

Bronchial Exudate of Serum Proteins During Asthma Attack

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OBJECTIVE: Although altered vascular permeability and edema of the bronchial mucosa are associated with asthma attack, their influence on its severity remains unknown. We address this issue by comparing relative indices for the concentration of albumin (RIAlb) and α_2 -macroglobulin (RI α_2 M) in induced sputum and peripheral blood from patients with exacerbated asthma, patients with stable asthma, and control subjects.

PATIENTS AND METHODS: Forty-six volunteers participated in the study: 14 with exacerbated asthma (forced expiratory volume in the first second [FEV₁] 74.3% [SD, 20.8%] of reference), 23 with stable asthma (FEV₁ 93.6% [7.5%]), and 9 controls (FEV₁ 101.1% [9.9%]). The concentrations of albumin and α_2 -macroglobulin were quantified by immunoturbidimetry and immunonephelometry, respectively. The relative index was then calculated by dividing the concentration in sputum supernatant by the concentration in peripheral blood.

RESULTS: The mean RIAIb was 1.2 (1.1) in the control group, 2.9 (3.1) in the stable asthma group, and 6.0 (6.7) in the exacerbated asthma group. The RI α_2 M values were 11.7 (10.9), 11.9 (14.7), and 3.2 (3.8) for the control group and stable and exacerbated asthma groups, respectively. The increases in the RIAIb values between all groups, and the decrease in the RI α_2 M value between the exacerbated asthma and control groups were statistically significant ($P < .05$). The percentage of neutrophils, but not of eosinophils, in sputum was correlated with the RIAIb ($r = 0.39$; $P = .008$) but not the RI α_2 M ($r = -0.035$; $P = .82$). FEV₁ displayed an inverse relationship with the RIAIb ($r = -0.43$; $P = .009$) but not with the RI α_2 M ($r = -0.206$; $P = .24$). No correlation was found between oxyhemoglobin saturation and either the RIAIb ($r = -0.33$; $P = .19$) or the RI α_2 M ($r = -0.12$; $P = .84$).

CONCLUSIONS: Vascular permeability is altered during asthma exacerbations and appears to be correlated with the presence of neutrophils and the degree of bronchial obstruction.

Key words: Albumin. α_2 -macroglobulin. Induced sputum. Eosinophils. Neutrophils. Crisis, asthmatic.

Exudado bronquial de proteínas séricas en las crisis de asma

OBJETIVO: La alteración de la permeabilidad vascular y el edema de la mucosa bronquial se asocian con las crisis de asma. Hay pocos datos publicados y además no se conoce su relación con la gravedad de éstas. Así se propuso comparar los índices relativos de albúmina (RIAlb) y de macroglobulina α_2 (IRM α_2), entre esputo inducido y sangre periférica, de asmáticos agudizados (AA), asmáticos estables (AE) y controles.

PACIENTES Y MÉTODOS: Se estudió a 46 voluntarios: 14 del grupo AA (volumen espiratorio forzado en el primer segundo [FEV₁]: 74,3 \pm 20,8), 23 del AE (FEV₁: 93,6 \pm 7,5) y 9 controles (FEV₁: 101,1 \pm 9,9). Se cuantificó la concentración de albúmina (turbidimetría inmunoquímica) y de macroglobulina α_2 (nefelometría inmunoquímica) en el sobrenadante del esputo y en sangre venosa periférica y se calcularon los índices relativos.

RESULTADOS: La media \pm desviación estándar del RIAIb fue de 1,2 \pm 1,1 en el grupo control, de 2,9 \pm 3,1 en AE y de 6,0 \pm 6,7 en AA. El IRM α_2 fue 11,7 \pm 10,9, 11,9 \pm 14,7 y 3,2 \pm 3,8, respectivamente. El incremento del RIAIb entre los grupos AA, AE y control, y el descenso del IRM α_2 entre AA y control fueron estadísticamente significativos ($p < 0,05$). Se relacionó el porcentaje de neutrófilos, y no el de eosinófilos, con el RIAIb ($r = 0,39$; $p = 0,008$), pero no con el IRM α_2 ($r = -0,035$; $p = 0,82$). El FEV₁ se relacionó inversamente con el RIAIb ($r = -0,43$; $p = 0,009$) y no con el IRM α_2 ($r = -0,206$; $p = 0,24$), y tampoco se relacionó la saturación de oxihemoglobina con el RIAIb ($r = -0,33$; $p = 0,19$) o el IRM α_2 ($r = -0,12$; $p = 0,84$).

