



Bronchoalveolar lavage causes decrease in PaO_2 , increase in $(A-a)$ gradient value and bronchoconstriction in asthmatics

A. SPANEVELLO*, G. B. MIGLIORI*, A. SATTA*, A. SHARARA[†], L. BALLARDINI, P. W. IND[‡] AND M. NERI*

*Division of Pneumology, Fondazione Salvatore Maugeri, Clinica del Lavoro e della Riabilitazione, Care and Research Institute, Tradate, Italy

[†]Respiratory Medicine, Royal Postgraduate Medical School, Hammersmith Hospital, London, U.K.

[‡]Bioengineering Department, Fondazione Salvatore Maugeri, Tradate, Italy

The aims of this study were to (1) record the changes of (arterial oxygen partial pressure) PaO_2 , (arterial carbon dioxide partial pressure) $PaCO_2$, (percentage saturation of haemoglobin with oxygen in arterial blood) SaO_2 and alveolar-arterial $(A-a)$ oxygen gradient resulting from bronchoalveolar lavage (BAL) in asthmatic and normal subjects; (2) measure changes in forced expiratory volume in 1 s (FEV_1), vital capacity forced (FVC) associated with BAL; and (3) assess possible predictive factors for the degree of hypoxaemia and impairment of spirometry resulting from BAL.

Bronchoscopy and BAL (150 ml) were performed in 24 asthmatics and 15 healthy subjects. Serial arterial blood samples (radial artery) were obtained in all subjects: T_1 and before T_2 after local anaesthesia; T_3 at end of bronchoscopy; T_4 after BAL and 5 min, 15 min, 1 h, 2 h, 8 h and 24 h (T_5 - T_{10}) after the procedure, FEV_1 and FVC were measured immediately before and 5 min after bronchoscopy.

Baseline PaO_2 was lower in asthmatics (10.2 ± 0.8 kPa) than in healthy subjects (10.8 ± 0.8). Both groups showed a significant decrease in PaO_2 , and a significant widening in $(A-a)$ oxygen tension gradient at T_{3-9} , with respect to T_1 ($P < 0.05$). PaO_2 reached a significantly lower value in asthmatics (7.1 ± 0.6 kPa) than in HS (7.7 ± 0.5 ; $P < 0.05$). In asthmatics, FEV_1 , FVC and the ratio FEV_1/FVC decreased significantly after BAL ($P < 0.001$). In healthy subjects, FEV_1 and FVC decreased significantly ($P < 0.001$), whereas FEV_1/FVC did not. The fall in FEV_1 after BAL was significantly greater in asthmatics ($32.4 \pm 10.0\%$) than in healthy subjects (17.7 ± 4.6 ; $P < 0.001$). Severity of asthma, baseline FEV_1 or initial PaO_2 did not predict the degree of hypoxaemia or the fall of FEV_1 .

It is concluded that BAL causes more severe hypoxaemia and a greater decrease in FEV_1 in asthmatics compared to healthy subjects, strongly supporting the recommendation of special caution and careful monitoring when BAL is undertaken in asthmatics.

RESPIR. MED. (1998) 92, 191-197

Introduction

Fibre-optic bronchoscopy with bronchoalveolar lavage (BAL) is commonly used in the investigation of various pulmonary diseases (1,2). In recent years, BAL has been used increasingly to investigate the basic mechanisms of bronchial asthma, and numerous publications have been devoted to the use of bronchoscopy with BAL in asthmatic patients (3-8).

The safety of bronchoscopic procedures in the asthmatic patient has been a matter of obvious concern. Short-term reduction in pulmonary function (forced expiratory volume in 1 s = FEV_1 ; forced vital capacity, FVC) has been demonstrated after the procedure (9-13). Some authors have suggested that bronchoconstriction is the principal mechanism causing a more marked fall in FEV_1 in asthmatic subjects (11); whereas others, in contrast, suggested a more restrictive reduction in lung function (13). Some investigators have found a more marked decrease in oxygen saturation in patients with mild asthma than in normal subjects (11), whereas others have shown no differences in BAL-associated desaturation between asthmatics and normal subjects (13). In these studies (11,13), using digital oximetry, oxygen saturation returned to normal soon after termination of the procedure. The analysis of arterial blood

Received 20 November 1996 and accepted in revised form 26 March 1997.

