Eur Respir J
1993, 6, 110–115

Changes in epithelial lining fluid albumin associated with smoking and interstitial lung disease

C.M. Roberts*, D.Cairns**, D.H. Bryant*, W.M.J. Burke*, M.Yeates+, H. Blake++, R. Penny++, L. Shelley++, J.J. Zaunders++, S.N. Breit++

Changes in epithelial lining fluid albumin associated with smoking and interstitial lung disease. C.M. Roberts, D. Cairns, D.H. Bryant, W.M.J. Burke, M. Yeates, H. Blake, R. Penny, L. Shelley, J.J. Zaunders, S.N. Breit.

ABSTRACT: Albumin is an important plasma protein which is useful in the assessment of *in vivo* membrane permeability in the lung. In subjects with interstitial lung disease (ILD) the relationship between albumin recovered from bronchoalveolar lavage (BAL) and other markers of inflammatory activity may provide useful information of the pathogenesis of the disease process. Unfortunately, its measurement is hampered by the variable dilution in BAL fluid. In this study, urea was used as a marker of epithelial lining fluid (ELF) dilution allowing the calculation of an apparent epithelial lining fluid volume and adjusted albumin content.

We examined the relationship between ELF albumin content and BAL cell counts, immunoglobulin content, respiratory function tests and gallium lung scans in both smokers and nonsmokers with and without interstitial lung disease. Forty seven subjects with connective tissue disease and interstitial lung disease and 51 subjects with either connective tissue disease but no pulmonary involvement or non pulmonary malignancy (18 current smokers) underwent BAL, gallium lung scans and respiratory function tests. The subjects with ILD were further subdivided into those with active ILD or bronchiolitis using cluster analysis.

In smokers without ILD an increased ELF volume and a decrease in ELF albumin were found. Increased ELF albumin was related to increased age. In subjects with ILD, increased albumin was strongly correlated with increased BAL lymphocyte absolute and differential counts, which overwhelmed any age or smoking effect.

These findings suggest a possible causal relationship between lung vascular permeability and lymphocyte numbers in subjects with interstitial lung disease and reinforce the need to consider smoking and age as confounding factors in BAL analysis.

Eur Respir J., 1993, 6, 110–115.

*Thoracic and +Nuclear Medicine Depts, St Vincent's Hospital, NSW, Austrialia. **School of Behavioural Science, Macquarie University, Sydney, Australia, ++Centre for Immunology, St Vincent's Hospital and University of NSW, Australia

Correspondence: S.N. Breit
Centre for Immunology
St Vincent's Hospital
Victoria St
Sydney 2010
Australia

Keywords: Bronchoalveolar lavage
epithelial lining fluid albumin
interstitial lung disease
smoking

Received: January 17, 1992
Accepted after revision August 19, 1992

This work was supported in part by a grant from the National Health and Medical Research Council of Australia.

Albumin is an important plasma protein that has been extensively used as a marker of membrane permeability *in vivo*. In subjects with interstitial lung disease (ILD), bronchoalveolar lavage (BAL) is a useful method for sampling the alveoli in order to obtain material, including albumin, for the evaluation of the interstitial disease process [1–4]. Unfortunately, the assessment of lung vascular permeability using albumin recovered from BAL fluid is limited by the variable dilution of albumin and epithelial lining fluid (ELF) with the infused lavage fluid. One suggested method for overcoming this problem is to use urea as an endogenous marker of ELF dilution [5], thus allowing the estimation of an apparent ELF volume. This then provides a frame of reference to measure protein concentration, as well as a more useful method for expressing cell numbers [6, 7]. Although imperfect, this method allows consistent compensation to be made for the variable dilution of the ELF.

In this study, the same approach has been used to determine the albumin content of the ELF in subjects with and without interstitial lung pathology secondary to connective tissue disease. In addition, the relationship between albumin content and other measures of pulmonary function and inflammation, including respiratory function tests, lavage cell numbers and gallium index has been examined.

