

Comparison of cell profiles in separately evaluated fractions of bronchoalveolar lavage (BAL) fluid in children

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Abstract

Background – Bronchoalveolar lavage (BAL) has been widely accepted as a routine procedure in the differential diagnosis of respiratory diseases in adults. However, there is only limited information about the value of BAL in children and there is no standardisation of the procedures.

Methods – The difference in cellular profiles of sequential BAL fractions from children was analysed to assess the effect of evaluating bronchial and alveolar fractions separately. Twenty five children (mean age 13.4 years) were examined by fiberoptic bronchoscopy under general anaesthesia, combined with local application of lignocaine. The investigation was part of the clinical evaluation of an infective pulmonary problem. Bronchoalveolar lavage was performed from the right middle lobe using four 20 ml aliquots of normal saline and the four fractions of recovered fluid were evaluated separately.

Results – The total cell count was lowest in the first aspirate (1.8×10^6) and increased gradually from fraction 1 to fraction 4. Similarly, the viability of the cells was lowest in fraction 1 and gradually improved from fraction 1 to fraction 4. In the differential count the number of neutrophils decreased from fraction 1 (8.3%) to fraction 4, with fractions 2 (5.4%), 3 (3.4%), and 4 (2.4%) being significantly different from fraction 1. There were no differences in the numbers of eosinophils, lymphocytes, or macrophages between individual fractions.

Conclusions – These results show that the cell profile of the first “bronchial” fraction is different from subsequent samples. It should be evaluated separately while the second and third aliquots may be pooled.

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Bronchoalveolar lavage (BAL) has become a widely used procedure in various clinical settings including the differential diagnosis and monitoring of interstitial lung disease,¹ and for the detection of opportunistic infections in immunocompromised patients.²³ It has also been frequently applied in research to study bronchial and alveolar pathology.

The availability of paediatric flexible bronchoscopes has extended the usefulness of BAL

into paediatric pulmonology.⁴⁻⁸ In adults the basic methodological recommendations have been summarised by the BAL Task Group within the European Respiratory Society,^{9,10} but there is no generally accepted standardisation in children.

Some authors report using the lavage volume related to body weight. Typically, volumes have ranged from 0.75 to 1 ml/kg per aliquot and the number of aliquots has also varied.¹¹⁻¹³ Furthermore, the methods of processing the recovered lavage fluid vary. The aspirated fractions of lavage fluid are usually pooled but, to our knowledge, only two groups have looked at the possibility of fractional processing of BAL fluid from children to distinguish bronchial from alveolar material. However, one of these studies was performed in intubated newborn infants by a non-bronchoscopic approach,¹² while the other was performed in healthy children undergoing elective surgery with only the first fraction separated.¹⁴ In the recently published study by Midulla *et al* the authors analysed the cellular and non-cellular components of BAL fluid in small children using only the second fraction whilst the first fraction was used for microbiological analysis.¹⁵

We have therefore assessed the differences in cytological findings in sequential fractions of BAL fluid in order to resolve the difficulties in the application and evaluation of BAL in children.

Methods

STUDY SUBJECTS

The study subjects comprised 24 patients of mean (SD) age 13.5 (2.7) years (range 8-18) and mean (SD) weight 40.1 (14.6) kg (range 25-80) who were undergoing bronchoscopic examination with BAL for investigation of clinical symptoms. The clinical diagnoses of the subjects were recurrent productive cough (14), chronic productive cough (5), chronic dry cough (4), and recurrent pneumonia (1).

Informed parental consent was obtained for each child to participate in the study.

BRONCHOSCOPY

All of the children were examined with a Pentax FB 15X bronchoscope (outer diameter 4.9 mm and 2.2 mm working channel) while spontaneously breathing under ketamine general anaesthesia combined with the topical application of 1% or 2% lignocaine. No muscle relaxing drugs were used. Pulse rate, ECG, and

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oxygen saturation were monitored during the procedure and for at least 30 minutes afterwards. After the routine examination the bronchoscope was wedged either into the right middle lobe bronchus or into one of the segmental bronchi.

BRONCHOALVEOLAR LAVAGE (BAL)

BAL was performed using four 20 ml aliquots of normal saline warmed to 37°C. The fluid was injected through the channel of the bronchoscope and each fraction was immediately gently aspirated back by the same syringe. Each aspirate was immediately transferred from the syringe into a plastic vial, labelled, and stored separately for evaluation.

PROCESSING OF BAL FLUID

Each fraction of BAL fluid was filtered through two layers of gauze and the recovered fluid was centrifuged separately (10 minutes at 1700 g). The cells were then counted in a haemocytometer. Air dried smears were stained with May-Grunwald-Giemsa stain for differential cell counts. At least 300 cells were counted, excluding epithelial cells. The viability of the cells was determined by trypan-blue exclusion and the phagocytic activity by the ingestion of synthetic methacrylate particles.