Correspondence should be addressed to: A. Spanevello, Fondazione Salvatore Maugeri, Via Roncaccio 16, 21049 Tradate, Italy.

TABLE 1. Subject characteristics: asthmatics and healthy subjects

	Number	Age (years)	Sex (male/female)	FEV ₁ (% predicted)	FVC (% predicted)
Asthmatics	24	58 ± 10	18M/6F	79.1 ± 16.3	94.0 ± 10.4
Healthy subjects	15	54 ± 12	3M/12F	105.3 ± 10.8	110.1 ± 12.7

gases represents a more precise technique for examining the effects of BAL on lung function. Moreover the analysis of arterial oxygen partial pressure (P_{aO_2}) and arterial carbon dioxide (P_{aCO_2}) gives information on the perfusion-ventilation relationship through the behaviour of alveolar arterial ($A-a$) oxygen tension gradient. To the authors' knowledge, no studies have analysed the behaviour of arterial blood gases and ($A-a$) gradient associated with BAL in asthmatics, and monitored arterial blood gases for a period of up to 24 h after BAL.

The aims of this study were to (1) record the changes of P_{aO_2} , P_{aCO_2} and ($A-a$) gradient resulting from BAL in asthmatics during and up to 24 h after the procedure; (2) measure changes in FEV₁ and FVC associated with BAL; and (3) assess possible predictive factors for the degree of hypoxaemia and impairment of spirometry resulting from BAL in asthmatics.

Methods

STUDY POPULATION

Twenty-eight asthmatics were enrolled in the study. Two of them were excluded from data analysis because the endoscopic procedure was suspended due to severe dyspnoea, and they were given inhaled salbutamol and intravenous theophylline. Two more subjects were excluded because it proved impractical to collect arterial blood sample. The data of 24 asthmatic subjects aged 58 ± 10 years were analysed (Table 1). Asthma was defined according to the criteria of the American Thoracic Society (14). All subjects had a clinical history of intermittent wheeze, cough, chest tightness or dyspnoea, and documented reversible airway limitation either spontaneously or with treatment in the preceding year.

Subjects' FEV₁ and FVC was, respectively, 79.1 ± 16.3 and 94.0 ± 10.4, expressed as mean ± SD of predicted value % (15). None of the subjects were current smokers and none had smoked within the previous 3 years. None of the subjects were taking oral steroids or long-acting β_2 -agonists. Nine subjects were taking inhaled steroids. The clinical severity of asthma was assessed according to the scoring system of Aas (16). Fifteen non-smoking subjects aged 54 ± 12 years were studied (Table 1), recruited from subjects who were referred to the asthma clinic for subjective breathless or periodic cough and had negative investigations. They came to the laboratory on three occasions. On Day 1, spirometry, chest X-ray and peak expiratory flow (PEF) monitoring were performed and all subjects

gave their written consent. On Day 2, methacholine challenge was performed and 1 week later (Day 3), bronchoscopy was performed. No subject presented any respiratory symptom at the time of bronchial challenge or bronchoscopy. Mean FEV₁ was 105.3 ± 10.8 (% predicted), FEV was 110.1 ± 12.7, and mean PEF daily variability (highest PEF – lowest PEF/highest PEF × 100) was <10%, over a monitoring period of 2 weeks before the endoscopic procedure, using a Wright peak flow meter (Markos, Monza, Italy) (17). Provocative dose of methacholine for a 20% fall in FEV₁ (PD₂₀) was >1.5 mg in all healthy subjects.

Bronchial challenge with methacholine was performed according to a method modified from the SEPCR working group guidelines to calculate the PD₂₀ (15). The solutions were administered by nebulizer (Mefar MB3, Bavezzo, Brescia, Italy), and PD₂₀ was calculated by interpolation. The study was approved by the Ethics Committee of Fondazione Salvatore Maugeri, and all subjects gave written informed consent.

PULMONARY FUNCTION

Pulmonary function was assessed with a dry wedge spirometer (Vitalograph, Buckinghamshire, U.K.) by measuring FEV₁ and FVC. At least three measurements were obtained and the values associated with the best FEV₁ were recorded by the same operator (PG). Spirometry was performed just before and 5 min after endoscopic procedure was completed. β_2 agonist (Salbutamol) was withheld for at least 8 h before testing.