Methods

Patient selection

The study population consisted of a large group of patients with connective tissue diseases (both with and

without respiratory symptomatology) and a group of subjects without either pulmonary or systemic inflammatory disorders. There was a total of six subjects with non-pulmonary malignancies (early carcinoma of the breast), and 92 patients with connective tissue diseases, 47 with and 45 without clinical or lung function evidence of interstitial lung involvement. The subject groups, therefore, consisted of 47 subjects with clinical or pulmonary function evidence of interstitial lung disease and 51 subjects without such evidence. All patients with connective tissue diseases attending an immunology clinic were asked to participate in this study, irrespective of whether they had pulmonary symptoms, and thus to this extent were unselected. The connective tissue diseases included scleroderma alone (16), scleroderma with Sjogren's syndrome (29), Sjogren's syndrome alone (15), mixed connective tissue disease (2), mixed connective tissue disease with Sjogren's syndrome (4), systemic lupus erythematosus with (7), or without Sjogren's syndrome (7), rheumatoid arthritis (1), polymyositis (1) and 10 patients with vasculitis or variety of other less well-defined connective tissue diseases. Patients with scleroderma, systemic lupus erythematosus, rheumatoid arthritis or mixed connective tissue disease each fulfilled the American Rheumatism Association criteria for that diagnosis. For the purposes of this study, patients were considered to have Sjogren's syndrome when they gave a definite positive response to at least two out of three questions concerning the presence of dry eyes, dry mouth or recurrent salivary gland enlargement, and had additional objective confirmatory evidence for that diagnosis. The latter consisted of abnormalities of at least one of the following: Schirmer's test, salivary scan, minor salivary gland biopsy or antibodies to nuclear antigens SSA and/or SSB. Smoking history was defined in terms of pack years, 20 cigarettes smoked a day for one year being one pack year. Ex-smokers who had abstained for more than 2 yrs were defined as nonsmokers for the purpose of analysis of acute inflammatory indicators.

In order to define the pattern of associated lung involvement, an approach was adopted that was based on the use of clustering techniques to define the multivariate structure inherent in the data, then discriminant analysis to group patients into distinct clusters and, finally, an algorithm derived from the discriminant analysis was developed that allowed the assignment of any new patient into one of these clusters [8]. Discriminant analysis allowed grouping of all subjects into four distinct categories on the basis of respiratory function tests, gallium lung scans, and BAL cell counts and differentials, but not including albumin content or ELF volume [8]. The profile for these four groups is presented in table 1. In summary, cluster 1 represents nonsmokers without ILD and cluster 2 current smokers without ILD. Cluster 3 represents patients with active ILD and cluster 4 patients with a depressed maximum mid-expiratory flow rate (MMEFR), high lavage lymphocyte count and mildly elevated gallium index, who probably had a bronchiolitis. Smokers and nonsmokers were combined for clusters 3 and 4.

Respiratory evaluation

Respiratory physiology. A comprehensive set of respiratory function parameters was obtained, including vital capacity (VC), alveolar volume (V_A), maximal mid-expiratory flow rate (MMEFR), and diffusing capacity of the lung for carbon monoxide (D_{LCO}).

Gallium Scan of the Lung. Gallium scans were performed on each patient. Two hundred to two hundred and sixty MBq of ^{67}gallium citrate was injected intravenously and scans were obtained 48 h later, using a posterior projection. Using published methods and computerized data acquisition, a lung/liver gallium uptake index was calculated for the upper and lower regions of each lung, and then a mean value derived [9].

Bronchoalveolar lavage. This was carried out under local anaesthesia as described previously [10]. Briefly, it consisted of the passage, through the mouth, of a flexible fibreoptic bronchoscope, which was wedged in turn into three subsegmental bronchi and, into each, 100 ml of room temperature normal saline was instilled in four 25 ml aliquots. Each aliquot was aspirated immediately after instillation and the material was collected into a chilled, siliconized glass container and kept on ice until processed. A maximum dwell- time of 3 min was allowed for each segment lavaged. Patients with a history of chronic bronchitis or an acute respiratory tract infection within the preceding two weeks were excluded from the study.

