, Scheinmann P, Warner JO, Zach M, eds. The Bronchoscope - Flexible and Rigid - in Children. Treviso/Italy, Arcari, 1995; pp. 55–60.
2. De Blasio F, Daughton D, Thompson A, *et al*. General *versus* local anaesthesia. *Chest* 1993; 104: 1032–1037.
3. Klech H, Pohl W and the European Society of Pneumology Task Group on BAL. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). *Eur Respir J* 1989; 2: 561–585.
4. Wood RE. Pitfalls in the use of the flexible bronchoscope in paediatric patients. *Chest* 1990; 97: 190–203.
5. Zetter LK, Jay SM, Fisher NM. The management of pain associated with paediatric procedures. *Pediatr Clin North Am* 1989; 36: 941–964.
6. Bahal-O'Mara N, Nahata MC, Murray RD, *et al*. Sedation with meperidine and midazolam in paediatric patients undergoing endoscopy. *Eur J Clin Pharmacol* 1994; 47: 319–323.
7. Chuang E, Wenowe WJ, Piccoli DA, Altschuler SM, Liacouras CA. Intravenous sedation in paediatric upper gastrointestinal endoscopy. *Gastrointest Endosc* 1995; 42: 156–160.
8. Clarkson K, Power CK, O'Connell F, Pathmakanthan S, Burke CM. A comparative evaluation of propofol and midazolam as sedative agents in fiberoptic bronchoscopy. *Chest* 1993; 104: 1029–1031.
9. Raeder JC, Misvaer G. Comparison of propofol induction with thiopentone in short outpatient general anaesthesia. *Acta Anaesthesiol Scand* 1988; 32: 607–613.
10. Burkle H, Dunbar S, Van Aken H. Remifentanil: a novel short-acting,mu-opioid. *Anesth Analg* 1996; 83: 646–651.
11. Barbato A, Magarotto M, Crivellaro M, *et al*. Use of paediatric bronchoscope, flexible and rigid, in 51 European Centres. *Eur Respir J* 1997; 10: 1761–1766.
12. Kanto J, Klotz U. Pharmacokinetic implications for the clinical use af atropine, scopolamine and glycopyrrolate. *Acta Anaesthesiol Scand* 1988; 32: 69–78.

13. Pirozynski M, Siwinski P, Polubiez M, Zieiinski J, Radwan L. Atropine influences bronchoalveolar lavage induced arterial oxygen desaturation. *Eur Respir J* 1988; 1 (Suppl. 2): 312s.
14. Amitai Y, Zylber KE, Avital A, Zangen O, Noviski N. Serum lidocaine concentrations in children during bronchoscopy with topical anesthesia. *Chest* 1990; 98: 1370–1373.
15. Pohunek P, Pokorna H, Striz I. Comparison of cell profiles in separately evaluated fractions of bronchoalveolar lavage (BAL) fluid in children. *Thorax* 1996; 51: 615–618.
16. Pingleton AK, Harrison GF, Stechschulte DJ, Wesselius LJ, Kerby GR, Ruth WE. Effect of location, pH and temperature of instillate in bronchoalveolar lavage in normal volunteers. *Am Rev Respir Dis* 1983; 128: 1035–1037.
17. Wilmott RW, Kassab JT, Kilian PL, Benjamin WR, Douglas SO, Wood RE. Increased levels of interleukin-1 washing from children with bacterial pulmonary infections. *Am Rev Resp Dis* 1990; 142: 365–368.
18. de Blic J, McKelvie P, Le Bourgeois M, Blanche S, Benoist MR, Scheinmann P. Value of bronchoalveolar lavage in the management of severe acute pneumonia and interstitial pneumonitis in the immunocompromised child. *Thorax* 1987; 42: 759–765.
19. Riedler J, Grigg J, Stone C, Tauro G, Robertson CF. Bronchoalveolar lavage cellularity in healthy children. *Am J Respir Crit Care Med* 1995; 152: 163–168.
20. Grigg J, van den Borre C, Malfroot A, Pierard D, Wang D, Dab I. Bilateral fiberoptic bronchoalveolar lavage in acute unilateral lobar pneumonia. *J Pediatr* 1993; 122: 606–608.