ENDOSCOPIC PROCEDURE

Pre-medication consisted of intramuscular atropine (0.5 mg), intramuscular diazepam (5 mg) and local anaesthesia with 2% lignocaine applied to the upper respiratory tract in all subjects. With the subjects in a supine position, an Olympus BF1T20 bronchoscope (Olympus Optical Co., Tokyo, Japan) was introduced through the mouth, and 1% lignocaine solution was administered through the bronchoscope channel to provide anaesthesia for the airways below the vocal cords. Duration of the procedure was timed from intubation of the vocal cords until extraction of the bronchoscope using an electric timer (Casio 376, ARW-320, Japan). The airways were systematically examined, the bronchoscope was wedged into one of the subsegmental bronchi of the middle lobe and BAL was performed using three aliquots of 50 ml saline pre-warmed at 37°C,

re-aspirated by gentle 50 ml syringe suction by the same operator (PG) for all bronchoscopies. During bronchoscopy, oxygen, electrocardiographic monitoring and full resuscitation were readily available and the patient had an intravenous infusion to provide venous access. During the procedure, HbO₂ saturation was continuously monitored in all subjects using oxymetry (Pulsox 7—Minolta; AVL AG, Schaffhausen, CH) with a finger probe. All subjects were given salbutamol 300 µg by metered-dose inhaler, immediately after the collection of arterial blood sample, 15 min after endoscopic procedure. Asthmatics restarted therapy that they were taking after the arterial blood gas analysis performed 8 h after the end of the endoscopic procedure. The protocol called for the procedure to be terminated if either the patient had severe asthma symptoms or HbO₂ saturation (monitored by oximetry) fell below 80%. In this case, oxygen would have been administered and bronchodilator therapy given.

ARTERIAL BLOOD GAS ANALYSES

Ten arterial blood samples were obtained for each subject at the following times (T): T₁ before and T₂ after local anaesthesia with 2% lignocaine; T₃ at end of routine bronchoscopy when the bronchoscope was wedged ready for BAL, T₄ at end of BAL with the bronchoscope still wedged, T₅ 5 min and T₆ 15 min after the end of the procedure; and T₇, T₈, T₉, T₁₀ (respectively) 1, 2, 8 and 24 h after the end of the procedure. All the arterial blood samples were obtained by the same operator (GBM) using a syringe (Microsample—AVL, Schaffhausen, CH) with a thin needle (Microlance 256, 0.5 × 16, Dublin, Ireland). Arterial blood gas analyses were performed immediately on an ABL 330 (Radiometer, Copenhagen, Denmark) blood gas analyser situated in the same room where bronchoscopy was performed.

PREDICTIVE FACTORS FOR HYPOXAEMIA AND IMPAIRMENT OF SPIROMETRY

For the analysis of predictive factors, the authors correlated PaO₂ at time T₁, basal FEV₁ measured before bronchoscopy and Aas score with the lowest value of PaO₂ occurring in asthmatics during the procedure, and the percentage fall in FEV₁ caused by the procedure.

STATISTICAL ANALYSIS

All the evaluated parameters were analysed by descriptive statistics. FEV₁ and FVC were expressed as a percentage of predicted value (15). SaO₂ as percentage, PaO₂, PaCO₂ and (A-a) gradient are in kiloPascals (kPa). The distribution of the different parameters (FEV₁; FVC; PaO₂; PaCO₂) was evaluated for normality using the Statgraphics Statistical Package, version 7. Since the data were normally distributed, parametric tests were used. To detect differences concerning a variable measured at different times, ANOVA for repeated measurements was applied (multifactor ANOVA procedure of Statgraphics). Student's *t*-test with

Bonferroni correction was applied to detect significant changes in PaO₂, PaCO₂, SaO₂ and (A-a) gradient in the same subject (paired) or between two groups (unpaired). To detect differences concerning FEV₁, FVC and FEV₁/FVC in the same subjects, Student's paired *t*-test was used. To evaluate the difference between groups, Student's unpaired *t*-test was applied. Correlation coefficients, to study the association between two variables, were calculated by linear regression. A *P* value < 0.05 was considered statistically significant.

Results

BAL FLUID RECOVERY AND DURATION OF THE PROCEDURE

BAL fluid recovery was significantly lower at 56.6 ± 11.0 ml in asthmatic patients compared with 73.0 ± 13.3 ml in controls (*P* < 0.001). Total duration of procedure did not differ in asthmatics (641 ± 80 s) from that in healthy subjects (603.3 ± 45.9 s) (*P* > 0.05).