Evaluation of bronchoalveolar cells. Enumeration of different cell types in BAL fluid is frequently carried out using a cytocentrifuge preparation, which substantially distorts the estimation of lymphocyte numbers [10]. The method described here [10] gives substantially higher estimates of lymphocyte numbers, that are more accurate. In brief, this consisted of collection of an aliquot of unprocessed lavage fluid, which was deposited onto a millipore filter. This was washed, fixed and stained with haematoxylin and eosin, and then further for nonspecific esterase activity, in order to accurately differentiate macrophages from lymphocytes. Total cell counts were obtained using unprocessed lavage fluid and a counting chamber.

Evaluation of ELF volumes and albumin content. The volume of ELF collected in the lavage procedure was estimated as described previously [5]. Briefly, the urea and albumin levels of the unconcentrated lavage fluid and a simultaneously collected plasma sample were determined by standard methods.

Table 1. – Respiratory test profiles of subjects according to cluster grouping

	Cluster 1 No ILD		Cluster 2 No ILD Smk		Cluster 3 Active ILD		Cluster 4 Bronchiolitis	
number	33		18		22		25	
Age yrs	49[a]	(14)	45[a]	(13)	57[b]	(12)	52[ab]	(11)
Smoking pack yrs	6[a]	(8.2)	27[b]	(13.9)	12[a]	(23.7)	8[a]	(13.8)
RFTs								
VC %	91[a]	(19.3)	93[a]	(13.0)	67[b]	(18.9)	89[a]	(12.6)
MMEFR %	107[a]	(25.4)	95[ab]	(28.6)	83[b]	(19.7)	63[c]	(16.6)
DLCO %	88[a]	(15.6)	72[bd]	(18.3)	50[c]	(16.9)	76[d]	(12.3)
Gallium index	3.0[a]	(2.4)	3.2[a]	(2.6)	5.7[b]	(2.3)	4.1[a]	(2.7)
Bronchoalveolar lavage								
BAL volume recovered %	46[a]	(14.5)	50[a]	(13.4)	50[a]	(16.3)	41[a]	(11.6)
ELF volume ml·300 ml^{-1} BAL	1.29[a]	(0.81)	2.43[b]	(1.47)	2.52[bc]	(1.32)	1.11[a]	(0.54)
Lavage cells								
Total cells×10^{-6}	34.5[a]	(22.7)	94.2[b]	(46.0)	53.1[ca]	(34.1)	25[a]	(21.6)
Cells·ml^{-1} ELF×10^{-6}	25.1[a]	(10.8)	39.4[b]	(21.2)	25.7[a]	(15.7)	32.9[ab]	(21.2)
Mac %	68[a]	(12.2)	77[b]	(9.8)	59[c]	(11.9)	47[d]	(15.6)
Lym %	31[a]	(12.5)	21[b]	(8.6)	34[a]	(11.8)	50[c]	(14.9)
Neut %	1[a]	(2.8)	2[a]	(2.3)	6[b]	(10.3)	3[a]	(5.4)
Mac·ml^{-1} ELF×10^{-6}	17.9[a]	(8.5)	31.0[b]	(20.1)	15.2[a]	(9.0)	16.5[a]	(10.5)
Lym·ml^{-1} ELF×10^{-6}	6.9[a]	(3.4)	7.9[a]	(3.1)	7.2[a]	(4.2)	15.7[b]	(13.7)
Neu·ml^{-1} ELF×10^{-6}	0.4[a]	(1.0)	0.6[a]	(0.6)	3.2[b]	(7.0)	0.7[b]	(1.1)
Lavage protein								
ELF ALB g·dl^{-1}	0.56[a]	(0.2)	0.32[b]	(0.19)	0.42[ab]	(0.32)	0.76[c]	(0.39)
ALB RAT	0.015[a]	(0.005)	0.009[b]	(0.005)	0.011[ab]	(0.009)	0.021[c]	(0.011)
IgG RAT	0.112[a]	(0.059)	0.086[a]	(0.063)	0.176[a]	(0.210)	0.241[a]	(0.246)
IgM RAT	0.031[a]	(0.025)	0.008[b]	(0.004)	0.028[abc]	(0.022)	0.047[ac]	(0.039)