CONCLUSIONES: La permeabilidad vascular está alterada en las agudizaciones de asma y parece relacionarse con la presencia de neutrófilos y el grado de obstrucción bronquial.

Palabras clave: Albúmina. Macroglobulina α_2 . Esputo inducido. Eosinófilos. Neutrófilos. Crisis de asma.

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Introduction

Asthma is a disease in which periods of clinical stability alternate with exacerbations that appear to be related to the presence of underlying inflammation.¹ Although the inflammatory response has not been extensively studied in vivo, experimental models using bronchial provocation with allergens suggest that it is

biphasic.² The early phase would be characterized by bronchoconstriction and altered vascular permeability caused by the release of preformed mediators. This would then be followed by a late phase characterized by the presence of cellular infiltrates induced by the prior release of mediators and would represent the more chronic phase of inflammation. Thus, altered vascular permeability during asthma attack would cause leakage of serum components into the interstitial space (edema) and the bronchial lumen.³ Although it is assumed that this phenomenon participates in the appearance of symptoms,⁴ it is not known whether these changes have any effect on gas exchange or bronchial obstruction during asthma attack.

One of the reasons for this gap in our understanding is probably the absence of a safe and reliable method that would allow samples of respiratory secretions to be obtained in the acute phase of asthma for the measurement of markers of pulmonary vascular permeability. Nowadays this should not present a problem. Firstly, various markers of pulmonary vascular permeability have been described in patients with stable asthma.³ Probably the best known example, due to its simplicity and widespread clinical availability, is the concentration of plasma proteins in respiratory secretions. Secondly, noninvasive techniques have been developed with which to obtain respiratory secretions during the acute phase. Of these, sampling of induced sputum obtained using hypertonic saline is an appropriate method for the analysis of serum protein level as a marker of pulmonary vascular permeability.⁵ Although it is difficult to control sample dilution by nebulized saline,⁶ its advantage over methods such as bronchoalveolar lavage is that it can be used safely in asthma patients during severe exacerbations.⁷⁻¹⁰

Given the lack of data confirming altered vascular permeability in the acute phase of asthma, the aim of this study was to compare the degree of bronchial exudate of serum proteins through the relative indices of the albumin concentration (RIAlb) and α_2 -macroglobulin concentration (RI α_2 M) in 3 groups of patients: control subjects, patients with stable asthma, and patients with exacerbated asthma. In addition, in patients with exacerbated asthma, we analyzed the relationship between the level of exudate and sputum cytology, the degree of obstruction, based on the forced expiratory volume in the first second (FEV₁), and oxygen saturation to assess the extent to which it participates in the severity of asthma attack.

Patients and Methods

Patients

The study included 49 volunteers, all of whom were nonsmokers: 9 in the control group, 25 with stable asthma, and 15 with exacerbated asthma experiencing a mild to moderate attack. The patients in the exacerbated asthma group were recruited consecutively from among the patients

who attended our respiratory emergency day hospital and presented intensified asthma symptoms for at least 48 hours along with a reduction of FEV₁ or peak expiratory flow of at least 20% when compared with their best recorded result. Patients in the stable asthma group were asymptomatic and their asthma was controlled according to the results of a specific questionnaire¹¹; they remained clinically stable throughout the study period and their treatment had not been altered in the previous 2 months. Individuals in the control group did not suffer from any known disease, had normal spirometry results, and a negative methacholine provocation test. The study was performed in the Asthma and Allergies Clinic (Department of Pulmonology) of the Hospital de la Santa Creu i de Sant Pau in Barcelona, Spain, and all of the subjects gave informed consent.

Patients with asthma were defined based on the current or prior existence of asthma symptoms and a methacholine provocation test recorded in the patient's chart in which a maximum of 8 mg/mL was required to provoke a 20% decrease in FEV₁ compared with basal values (PC₂₀FEV₁).⁴ In the control group, a PC₂₀FEV₁ of at least 32 mg/mL⁴ was required along with the absence of clinical manifestations.