ARTERIAL BLOOD GASES

PaO₂, PaCO₂, SaO₂ and (A-a) gradient values at the different times before, during and after the procedure are shown in Figs 1 and 2. A significant fall in PaO₂ was observed from T₁ to T₃₋₉ in asthmatic and healthy subjects. The mean value of minimum PaO₂ values (7.10 ± 0.60) reached in asthmatics (at T₄ or T₅) was significantly lower than that in control subjects compared (7.75 ± 0.47; *P* < 0.05). A significant fall in PaCO₂ was observed from T₁ to T₃ in asthmatic and healthy subjects. A significant increase in (A-a) gradient was observed from T₁ to T₃₋₉ in asthmatic and healthy subjects. The width of (A-a) gradient was not significantly different between the two groups at different times (T₁–T₁₀). There was no significant difference between the mean values of minimum PaO₂ reached during bronchoscopy in asthmatics treated with inhaled steroids (PaO₂ = 7.0 ± 0.5) and asthmatics without inhaled steroids (PaO₂ = 7.2 ± 0.6).

PULMONARY FUNCTION

In asthmatic patients, FVC decreased significantly from 94.0 ± 10.4 to 70.5 ± 12.4% predicted (*P* < 0.001), FEV₁ decreased significantly from 79.1 ± 16.3 to 53.5 ± 14.1% predicted (*P* < 0.001) and the ratio FEV₁/FVC decreased significantly from 68.0 ± 9.8 to 61.5 ± 10.1% (*P* < 0.001; Fig. 1). In healthy subjects, FVC decreased significantly from 110.1 ± 12.7 to 92.1 ± 13.7% predicted (*P* < 0.001) and FEV₁ decreased significantly from 105.3 ± 10.8 to 86.2 ± 10.0% predicted (*P* < 0.001) with the procedure. However, the ratio FEV₁/FVC did not change significantly from 80.2 ± 6.6 to 78.7 ± 7.7% (*P* > 0.05; Fig. 3). The fall in FEV₁ after the procedure was significantly higher in asthmatics than in healthy subjects (32.4 ± 10.0 vs 18.2 ± 4.6%;

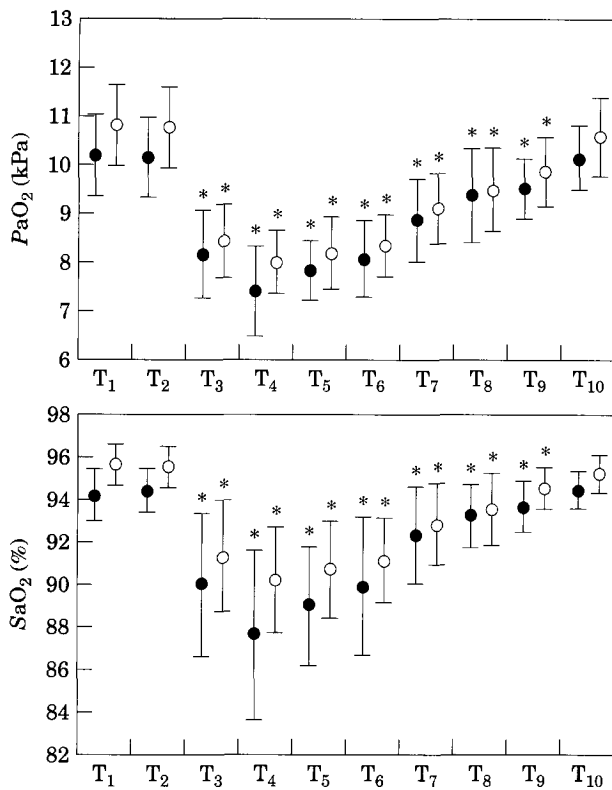


FIG. 1. Evolution of changes in PaO₂ (kPa) and SaO₂ (%) presented as mean ± SD in asthmatics (AS, ●) and healthy subjects (HS, ○) at different times before, during and after the procedure. *Significant difference ($P < 0.05$) when comparing values of the different times with respect to the value at baseline (T₁). T₁ before and T₂ after local anaesthesia with lignocaine; T₃ at end of bronchoscopy; T₄ at end of BAL; T₅ 5 min and T₆ 15 min after the end of the procedure; T₇–T₁₀ (respectively) 1, 2, 8 and 24 h after the end of the procedure.