Values are mean (±SD). Each mean value is followed by a superscript letter which identifies its similarities and differences to the other values in that row using the ANOVA and a significance level of at least $p<0.05$. For example, for age, clusters 1, 2 and 4 all bear the letter "a" and are not significantly different from each other. Cluster 3, bearing the letter "b", differs from clusters 1 and 2, but not from cluster 4 which also bears the letter "b". VC: vital capacity; MMEFR: maximal mid-expiratory flow rate; DLCO: diffusion capacity of the lungs for carbon dioxide; Mac: macrophage; Lym: lymphocyte; Neu: neutrophil; ELF: epithelial lining fluid; ALB: albumin; BAL: bronchoalveolar lavage; RAT: ratio; ILD: interstitial lung disease; Smk: current smokers; IgG: immunoglobulin G; IgM: immunoglobulin M; RFT: respiratory function test; ANOVA: analysis of variance.

Urea levels were determined using the urease method according to manufacturer's instructions (Roche, Switzerland), by measuring absorbance at 340 nm on a Kone Progress Selective Chemistry Analyser (Kone, Finland). Serum albumin was measured on the basis of bromcresol green dye binding (Technicon, USA) using absorbance at 628 nm on the Kone analyser. Lavage fluid albumin was determined using a turbidometric method employing anti-albumin antibody (Dako, Denmark) and absorbance at 340 nm on a Roche Centrifichem 400 analyser (Roche, Switzerland). The ELF volume was then calculated using the formula: ELF=[(urea concentration of lavage fluid/urea concentration of plasma)(recovered lavage volume)]. The albumin result was then corrected for the actual ELF volume recovered. To take into account changes in lavage ELF albumin concentration due to variation in plasma albumin, the result was expressed as the ratio of ELF to plasma albumin (ALB RAT).

Evaluation of epithelial lining fluid immunoglobulin G and M (IgG and IgM) content. The plasma levels of IgG and IgM were measured by standard nephelometric method using a Beckman Nephelometer. Levels of the immunoglobulins were determined in unconcentrated lavage fluid using a radioimmunoassay method similar to that described previously [11]. ELF IgG and IgM levels were calculated as described above for albumin. Results were also expressed as the ratio of ELF to plasma levels (IgG RAT; IgM RAT) in order to correct for changes in the ELF, due simply to changes in plasma levels.

Statistical analysis

For analytical purposes the clusters were pooled so that clusters 1 and 2 became the group without interstitial lung disease and clusters 3 and 4 became the ILD group. Essentially this pooling makes no difference to the result outcome. Spearman rank correlation coefficients (r_s) were used, in particular to allow the calculation of significance levels. All r_s values, unless otherwise stated, are significant to at least $p<0.05$. The data in table 1 have been analysed by analysis of variance comparing all four cluster groups.

Results

Relationship of lavage albumin to cells recovered during BAL

There is a moderate correlation ($r_s=0.45$, $p=0.019$) between the total lavage cell numbers·ml^{-1} of ELF and the ALB RAT in the ILD group but not in those subjects without ILD (table 2). When this is further examined by looking at the relationship with the numbers of macrophages, lymphocytes or neutrophils recovered·ml^{-1} of ELF, then it becomes apparent that the primary relationship is with the lymphocytes·ml^{-1} of ELF ($r_s=0.65$, p 0.008) (table 1). The relationship between the percentage of the different cell types in the BAL similarly reflects this association, with a correlation in the disease group, between ALB RAT and percentage of lymphocytes ($r_s=0.60$, $p=0.002$) and a reciprocal negative correlation with the percentage of neutrophils ($r_s=-0.44$, $p=0.02$) (table 2).

Relationship of lavage albumin to gallium index

In the subjects without evidence of ILD there appears to be a weak relationship between the gallium index and the ALB RAT ($r_s=0.33$, $p=0.04$). In the ILD group the correlation between the gallium index and ALB RAT is more variable ($r_s=0.31$) with a non-significant p value (tables 1 and 2).

Relationship of lavage albumin to lavage immunoglobulins

The lavage albumin as measured by the ALB RAT and the lavage IgG and IgM as measured by the IgG RAT (no ILD $r_s=0.85$, $p=0.003$; ILD group $r_s=0.91$, $p=0.001$) and IgM RAT (no ILD $r_s=0.54$, $p=0.018$; ILD group $r_s=0.82$, $p=0.003$) appear to be closely related in both those with and without ILD (table 2).