21. Ratjen F, Bruch J. Adjustment of bronchoalveolar lavage volume to body weight in children. *Pediatr Pulmonol* 1996; 21: 184–188.
22. Rennard SI, Basset G, Lecossier D, *et al.* Estimation of volume of epithelial lining fluid recovered by lavage using urea as marker of dilution. *J Appl Physiol* 1986; 60: 532–538.
23. Ghows M, Rosen M, Chciang M, Sacks H. Transcutaneous oxygen monitoring during fiberoptic bronchoscopy. *Chest* 1986; 89: 543–54.
24. Antonelli M, Conti G, Riccioni L, Meduri GU. Noninvasive positive pressure ventilation *via* face mask during bronchoscopy with BAL in high-risk hypoxemic patients. *Chest* 1996; 110: 724–728.
25. Spach DH, Silverstein FE, Stamm WE. Transmission of infection by gastrointestinal endoscopy and bronchoscopy. *Ann Inf Med* 1993; 118: 117–128.
26. Prakash UBS. Does the bronchoscope propagate infection? *Chest* 1993; 104: 552–559.
27. Albertini RE, Harrel JH, Kurihara N. Arterial hypoxemia induced by fiberoptic bronchoscopy. *Chest* 1974; 65: 117–118.
28. Montravers P, Ganzit R, Flombret MC, Blanchet F, Desmonts JM. Cardiopulmonary effects of bronchoalveolar lavage in critically ill patients. *Chest* 1993; 104: 1541–1547.
29. Kozak EA, Brath LK. Do "screening" coagulation tests predict bleeding in patients undergoing fiberoptic bronchoscopy with biopsy? *Chest* 1994; 106: 703–705.
30. Weiss S, Hert R, Gianola F, Clark J, Crawford S. Complications of fiberoptic bronchoscopy in thrombocytopenic patients. *Chest* 1993; 104: 1025–1108.
31. Malfroot A, Lanciers S, Ahlenajafi N, Dab I. Is bronchoalveolar lavage safe in acute airway infection in children? *Eur Respir J* 1995; 8: 521A.
32. Burns DM, Shure D, Francoz R, *et al.* The physiologic consequences of saline lobar lavages in healthy human adults. *Am Rev Respir Dis* 1983; 127: 695–701.
33. Ratjen F, Bredendiek M, Brendel M, Meltzer J, Costabel U. Differential cytology of bronchoalveolar lavage fluid in normal children. *Eur Respir J* 1994; 7: 1865–1870.
34. Midulla F, Villani A, Merolla R, Bjermer L, Sandstrom T, Ronchetti R. Bronchoalveolar lavage studies in children without parenchymal lung disease: cellular constituents and protein levels. *Pediatr Pulmonol* 1995; 20: 112–118.
35. Mordelet-Dambrine M, Arnoux A, Stanislas-Leguern G, Sandron D, Chretien J, Huchon G. Processing of lung lavage fluid causes variability in bronchoalveolar cell count. *Am Rev Respir Rev* 1984; 130: 305–306.
36. The BAL Cooperative Group Steering Committee. Bronchoalveolar lavage constituents in healthy individuals, idiopathic pulmonary fibrosis and selected comparison groups. *Am Rev Respir Dis* 1990; 141: S169–S201.
37. Ratjen F, Bredendiek M, Zheng L, Brendel M, Costabel U. Lymphocytes subsets in bronchoalveolar lavage fluid of children without bronchopulmonary disease. *Am J Respir Crit Care Med* 1995; 152: 174–178.
38. Baughman R, Strohofer S, Kim K. Variation of differential cell counts of bronchoalveolar lavage fluid. *Arch Pathol Lab Med* 1986; 110: 341–343.
39. Padovan CS, Behr J, Allmeling AM, Tilman Gerlach J, Vogelmeier C, Krombach FP. Immunophenotyping of lymphocyte subsets in bronchoalveolarlavage fluid- comparison of flow cytometric and immunocytochemical techniques. *J Immunol Methods* 1992; 147: 27–32.