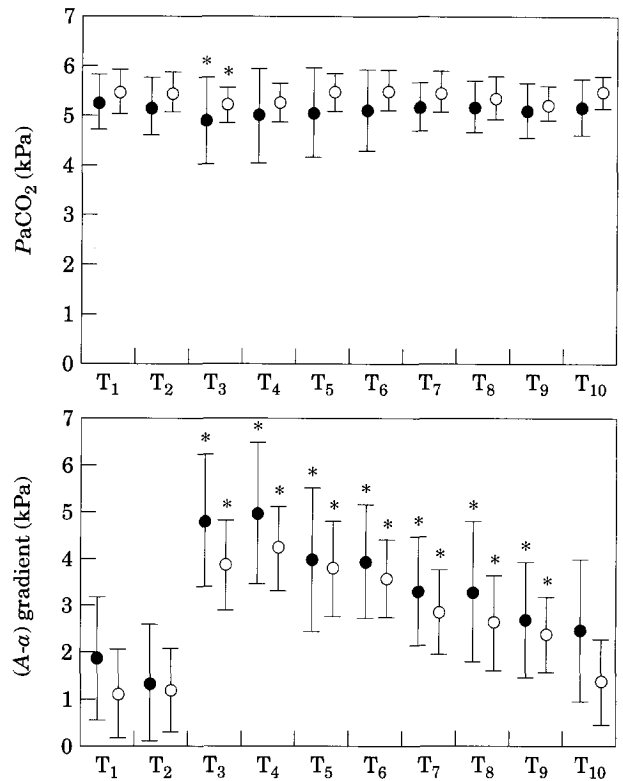


FIG. 2. Evolution of changes in PaCO₂ and (A-a) gradient (kPa) presented as mean ± SD in asthmatics (AS, ●) and healthy subjects (HS, ○) at different times before, during and after the procedure. *Significant difference ($P < 0.05$) when comparing values of the different times with respect to the value at baseline (T₁). T₁ before and T₂ after local anaesthesia with lignocaine; T₃ at end of bronchoscopy; T₄ at end of BAL; T₅ 5 min and T₆ 15 min after the end of the procedure; T₇–T₁₀ (respectively) 1, 2, 8 and 24 h after the end of the procedure.

$P < 0.001$). The same was observed for FVC (24.9 ± 11.0 vs $16.5 \pm 5.9\%$; $P < 0.05$). The fall in FEV₁, expressed as percentage of the initial value, was significantly greater than the fall in FVC in asthmatics ($P < 0.001$), whereas it did not differ significantly in healthy subjects ($P > 0.05$). There was no significant difference between the fall in either FEV₁ (%) or FVC (%) determined by the endoscopic procedure in asthmatics treated with inhaled steroids (FEV₁ = 33.1 ± 8.8 ; FVC = 23.2 ± 11.2) and asthmatics without inhaled steroids (FEV₁ = 32.0 ± 10.9 ; FVC = 26.0 ± 11.2).

PREDICTIVE FACTORS FOR HYPOXEMIA AND IMPAIRMENT OF SPIROMETRY

There was no significant correlation between the lowest value of PaO₂ reached in asthmatics during the procedure and PaO₂ at time T₁ ($r = -0.16$), or basal FEV₁ ($r = 0.07$) or Aas score ($r = -0.04$; $P > 0.05$). Similarly, there was no significant correlation between the same factors and the percentage fall in FEV₁ caused by the procedure in

asthmatics (PaO₂ at the time T₁; $r = 0.29$; basal FEV₁; $r = 0.02$; Aas score: $r = 0.03$; $P > 0.05$).

Discussion

This study assessed the behaviour of PaO₂, PaCO₂ and (A-a) gradient during and up to 24 h after BAL, and showed changes in FEV₁ and FVC caused by BAL in both asthmatics and healthy subjects. In addition, possible factors predictive of the magnitude of O₂ desaturation and impairment of spirometric values were assessed. There was a significant fall in PaO₂ and a significant widening of (A-a) gradient in both groups of subjects during and up to 8 h after the procedure. Asthmatics reached a significantly lower value of PaO₂. PaCO₂ did not increase significantly at any time during the procedure. There was a decrease of FEV₁ and FVC after BAL in asthmatic and healthy subjects, but in asthmatics, the reduction in FEV₁ and FVC was significantly greater than in healthy subjects. In