Table 2. – Correlation of ELF albumin content with BAL cell recovery, immunoglobulin content and respiratory function tests

Measured value	Spearman rank correlation coefficient			
	No ILD		ILD	
Total cells recovered	-0.152		0.45	(0.019)
Macrophages·ml^{-1} ELF	-0.178		0.36	(0.05)
Lymphocytes·ml^{-1} ELF	-0.003		0.647	(0.0008)
Neutrophils·ml^{-1} ELF	-0.117		-0.299	
BAL macrophage %	-0.218		-0.33	
BAL lymphocytes %	0.22		0.598	(0.0019)
BAL neutrophils %	-0.18		-0.444	(0.002)
IgM ratio	0.58	(0.01)	0.956	(0.0006)
IgG ratio	0.522	(0.02)	0.511	(0.02)
Vital capacity	0.208		0.374	(0.05)
Alveolar volume	0.274		0.464	(0.016)
MMEFR	0.16		-0.084	
DLCO	0.337	(0.04)	0.524	(0.007)

The values in brackets represent the level of significance where $p\leq0.05$. For abbreviations see legend to table 1.

Relationship of lavage albumin to respiratory function tests

There appears to be a variable relationship between improvement in respiratory function tests and ALB RAT in the ILD group (table 2). The strongest relationship is with the DLCO ($r_s=0.52$, $p=0.007$).

Relationship of lavage albumin to smoking status

Within the group without ILD, there appears to be a correlation between the duration of cigarette consumption (pack years) and a decrease in the ALB RAT($r_s=-0.47$, $p=0.004$). Within the disease groups no such relationship is discernible.

Relationship of lavage albumin to age and ELF volume

In those without ILD, there is a tendency for the ALB RAT to rise with age ($r_s=0.42$, $p=0.01$), but no such association is discernible in the ILD group. There is also a strong negative correlation between the ELF volume and the ALB RAT in those without ILD ($r_s=-0.64$, $p=0.001$). There is, however, no clear relationship between the ALB RAT and either age or ELF volume in the ILD group.

Discussion

The accurate assessment and interpretation of data on the protein content of BAL fluid is dependent on the capacity to measure it in small quantities, without

the need to concentrate the sample [12], and on being able to obtain a suitable frame of reference. The variable dilution of the ELF during the lavage procedure makes it necessary to find a method of determining this dilution factor in order to be able to standardize measurement of both protein and cellular elements. A number of different markers have previously been used in addition to urea. These include the use of albumin itself as a reference standard, or calcium, potassium, technetium pertechnetate or methylene blue as markers of dilution [13, 14]. Although all methods have their limitations, there are both theoretical and practical considerations that suggest that urea provides the best estimate for an apparent ELF volume currently available [5, 13]. Although it agrees well with other direct measurement methods, it also has limitations and tends to overestimate the actual ELF volume [13, 15, 16]. This effect can be minimized by undertaking relatively small volume lavages, and minimizing the dwell-time of the saline prior to re-aspiration [13] as practised in this study.

The use of urea as a marker for ELF dilution remains controversial. The recent study of SCHMEKEL and VENGE [17] performed in normal volunteers, reported only that urea and albumin quantities retrieved from BAL fluid continued to rise proportionally with successive lavage aliquots. This finding implied continued movement of these substances across the epithelial capillary barrier during lavage, with the authors concluding that neither albumin nor urea were suitable markers of ELF dilution. Delayed recovery of initial aliquots and increased dwell-time are known to increase urea leakage, whilst both osmotic effects and disruption of the barrier by the trauma of lavage have been proposed as hypothetical mechanisms for such a finding [18]. Several other recent studies of subjects with ILD have reached the opposite conclusion, *i.e.* that urea is a satisfactory marker of ELF dilution in the diseased lung. Both JONES *et al.* [15] and VALEYRE *et al.* [19] recovered increased quantities of albumin in lavage fluid from subjects with ILD but observed no such increase in urea.