DATA ANALYSIS

For the statistical analysis Friedman two-way ANOVA was used. Post-hoc significances of differences were determined by the Nemenyi method. A p value of <0.05 was considered significant.

Results

The total and differential cell counts, viability of cells, and phagocytosis of alveolar macrophages are shown in the table.

TOTAL NUMBER AND VIABILITY OF BAL CELLS

The total cell count was lowest in the first aspirate (1.8×10^6) and increased gradually from fraction 1 to fraction 4 up to a value of 3.6×10^6 (fig 1). Statistical significance ($p < 0.01$) was reached between the first and second aspirates and increased with further samples ($p < 0.001$). Similarly, the viability of the cells was lowest in fraction 1 and gradually improved with each fraction (fig 2), the differ-

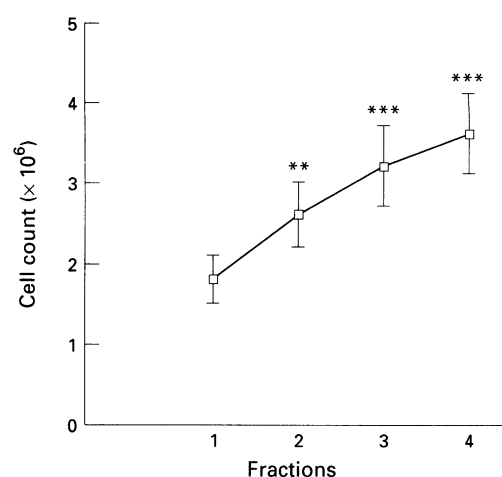


Figure 1 Total cell count. ** $p < 0.01$; *** $p < 0.001$.

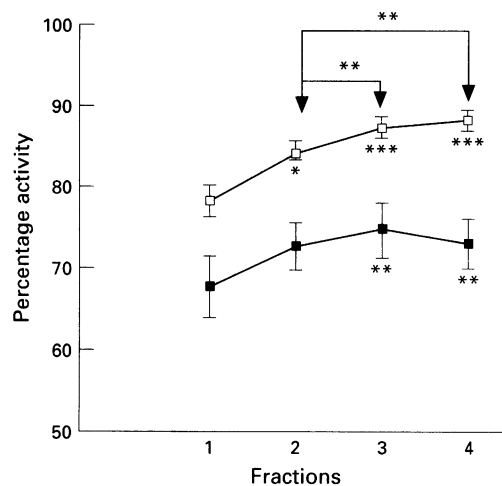


Figure 2 Percentage viability (\square) and phagocytic (\blacksquare) activity. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

ence becoming significant in fractions 2 ($p < 0.05$), 3 ($p < 0.001$), and 4 ($p < 0.001$).

DIFFERENTIAL COUNT OF FRACTIONATED BAL CELLS

The most striking difference in the differential cell count between individual aliquots was the percentage of neutrophils (fig 3). The highest number of neutrophils was found in fraction 1 (8.3%) and the percentage decreased from fraction 1 to 4, with fractions 2 (5.4%), 3

Mean (SE) results of analysis of sequential fractions of BAL fluid

	Fraction			
	1	2	3	4
Cell count ($\times 10^6$)	1.8 (0.3)	2.6 (0.4)**	3.2 (0.5)***	3.6 (0.5)***
Viability (%)	78.3 (2.0)	84.1 (1.6)*	87.4 (1.3)***	88.3 (1.3)***
Phagocytosis (%)	67.7 (3.8)	72.7 (2.9)	74.6 (3.4)**	72.9 (3.1)**
Macrophages (%)	77.1 (2.9)	78.7 (2.5)	82.0 (3.0)	83.3 (2.5)
Neutrophils (%)	8.3 (1.9)	5.4 (1.7)**	3.4 (1.7)***	2.4 (0.9)***
Lymphocytes (%)	13.3 (1.9)	14.5 (1.9)	14.0 (2.2)	13.5 (2.1)
Eosinophils (%)	1.4 (0.6)	1.4 (0.4)	0.6 (0.2)	0.6 (0.2)