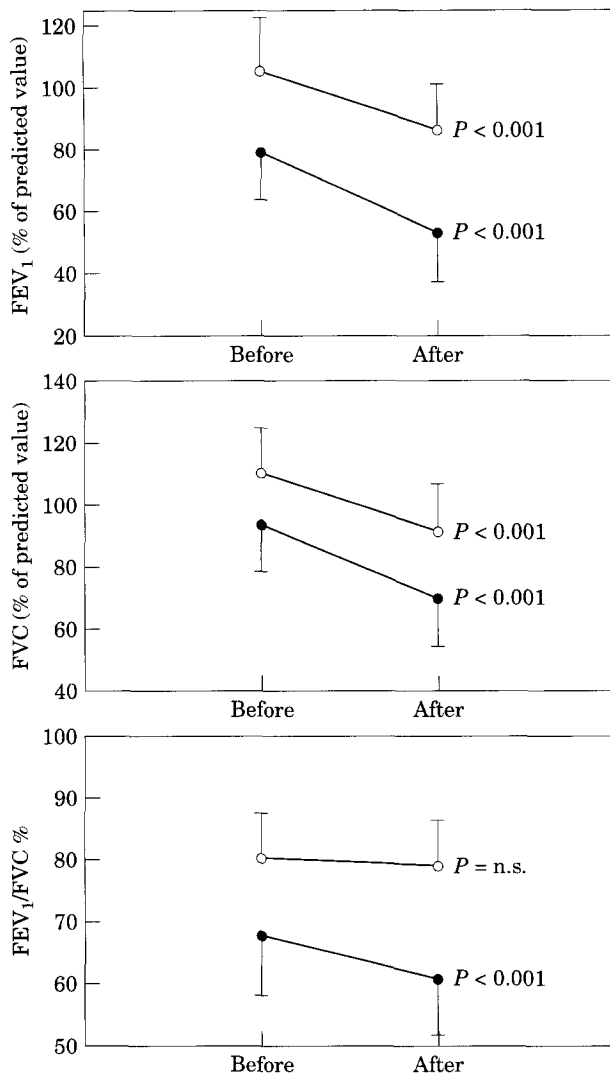


FIG. 3. Evolution of changes in pulmonary function (FEV₁; FVC; FEV₁/FVC) before and after endoscopic procedure in asthmatic (●) and healthy (○) subjects.

asthmatics, the magnitude of decrease of PaO₂ and FEV₁ was not predicted by the severity of asthma, baseline PaO₂ or basal FEV₁.

In the present study, a significant fall in PaO₂ was observed at the end of routine bronchoscopy (T₃) and at end of BAL (T₄) with respect to the value of PaO₂ at baseline (T₁) in both asthmatic and healthy subjects. BAL was performed using three 50 ml aliquots of sterile saline, prewarmed at 37°C, without bronchial biopsies, β₂-agonist pre-treatment and oxygen administration. The present results differ from those obtained by Djukanovic *et al.* (11) who performed BAL (eight 20 ml aliquots of saline prewarmed to 37°C) and bronchial biopsies in asthmatics after albuterol and ipratropium bromide pre-medication and oxygen delivery during the procedure. They did not observe a significant decrease in oxygen saturation after BAL in asthmatic and normal subjects. Van Vyne *et al.* (13) performed BAL (five 50 ml aliquots of saline at room temperature) and bronchial biopsies in asthmatics and normals

without bronchodilator pre-medication and oxygen delivery. They demonstrated a significant decrease in oxygen saturation after BAL in asthmatic and normal subjects. The lack of oxygen administration in Van Vyne's and the present study could explain the differences between those results and Djukanovic's data. This observation is supported by Dubrawsky's study (18), in which patients with various pulmonary diseases, given supplemental oxygen, did not show a significant reduction in PaO₂, while this occurred in patients who did not receive supplemental oxygen during two bronchial washes, totally 80 ml of normal saline at room temperature. In the present study, the minimum value of PaO₂ reached in asthmatics was significantly lower than that observed in healthy subjects. The authors can suggest the possible role played by the lower baseline value of PaO₂, and lower fluid recovery observed in asthmatics. In fact, the difference in fluid recovery of asthmatics and healthy subjects might be one of the reasons determining different falls of PaO₂, as suggested by Pirozynsky *et al.* (19).