40. Reynolds HY, Newball HH. Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med* 1974; 84: 559–574.
41. Marcy TW, Merrill WW, Rankin JA, Reynolds HY. Limitations of urea to quantify epithelial lining fluid by bronchoalveolar lavage. *Am Rev Respir Dis* 1987; 135: 1276–1280.
42. Ward C, Duddridge M, Fenwick J, *et al.* Evaluation of albumin as a refenrence marker of dilution in bronchoalveolar lavage fluid of asthmatic and control subjects. *Thorax* 1993; 48: 518–522.
43. Von Wichert P, Joseph K, Mueller B, Franck WM. Bronchoalveolar lavage. Quantification of intraalveolar fluid? *Am Rev Respir Dis* 1993; 147: 148–152.
44. Ward C, Duddridge M, Fenwick J, *et al.* The origin of water and urea sampled at bronchoalveolar lavage in asthmatic and control subjects. *Am Rev Respir Dis* 1992; 146: 444–447.
45. Report of the European Task Force for noncellular components of bronchoalveolarlavage: special considerations in children. *Eur Respir Rev* 1999; 66: 1–157.
46. Stokes DC, Shenep JL, Parham D, Bozeman PM, Marienchek W, Mackert PW. Role of flexible bronchoscopy in the diagnosis of pulmonary infiltrates in pediatric patients with cancer. *J Pediatr* 1989; 115: 561–567.
47. de Blic J, Blanche S, Danel C, Le Bourgeois M, Caniglia M, Scheinmann P. Bronchoalveolar lavage in HIV infected patients with interstitial pneumonitis. *Arch Dis Child* 1989; 64: 1246–1250.
48. McCubbin MM, Trigg ME, Hendricker CM, Wagener JS. Bronchoscopy with bronchoalveolar lavage in the evaluation of pulmonary complications of bone marrow transplantation in children. *Pediatr Pulmonol* 1992; 12: 43–47.
49. Abadco DL, Amaro-Galvez R, Rao M, Steiner P. Experience with flexible fiberoptic bronchoscopy with bronchoalveolar lavage as a diagnostic tool in children with AIDS. *Am J Dis Child* 1992; 146: 1056–1059.

50. Kurland G, Noyes BE, Jaffe R, Atlas AB, Armitage J, Orenstein DM. Bronchoalveolar lavage and transbronchial biopsy in children following heart-lung and lung transplantation. *Chest* 1993; 104: 1043–1048.
51. Perez CR, Wood RE. Update on pediatric flexible bronchoscopy. *Pediatr Clin North Am* 1994; 41: 385–400.
52. Riedler J, Grigg J, Robertson CF. Role of bronchoalveolar lavage in children with lung disease. *Eur Respir J* 1995; 8: 1725–1730.
53. Rock MJ. The diagnostic utility of bronchoalveolar lavage in immunocompetent children with unexplained infiltrates on chest radiograph. *Pediatrics* 1995; 95: 373–377.
54. Eber E. Bronchoalveolar lavage in pediatric patients. *J Bronchology* 1998; 5: 227–241.
55. Naber SP. Molecular medicine. Molecular pathology - diagnosis of infectious disease. *N Engl J Med* 1994; 331: 1212–1215.
56. Schluger NW, Rom WN. The polymerase chain reaction in the diagnosis and evaluation of pulmonary infections. *Am J Respir Crit Care Med* 1995; 152: 11–16.
57. Eber E, Zach M. Pediatric flexible fiberoptic bronchoscopy – an analysis of 420 investigations. *Wien Klin Wochenschr* 1995; 107: 246–251.
58. Bjermer L, Rust M, Heurlin N, Rennard S, Klech H. The clinical use of bronchoalveolar lavage in patients with pulmonary infections. *Eur Respir J* 1992; 2: 106–113.
59. Meduri GU, Beals DH, Maijub AG, Baselski V. Protected bronchoalveolar lavage. A new bronchoscopic technique to retrieve uncontaminated distal airway secretions. *Am Rev Respir Dis* 1991; 143: 855–864.
60. Baker AM, Bowton DL, Haponik EF. Decision making in nosocomial pneumonia. An analytic approach to the interpretation of quantitative bronchoscopic cultures. *Chest* 1995; 107: 85–95.