In our own study, it would be difficult to explain the findings presented simply in terms of increased leakage of urea and albumin as an artefactual consequence of the BAL itself. Firstly, there was no difference between the BAL volumes recovered in the four cluster groups, whilst the ELF volumes (and hence urea quantities) were significantly different (table 1). In smokers without ILD, the ELF volume was increased, whilst the albumin concentration was reduced compared with nonsmokers. This is likely to be a result of either increased ELF production or reduced clearance [7]. In contrast, in subjects with ILD, the ELF volume was increased whilst the albumin content was not significantly different from that of the nonsmokers without ILD (table 1). Finally, in the "bronchiolitis" group, the ELF volume was no different from nonsmokers without ILD and yet the albumin content was significantly higher (table 1). It is not possible to explain these various findings by one simple procedural effect.

Changes in ELF albumin are associated with cellular changes detectable in the lavage fluid in patients with ILD. The ALB RAT is strongly correlated with increase in lymphocytes recovered, whether this is measured in relative or absolute terms (table 2). Indeed, the correlation of the ALB RAT with total cells·ml^{-1} of ELF in the disease group (table 2) is probably only due to increase in the absolute number of lymphocytes recovered. Whilst the association may not be causal, it does suggest the possibility that the lymphocytes, but not the macrophages or neutrophils, are liberating substances that influence the permeability of the pulmonary capillary bed. What this substance might be is not clear, but one could speculate that it might be interleukin-2 (IL-2) or other lymphokines that are thought to mediate the non-cardiogenic pulmonary oedema well-known with the *in vivo* administration of IL-2 [20, 21]. In the lymphoid interstitial pneumonitic disorders, which are usually not associated with destruction of pulmonary architecture, changes in vascular permeability, with resultant lung oedema, may be an important cause of respiratory impairment. The association between BAL lymphocytosis, ELF albumin concentration and disease activity was also recently reported by VALEYRE *et al.* [19] in subjects with sarcoidosis, suggesting that the reporting of this finding is valid.

Smoking is an important factor that must be taken into account in the assessment of pulmonary pathology, and in subjects without ILD it is associated with a decrease in the ALB RAT (r_s=-0.47). This appears to be due largely to the increase in ELF that is seen in smokers (table 1) and, therefore, this represents a dilutional effect. There is no discernible relationship in the disease group with smoking, but this is likely to be due to the relatively small numbers of smokers, and to the presence of other factors that influence ALB RAT, making it difficult to define a relationship to smoking. Smokers, for the purposes of this study, are only identified as such if they have actually been smoking for the entire 2 yrs prior to the lavage. However, this does not take account of the length of time since the last cigarette, or measure the cigarettes consumed over a relatively short interval preceding the lavage. It may well be that the effects of smoking on ELF and ALB RAT are acute, and hence smoking immediately prior to the lavage may be much more relevant.

An increase in the lavage albumin content is also weakly associated with an increased gallium index in subjects without ILD (r_s=0.33). There is no overall association in the ILD cluster. The presence of immunoglobulin in the ELF, unlike albumin, may represent the net effect of both diffusion and local synthesis. Both IgG and IgM have a much higher molecular weight than albumin; IgG about 150,000 Da and IgM, which is usually in a pentameric form, about 800,000 Da. In the group without ILD, there is a high degree of concordance between the correlation

coefficients of ALB RAT to IgG RAT and ALB RAT to IgM RAT (table 2). However, this concordance is substantially less for the ILD group (r_s=0.54 for IgM RAT and r_s=0.82 for IgG RAT) (table 2), suggesting that in this group, local synthesis may play an important role.

Acknowledgements: The authors thank the Department of Biochemistry, St Vincent's Hospital for performing urea and albumin determinations.