PaO₂ was significantly reduced 1, 2 and 8 h after the procedure, and at 5 and 15 min. In a previous study, Burns *et al.* (20) showed that healthy subjects lavaged with 1000 ml of room temperature saline developed abnormalities in their ventilation-perfusion relationships, which persisted for 6–8 h after lobar lavage. This fact could explain the persisting significant decrease of PaO₂ until after 8 h of BAL in the present study. Moreover, the authors have found a significant widening of (A-a) gradient during and after BAL in asthmatic and healthy groups resulting in a reduction of efficiency of respiratory gases exchange, with a low ventilation-perfusion ratio, still present 8 h after the procedure. This could be explained by 'flooding' of bronchoalveolar spaces.

The present study showed a significant fall in FEV₁ and FVC after the endoscopic procedure with BAL in asthmatic and healthy subjects in line with other studies (9,11,13). Van Vyne *et al.* found no change in FEV₁/FVC ratio after the procedure in either asthmatics or control subjects, suggesting a more restrictive reduction in lung function. In the present study, all subjects experienced some restriction, demonstrated by a significant fall in FEV₁ and FVC in both asthmatics and healthy subjects. Moreover, a significant decrease in FEV₁/FVC ratio was observed after BAL in asthmatics, whilst the ratio did not change in healthy subjects, suggesting a more obstructive reduction in lung function in asthmatics. This is emphasized by the more marked fall in FEV₁ in asthmatics. The more marked fall in FEV₁ in asthmatics may be related to the increased tendency (non-specific bronchial responsiveness) to bronchoconstriction after lignocaine and forced expiratory manoeuvre, e.g. coughing (21), or physical stimulation by the bronchoscope and BAL. The present authors did not observe a significant correlation between the lowest value of PaO₂ in asthmatics and baseline FEV₁ or the clinical severity of asthma, characterized by the Aas core, as found in previous studies (11,13). Furthermore, it was found that baseline PaO₂ value cannot be considered a predictive factor for hypoxaemia determined by BAL. Previous studies have shown that arterial O₂ desaturation is

dependent upon the volume of saline instilled (19,20) and the duration of the procedure (19), but in the present study, the volume of lavage was constant in all subjects and there was no great variation in the time required to complete the procedure in any of the subjects or between the two groups studied. In conclusion, this study has shown that: (i) BAL produces a significant reduction of PaO_2 and an increased width of (A-a) gradient, without increasing $PaCO_2$ during and up to 8 h after BAL in asthmatic subjects with asthma of variable severity; (ii) the extent and time course of reduction of PaO_2 and the increased width of (A-a) gradient are similar in asthmatics and healthy subjects, but asthmatics reach a significantly lower value of PaO_2 ; (iii) the fall in FEV_1 and FVC after BAL is significantly greater in asthmatics than in healthy subjects; (iii) the fall in PaO_2 and FEV_1 cannot be predicted by the severity of asthma, baseline PaO_2 and FEV_1 . Although this study has confirmed previous findings that BAL can be performed without β_2 -agonist, pre-medication and oxygen delivery during and after the procedure in asthmatics, the results have shown a significant decrease of PaO_2 lasting up to 8 h, a lower value of PaO_2 during the procedure and a mean reduction in FEV_1 of 32% demonstrating significant adverse effects which may not be apparent clinically. Therefore, the authors strongly support the recommendations of oxygen administration during bronchoscopy, and special caution and careful monitoring when BAL is undertaken in asthmatic subjects (22,23); additionally the authors recommend that oxygen saturation should be frequently monitored after the end of the procedure, and that pre-medication with β_2 -agonists should be administered before the endoscopic procedure in asthmatics.

Acknowledgements

The authors wish to thank P. Vaghi, D. Brovelli and E. Radice (Department of Biostatistic, Clinica del Lavoro Foundation, Tradate, Italy) for the relevant contribution in data analysis, and P. Bridge, A. Bianchi and B. Beghè for their useful comments on the manuscript. This study was partially supported by the Fund for Current Research, Ministry of Health, Italy, 1993.