61. Jourdain B, Joly-Guillou M-L, Dombret M-C, *et al.* Usefulness of quantitative cultures of BAL fluid for diagnosing nosocomial pneumonia in ventilated patients. *Chest* 1997; 111: 411–418.
62. Speich R, Wüst J, Hess T, Kayser FH, Russi EW. Prospective evaluation of a semiquantitative dip slide method compared with quantitative bacterial cultures of BAL fluid. *Chest* 1996; 109: 1423–1429.
63. Kessler HH, Reinthaler FF, Pschaid A, *et al.* Rapid detection of Legionella species in bronchoalveolar lavage fluids with the Enviroamp Legionella PCR amplification and detection kit. *J Clin Microbiol* 1993; 31: 3325–3328.
64. Bretagne S, Costa JM, Vidaud M, Tran J, Nhieu V, Fleury-Feith J. Detection of *Toxoplasma gondii* by competitive DNA amplification of bronchoalveolar lavage samples. *J Infect Dis* 1993; 168: 1585–1588.
65. Leibovitz E, Pollack H, Rigaud M, *et al.* Polymerase chain reaction is more sensitive than standard cytologic stains in detecting *Pneumocystis carinii* in bronchoalveolar lavages from human immunodeficiency virus type 1-infected infants and children with pneumonia. *Pediatr Infect Dis J* 1995; 14: 714–716.
66. Delacourt C, Poveda JD, Chureau C, *et al.* Use of polymerase chain reaction for improved diagnosis of tuberculosis in children. *J Pediatr* 1995; 126: 703–709.
67. Kessler HH, Dodge DE, Pierer K, *et al.* Rapid detection of Mycoplasma pneumoniae by an assay based on PCR and probe hybridization in a nonradioactive microwell plate format. *J Clin Microbiol* 1997; 35: 1592–1594.
68. Goyal M, Shaw RJ, Banerjee DK, Coker RJ, Robertson BD, Young DB. Rapid detection of multidrug-resistant tuberculosis. *Eur Respir J* 1997; 10: 1120–1124.
69. Chaske J, Trouillet J-L. Nosocomial pneumonia: guidelines for initial management and empirical treatment. *Eur Respir Mon* 1997; 3: 101–117.
70. ATS Committee on Health Care Policy and Practice. Clinical role of bronchoalveolar lavage in adults with pulmonary disease (ATS statement). *Am Rev Respir Dis* 1990; 142: 481–486.
71. Pinar A, Ahkee S, Miller RD, Ramirez JA, Summersgill JT. Use of heteroduplex analysis to classify legionellae on the basis of 5S rRNA gene sequences. *J Clin Microbiol* 1997; 35: 1609–1611.
72. Skot J, Lerche AG, Kolmos HJ, Nielsen JO, Mathiesen LR, Lundgren JD. Pneumocystis carinii in bronchoalveolar lavage and induced sputum: detection with a nested polymerase chain reaction. *Scand J Infect Dis* 1995; 27: 363–367.
73. Roth A, Schaberg T, Mauch H. Molecular diagnosis of tuberculosis: current clinical validity and future perspectives. *Eur Respir J* 1997; 10: 1877–1891.
74. Crawford SW, Bowden RA, Hackman RC, Gleaves CA, Meyers JD, Clark JG. Rapid detection of cytomegalovirus pulmonary infection by bronchoalveolar lavage and centrifugation culture. *Ann Intern Med* 1988; 108: 180–185.
75. Eber E. Infection control in flexible fiberoptic bronchoscopy. *In*: Barbato A, Landau LI, Scheinmann P, Warner JO, Zach M, eds. The bronchoscope– flexible and rigid – in children. Treviso, Italy, Arcari, 1995; pp. 87–92.
76. Tessier V, Chadelat K, Baculard A, Housset B, Clement A. A controlled study of differential cytology and cytokine expression profiles by alveolar cells in pediatric sarcoidosis. *Chest* 1996; 109: 1430–1438.
77. Clement A, Chadelat K, Massliah J, *et al.* A controlled study of oxygen metabolise release by alveolar macrophages from children with interstitial lung disease. *Am Rev Respir Dis* 1987; 136: 1424–1428.