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

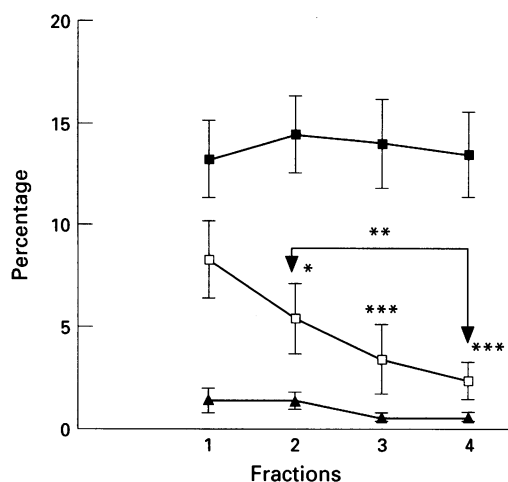


Figure 3 Percentage of neutrophils (□), lymphocytes (■), and eosinophils (▲) in bronchoalveolar fluid. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

(3.4%), and 4 (2.4%) being significantly different from fraction 1 ($p < 0.01$, $p < 0.001$, and $p < 0.001$, respectively). There were no significant differences in the numbers of eosinophils, lymphocytes, and macrophages between individual fractions.

PHAGOCYTOTIC ACTIVITY OF ALVEOLAR MACROPHAGES

The ingestion of methacrylate particles by alveolar macrophages ranged from 28% to 94%. Analysis of the phagocytic activity of the alveolar macrophages only became significant between fractions 1 (67.7%) and 3 (74.6%) ($p < 0.01$), and fractions 1 and 4 (72.9%) ($p < 0.01$).

Comparisons of fractions 2, 3, and 4 did not reveal any significant differences with the exception of the viability of the cells between fractions 2 and 3 ($p < 0.01$), and between fractions 2 and 4 ($p < 0.01$). A significant difference was also found in the percentage of neutrophils between fractions 2 and 4 ($p < 0.05$).

COMPLICATIONS

The only complication encountered was a short febrile reaction with pleurisy in one patient which occurred six hours after the procedure and subsided after a single dose of 500 mg paracetamol.

Discussion

Our study has shown that BAL, using 20 ml aliquots of saline and performed as part of a diagnostic bronchoscopic examination, is well tolerated in school age children and provides sufficient cell numbers for analysis. The first fraction of BAL fluid differed significantly from the subsequent samples, particularly in the lower number and viability of recovered cells, and the increased percentage of neutrophils. In contrast to recent studies of the differential cytology of BAL fluid in normal children,¹⁴ no change in the proportion of lymphocytes was found, and the percentage of granulocytes was

higher in our patients than in normal subjects. This reflected the disease groups studied.

Detailed fractional analysis of BAL fluid in adults¹⁶ using five 20 ml aliquots showed that the first fraction was significantly enriched by bronchial cellular material with an increased presence of bronchial epithelial cells, neutrophils, lactoferrin, lysozyme, and IgA. Similar results were also found in adult patients with sarcoidosis and with bronchial asthma, showing differences in cellular findings in the first ("bronchial") and subsequent fractions.¹⁷⁻¹⁹ It has been recommended therefore that the first fraction of BAL fluid should be processed separately, especially when the pathology of the bronchi is being considered.¹⁶

The differential count has been a widely used parameter in the routine investigation of inflammatory disorders in the lungs. From available data it is obvious that a pooled BAL specimen contains both airway and alveolar cells, and inflammation in either anatomical compartment leads to changes in the pooled BAL fluid. Separation of the first portion of the aspirated fluid could thus improve the information gained and could be adopted for routine protocols when bronchial disease is suspected.

As no significant differences were found between the second, third, and fourth aliquots, we conclude that separation of further aliquots would be of no further value.

BAL has been widely used in the paediatric age group for diagnostic and research purposes, but no standard values are available. The volumes used vary substantially with aliquots of 0.75-1 ml/kg often being given to small children, while in older children aliquots of 10-20 ml have been common.^{5,20} In our study we have used volumes of 20 ml for all children, mainly for technical reasons, which resulted in volumes of 0.25-0.80 ml/kg per aliquot. Despite this wide range, the cellular analysis was always satisfactory and the range of volume did not have any influence on the reliability of the results. This correlates with recently published results in normal children.¹⁵ Our data prove that, even in the separated first "bronchial" fraction, the number of cells was satisfactory and enabled reliable analysis of the differential count. The volume of 20 ml per aliquot used in this study seems to be sufficient for proper sampling of both the bronchial and alveolar compartments. With subsequent aliquots the differences in individual fractions diminished, indicating that the later lavage fluid apparently sampled primarily the alveolar space. Our results are in agreement with those of Rennard *et al*¹⁶ who used an aliquot volume for adults of 20 ml. The volume infused was independent of body weight and thus was lower per kg body weight than in our patients without any effect on cellular analysis. Volumes larger than 20 ml are less reliable for the separation of the bronchial and alveolar samples.

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