References

1. Reynolds HY. Bronchoalveolar lavage. State of the art. *Am Rev Respir Dis* 1987; **135**: 250–263.
2. Klech H, Hutter C. Clinical guidelines and indications for bronchoalveolar lavage (BAL): Report of the European Society of Pneumology Task Group on BAL. *Eur Respir J* 1990; **3**: 937–974.
3. Godard P, Aubas P, Calvayrac P *et al.* Endoscopie et lavage bronchiolo-alveolaire chez l'asthmatique allergique. *Nouv Presse Med* 1981; **10**: 3141–3148.
4. De Monchy JGR, Kaufmann HF, Venge P *et al.* Bronchoalveolar eosinophilia during allergen induced late asthmatic reactions. *Am Rev Respir Dis* 1985; **131**: 373–376.
5. Fick RB, Richerson HB, Zavala DC *et al.* Bronchoalveolar lavage in allergic asthmatics. *Am Rev Respir Dis* 1987; **135**: 1204–1209.
6. Beasley R, Roche WR, Roberts JA *et al.* Cellular events in the bronchi in mild asthma and after bronchial provocation. *Am Rev Respir Dis* 1989; **139**: 806–817.
7. Kirby JG, Hargreave E, Gleich G *et al.* Bronchoalveolar cell profiles of asthmatic and non asthmatic subjects. *Am Rev Respir Dis* 1987; **136**: 379–383.
8. Bousquet J, Chanez P, Lacoste JY *et al.* Eosinophilic inflammation in asthma. *N Engl J Med* 1990; **323**: 379–383.
9. Kelly C, Hendrick D, Walters H. The effect of bronchoalveolar lavage on bronchial responsiveness in patients with airflow obstruction. *Chest* 1988; **93**: 325–328.
10. Chetta A, Foresi A, Bertorelli G *et al.* Lung function and bronchial responsiveness after bronchoalveolar lavage and bronchial biopsy performed without pre-medication in stable asthmatic subjects. *Chest* 1992; **101**: 1563–1568.
11. Djukanovic R, Wilson RW, Lai CKW *et al.* The safety aspects of fiberoptic bronchoscopy, bronchoalveolar lavage, and endobronchial biopsy in asthma. *Am Rev Respir Dis* 1991; **143**: 772–777.
12. Gianorio P, Bonavia M, Crimi E *et al.* Bronchial responsiveness is not increased by bronchoalveolar and bronchial lavage performed after allergen challenge. *Am Rev Respir Dis* 1991; **143**: 105–108.
13. Van Vyve T, Chanez P, Bousquet J *et al.* Safety of bronchoalveolar lavage and biopsies in patients with asthma of variable severity. *Am Rev Respir Dis* 1992; **146**: 116–121.
14. Committee on Diagnostic Standards for Nontuberculous Respiratory Diseases. Chronic bronchitis, asthma, and pulmonary emphysema. *Am Rev Respir Dis* 1962; **85**: 762–768.
15. Quanjer PH, ed. Report of Working Party. European Community for Coal and Steel. Standardized lung function testing: 'Standardization of Lung Function Tests' *Bull Eur Physiopathol Respir* 1983; **19** (Suppl 5): 7–87.
16. Aas K. Heterogeneity of bronchial asthma: subpopulations or different stages of the disease. *Allergy* 1981; **36**: 3–10.
17. National Heart, Lung and Blood Institute, National Asthma Education Program. Expert Panel Report: Guidelines for the diagnosis and management of asthma. *J Allergy Clin Immunol* 1991; **88**: (Suppl 3): 425–434.
18. Dubrawsky C, Awe RJ, Jenkins DG *et al.* The effect of bronchofiberscopic examination on oxygenation status. *Chest* 1975; **67**: 137–140.
19. Pirozynski M, Sliwinski P, Zielinski J. Effect of different volumes of BAL fluid on arterial oxygen saturation. *Eur Respir J* 1988; **67**: 943–947.
20. Burns DM, Shure D, Francoz R *et al.* The physiologic consequences of saline lobar lavage in healthy human adults. *Am Rev Respir Dis* 1983; **127**: 695–701.

21. Peacock AJ, Benson-Mitchell R, Godfrey RC. Effect of fiberoptic bronchoscopy on pulmonary function. *Thorax* 1990; **45**: 38–41.
22. European Society of Pneumology Task Group on BAL. Technical recommendations and guidelines for bronchoalveolar lavage (BAL). *Eur Respir J* 1989; **2**: 561–585.
23. Summary and recommendations of a workshop on the investigative use of fiberoptic bronchoscopy and bronchoalveolar lavage in asthmatics. *Am Rev Respir Dis* 1985; **132**: 180–182.