78. Heaney G, Stevenson EC, Turner G, *et al.* Investigating paediateic airways by non-bronchoscopic lavage: normal cellular data. *Clin Exp Allergy* 1996; 26: 799–806.
79. Merchant RK, Schwartz DA, Helmers RA, Dayton CS, Hunninghake GW. Bronchoalveolar lavage cellularity. The distribution in normal volunteers. *Am Rev Respir Dis* 1992; 146: 448–453.
80. Braun J, Dalhof K, Ratjen F. Different protein composition of BALF in healthy children and adults. Technical and clinical implications. *Eur Respir J* 1994; 7: 228A.
81. Bjermer L, Rust M, Heurlin N, Rennard S, Klech H. The clinical use of bronchoalveolar lavage in patients with pulmonary infections. *Eur Respir Rev* 1992; 2: 106–113.
82. Ratjen F, KreuzLelder E. Differences in immunoglobulines and B2-microglobulin levels of bronchoalveolar lavage fluid between normal children and adults. *Lung* 1996; 174: 383–391.
83. Ratjen F, Rehn B, Costabel U, Brunch J. Age dependency of surfactant phospholipid and surfactant protein A in bronchoalveolar lavage fluid children without bronchopulmonary disease. *Eur Respir J* 1996; 9: 328–333.
84. Danel C, Israel-Biet D, Costabel U, Fabbbri LM, Klech H. Therapeutical applications of BAL. *Eur Respir J* 1990; 8: 960–961.
85. Wang B, Stern E, Schmidt RA, Pierson DJ. Diagnosing pulmonary alveolar proteinosis. *Chest* 1997; 111: 460–466.
86. Mahut B, Delacourt C, Mamou-Mani T, *et al.* Pulmonary alveolar proteinosis. *Pediatrics* 1996; 97: 117–122.
87. Milleron B, Costabel U, Teschler H, *et al.* Bronchoalveolar lavage cell data in alveolar proteinosis. *Am Rev Respir Dis* 1991; 144: 1330–1332.

88. Firth J, Gaedy S. Pulmonary haemorrhage and massive hemoptysis. *In*: Chernick V, ed. Kendig′s disorders of the respiratory tract in children. 1st Edn. Philadelphia, USA, WB Saunders Company, 1990: 966–976.
89. Stephan J. Histiocytoses. *Eur J Pediatr* 1995; 154: 600–609.
90. Réfabert L, Rambaud C, Mamou-Mani T, Scheinmann P, de Blic J. Cd la-positive cells in bronchoalveolar lavage samples from children with Langerhans cell histiocytosis. *J Pediatr* 1996; 129: 913–915.
91. Conference C. Activity of sarcoidosis: third WASOG meeting. *Eur Respir J* 1994; 7: 624–627.
92. Fireman E, Topilsky M. Sarcoidosis: an organized pattern of reaction from immunology to therapy. *Immunol Today* 1994; 15: 199–201.
93. Chadelat K, Baculard A, Grimfeld A, *et al*. Pulmonary sarcoidosis in children: serial evaluation of bronchoalveolar lavage cells during corticosteroid treatment. *Pediatr Pulmonol* 1993; 316: 41–47.
94. Peters MJ, Klein NJ, Gibb DM. HIV-related lung disorders in infants and children, including lymphocytic interstitial pneumonitis. *Eur Respir Mon* 1995; 2: 362–384.
95. Leigh MW, Henshaw NG, Wood RE. Diagnosis of *Pneumcystis carinii* pneumonia in pediatric patients using bronchoscopic bronchoalveolar lavage. *Pediatr Infect Dis* 1985; 4: 408–410.
96. Bye MR, Bernstein L, Shah K, Ellaurie M, Rubinstein A. Diagnostic bronchoalveolar lavage in children with AIDS. *Pediatr Pulmonol* 1987; 3: 425–428.
97. Mattey JE, Fitzpatrick SB, Josephs SH, Fink RJ. Bronchoalveolar lavage for pneumocystis pneumonia in HIV-infected children. *Ann Allergy* 1990; 64: 393–397.