References

1. Crystal RG, Reynolds HY, Kalica AR. – Bronchoalveolar lavage. The report of an international conference. *Chest*, 1986; 90: 122–131.
2. Daniele RP, Elias JA, Epstein PE, Rossman MD. – Bronchoalveolar lavage: role in the pathogenesis, diagnosis and management of interstitial lung diseases. *Anal Int Med*, 1985; 102: 93–108.
3. Gibson PG, Bryant DH, Robinson B, McLennan G, Breit SN. The role of bronchoalveolar lavage in the assessment of diffuse lung diseases. *Aust NZ J Med*, 1989; 19: 281–291.
4. Turner-Warwick ME, Haslam PL. Clinical Applications of bronchoalveolar lavage: an interim view. *Br J Dis Chest*, 1986; 80: 105–121.
5. Rennard SI, Basset G, Lecossier D, *et al.* – Estimation of volume of epithelial lining fluid recovered by bronchoalveolar lavage using urea as a marker of dilution. *J Appl Physiol*, 1986; 60: 532–538.
6. Thompson AB, Daughton D, Robbins RA, *et al.* – Intraluminal airway inflammation in chronic bronchitis. *Am Rev Respir Dis*, 1989; 140: 1527–1537.
7. Burke MJ, Roberts CM, Bryant DH, *et al.* – Smoking-induced changes in epithelial lining fluid volume, cell density and protein content. *Eur Respir J*, 1992; 5: 780–784.
8. Cairns D, Shelley L, Burke W, *et al.* – Interstitial lung disease associated with connective tissue diseases: the use of statistical structure analysis in model development. *Am Rev Respir Dis*, 1991; 143: 1235–1240.
9. Bisson G, Drapeau G, Lamoureux G, *et al.* – Computer-based quantitative analysis of gallium-67 uptake in normal and diseased lungs. *Chest*, 1983; 84: 513–517.
10. Saltini C, Hance AJ, Ferrans VJ, *et al.* – Accurate quantification of cells recovered by bronchoalveolar lavage. *Am Rev Respir Dis*, 1984; 130: 650–658.
11. Zaunders JJ, Ziegler JB, Penny R, Cooper DA. – A monoclonal antibody based radioimmunoassay for the *in vitro* production of IgE by lymphocyte cultures. *Int Arch Allergy Appl Immunol*, 1985; 78: 1–8.
12. Afford SC, Stockley RA, Kramps JA, Dijkman JH, Burnett D. – Concentration of bronchoalveolar lavage fluid by ultrafiltration: evidence of differential protein loss and functional inactivation of proteinase inhibitors. *Ann Biochem*, 1985; 151: 125–130.
13. Reynolds H, Newball H. – Analysis of proteins and respiratory cells obtained from human lungs by bronchial lavage. *J Lab Clin Med*, 1974; 84: 559–573.
14. Peterson TP, Idell S, Macarthur C, Gray LD, Cohen AB. – A modified bronchoalveolar lavage procedure that allows measurement of lung epithelial lining fluid volume. *Am Rev Respir Dis*, 1990; 141: 314–320.
15. Jones KP, Edwards JH, Peters TJ, Davies BH. – A comparison of albumin and urea as reference markers in bronchoalveolar lavage fluid from patients with interstitial lung disease. *Eur Respir J*, 1990; 3: 152–156.
16. Marcy TW, Merrill WW, Rankin JA, Reynolds HY. – Limitations of using urea to quantify epithelial lining fluid recovered by bronchoalveolar lavage. *Am Rev Respir Dis*, 1987; 135: 1276–1280.
17. Schmekel B, Venge P. – The distribution of myeloperoxidase, eosinophil cationic protein, albumin and urea in sequential bronchoalveolar lavage. *Eur Respir J*, 1991; 4: 517–523.
18. Walters EH, Gardiner PV. – Bronchoalveolar lavage as a research tool. *Thorax*, 1991; 46: 613–618.
19. Valeyre D, Soler P, Banet G, *et al.* – Glucose, K^+ and albumin concentrations in the alveolar milieu. *Am Rev Respir Dis*, 1991; 143: 1096–1101.
20. Glauser FL, DeBlois GG, Berchard DE, *et al.* – Cardiopulmonary effects of recombinant interleukin-2 infusion in sheep. *J Appl Physiol*, 1988; 64: 1030–1037.
21. Rosenstein M, Ettinghausen SE, Rosenberg SA. – Extravasation of intravascular fluid mediated by the systemic administration of recombinant interleukin-2. *J Immunol*, 1986; 137: 1735–1742.