98. Birriel JA Jr, Adams JA, Saldana MA, *et al*. Role of flexible bronchoscopy and bronchoalveolar lavage in the diagnosis of pediatric acquired immunodeficiency syndrome-related pulmonary disease. *Pediatrics* 1991; 87: 897–89.
99. Glaser JH, Schuval S, Burstein O, Bye MR. Cytomegalovirus and *Pneumocystis carinii* pneumonia in children with acquired immunodeficiency syndrome. *J Pediatr* 1992; 120: 929–931.
100. Allen KA, Markin RS, Rennard SI, *et al*. Bronchoalveolar lavage in liver transplant patients. *Acta Cyfol* 1989; 33: 539–543.
101. Colombo JL, Sammut PH, Langnas AN, Shaw BW Jr. The spectrum of *Pneumocystis carinii* infection after liver transplantation in children. *Transplantation* 1992; 54: 621–624.
102. Frankel LR, Smith DW, Lewiston NJ. Bronchoalveolar lavage for diagnosis of pneumonia in the immunocompromised child. *Pediatrics* 1988; 81: 785–788.
103. Pattishall EN, Noyes BE, Orenstein DM. Use of bronchoalveolar lavage in immunocompromised children. *Pediatr Pulmonol* 1988; 5: 1–5.
104. Winthrop AL, Waddell T, Superina RA. The diagnosis of pneumonia in the immunocompromised child: use of bronchoalveolar lavage. *J Pediatr Surg* 1990; 25: 878–880.
105. Ratjen F, Costabel U, Havers W. Differential count of bronchoalveolar lavage fluid in immunosuppressed children with pulmonary infiltrates. *Arch Dis Child* 1996; 74: 507–511.
106. Lanino E, Sacco O, Kotitsa Z, *et al*. Fiberoptic bronchoscopy and Bronchoalveolar lavage for the evaluation of pulmonary infiltrates after BMT in children. *Bone Marrow Transplantation* 1996; 18: 117–120.
107. Fauroux B, Meyer-Milsztain A, Boccon-Gibod L, *et al*. Cytotoxic Drung-induced pulmonary disease in infants and children. *Pediatr Pulmonol* 1994; 18: 347–355.
108. Prober CG, White H, Smith CR. Open lung biopsy in immunocompromised children with pulmonary infiltrates. *Am J Dis Child* 1984; 138: 60–63.
109. Shorter NA, Ross AJ, August C. The usefulness of open lung biopsy in the pediatric bone marrow transplant population. *J Pediatr Surg* 1988; 23: 533–537.
110. Guilinger RA, Paradis IL, Dauber JH, *et al*. The importance of bronchoscopy with transbronchial biopsy and bronchoalveolar lavage in the management of lung transplant recipients. *Am J Respir Crit Care Med* 1995; 152: 2037–2043.
111. Magnan A, Mege J-L, Reynaud M, *et al*. Monitoring of alveolar macrophage production of tumor necrosis factor-alpha and interleukin-6 in lung transplant recipients. *Am J Respir Crit Care Med* 1994; 150: 684–689.
112. Clelland C, Higenbottam T, Stewart S, *et al*. Bronchoalveolar lavage and transbronchial lung biopsy during acute rejection and infection in heart-lung transplant patients. Studies of cell counts, lymphocyte phenotypes, and expression of HLA-DR and interleukin-2 receptor. *Am Rev Respir Dis* 1993; 147: 1386–1392.
113. Koumbourlis AC, Kurland G. Nonbronchoscopic bronchoalveolar lavage in mechanically ventilated infants: technique, efficacy and applications. *Pediatr Pulmonol* 1993; 15: 257–262.
114. Grigg J, Amon S, Silverman M. Fractional processing of sequential bronchoalveolar lavage fluid from intubated babies. *Eur Respir J* 1992; 5: 727–732.
115. Alpert BE, O′Sullivan BP, Panitch HB. Non-bronchoscopic approach to bronchoalveolar lavage in children with artificial airways. *Pediatr Pulmonol* 1992; 13: 38–41.
116. Kotecha S, Wangoo A, Silverman M, Shaw RJ. Increase in transforming growth factor-p, concentration in bronchoalveolar lavage fluid obtained from infants with chronic lung disease of prematurity. *J Pediatr* 1996; 128: 464–469.
117. Kotecha S, Wilson N, Wangoo A, Silverman M, Shaw RJ. Increase in interleukin-lß and interleukin-6 in bronchoalveolar lavage fluid from infants with chronic lung disease of orematuritv. *Pediatr Res* 1996; 40: 250–256.
118. Bagchi A, Viscardi RM, Taciak V, Ensor JE, McCrea KA, Hasday JD. Increased activity of interleukin-6 but not tumour necrosis factor-alpha in lung lavage of premature infants is associated with the development of bronchopulmonary dysplasia. *Pediatr Res* 1994; 36: 244–252.
119. Vyas JR, Taub N, Tudor G, Rickett A, Field DJ, Kotecha S. Radiographic changes associated with serial NB-BAL of ventilated preterm infants. *Eur Respir J* 1998; 12: 7s.
120. Watts CL, Bruce MC. Comparison of secretory component for immunoglobulin A with albumin as reference proteins in tracheal aspirate from preterm infants. *J Pediatr* 1995; 127: 113–122.
121. Rennard SI, Ghafouri M, Thompson AB *et al*. Fractional processing of sequential bronchoalveolar lavage to separate bronchial and alveolar samples. *Am Rev Respir Dis* 1990; 141: 208–217.
122. Baumann H, Gauldie J. The acute phase response. *Immunol Today* 1994; 15: 74–80.
123. Pittet JF, Mackersie RC, Martin TR, Matthay MA. Biological markers of acute lung injury: prognosis and pathogenetic significance. *Am J Respir Crit Care Med* 1997; 155: 1187–1205.

124. Henderson W. The role of leukotrienes in inflammation. *Ann Intern Med* 1994; 121: 684–697.
125. Ricou B, Nicod L, Lacraz S, Welgus HG, Suter PM, Dayer JM. Matrix metalloproteinases and TIMP in acute respiratory distress syndrome. *Am J Respir Crit Care Med* 1996; 154: 346–352.
126. Clement A, Housset A. Role of free radicals in airways injury. *In*: Chretien J, Dusser D, eds. Lung biology in health and disease, Vol 93. Environmental impact of the airways: from injury to repair. New York, USA, Marcel Dekker, 1996; pp. 355–379.
127. Erjefalt J, Persson C. Airway epithelial repair: breathtakingly quick and multipotentially pathogenic. *Thorax* 1997; 52: 1010–1012.
128. Witschi H. Responses of the lung to toxic injury. *Environ Health Perspect* 1990; 85: 5–13.
129. Chadelat K, Boule M, Corroyer S, *et al*. Expression of insulin-like growth factors and their binding proteins by bronchoalveolar cells from children with and without interstitial lung disease. *Eur Respir J* 1998; 11: 1329–1336.
130. Barnes P. New therapies for chronic obstructive pulmonary disease. *Thorax* 1998; 53: 137–147.
131. Rogers D, Laurent G. New ideas on the pathophysiology and treatment of lung disease. *Thorax* 1998; 53: 200–203.
132. Voss T, Schafer KP, Nielsen PF, *et al*. Primary structure differences of surfactant-associated protein isolated from normal and proteinosis lung. *Biochim Biophys Acta* 1992; 1138: 261–267.
133. Richman-Eisenstat J. Cytokine soup: making sense of inflammation in cystic fibrosis. *Pediatr Pulmonol* 1996; 21: 3–5.
134. Holgate ST. Asthma: a dynamic disease of inflammation and repair. *Ciba found Symp* 1997; 206: 5–28.
135. Azevedo I, de Blic J, Schinmann P, Vargaftig BB, Bachelet M. Enhanced arachidonic acid metabolism in alveolar macrophages from wheezy infants. Modulation by dexamethasone. *Am J Respir Crit Care Med* 1995; 152: 1208–1214.
136. Azevedo I, de Blic J, Dumarey CH, Scheinmann P, Vargaftig BB, Bachelet M. Increased spontaneous release of tumor necrosis factor alpha by alveolar macrophages from wheezy infants. *Eur Respir J* 1997; 10: 1761–1766.