Methods

Participating subjects underwent a physical examination and a clinical history was taken in which symptoms, smoking history, history of asthma and allergies, and treatment were recorded. In the control and stable asthma groups, induced sputum was collected using the method described below and 10 mL of peripheral venous blood was extracted. In addition, a methacholine provocation test was performed in the control group on a different day. The exacerbated asthma group was treated with a solution of physiologic saline (3 mL) and 5 mg salbutamol (1 mL) nebulized with oxygen over a 20-minute period and after 10 minutes the induced sputum was obtained using a modification of the standard method described previously⁷; 10 mL of peripheral venous blood was also collected from these patients. The patients then received the remaining medication indicated for the treatment of their acute asthma according to the treatment guidelines of the Spanish Society of Pulmonology and Thoracic Surgery (SEPAR).¹²

The sputum samples were processed to obtain a cell pellet that was then used to determine the total and differential inflammatory cell counts. The blood samples were left to stand for 10 minutes and then centrifuged to obtain the serum. Serum and sputum supernatant were sent to the biochemistry laboratory for determination of albumin and α_2 -macroglobulin concentrations.

Spirometry and methacholine provocation test. Spirometry was performed using a Datospir 500 spirometer (Sibelmed S.A., Barcelona, Spain) according to the recommendations of the European Respiratory Society.¹³ Methacholine provocation was performed using an adaptation of the continuous inhalation method described by the European Respiratory Society.¹⁴

Induction and processing of sputum. Sputum induction with hypertonic saline for cell counts was performed in the control and stable asthma groups according to the standard procedure used in our laboratory.¹⁵ This consists of administration of an inhaled, short-acting β_2 -adrenergic agonist (2 inhalations of 100 μ g salbutamol in a pressurized cartridge with a spacer attachment) and, after 10 minutes of

bronchodilation, the subjects inhaled hypertonic saline at 3% and 4% for 7 minutes each. The patient was monitored by spirometry before and after each of the inhalations. Within 2 hours, the sputum was processed by selection of mucus plugs from saliva and treatment with dithiothreitol (Sputalysin, Calbiochem Corp, San Diego, CA, USA) and phosphate buffered saline. The cell suspension was filtered, trypan blue was added, and a hemocytometer was used to calculate the total number of cells per gram of sputum, cell viability, and the number of squamous cells due to contamination from the upper airways. After centrifugation, the cell pellet and supernatant were obtained. The cell pellet was used to perform a differential cell count (macrophages, eosinophils, neutrophils, basophils, lymphocytes, and bronchial epithelial cells) using the May-Grünwald-Giemsa stain. Reference values for the cell count were taken from Belda et al.¹⁶ For patients with exacerbated asthma, the modifications described elsewhere were applied.⁷

Determination of protein concentrations in sputum and serum. Sputum albumin concentration (mg/L) was measured by immunoturbidimetry (Tina-quant® Albumin, Roche Diagnostics GmbH, Mannheim, Germany) and serum albumin concentration (g/L) was measured by colorimetry (ALB plus, Roche Diagnostics GmbH, Mannheim, Germany). The concentration of α₂-macroglobulin in sputum (mg/L) and serum (g/L) was measured by immunonephelometry (Alpha-2-macroglobulin antiserum ref. SAM/15, Dade Behring, Marburg, Germany). The results were adjusted for the dilution (1:8) and expressed as relative indices calculated as the concentration of protein in sputum divided by the concentration in serum.

Although the technique of immunonephelometry used to determine the concentration of α₂-macroglobulin was in use in the biochemistry laboratory of our hospital, sputum samples had not been analyzed previously. Consequently, the variability of the measurements was evaluated in relation to storage times prior to processing—fresh (<4 hours) and 7, 15, 21, 28, 75, and 180 days—and to storage temperatures (4°C, -20°C, and -80°C). Comparison of 2 measurements separated by a period of storage revealed that, in general, the second measurement was lower than the first, irrespective of storage temperature. For instance, in samples kept at 4°C for 15 days, the concentration diminished by 21% and 83% for initial concentrations of 7.06 mg/L and 77.7 mg/L, respectively. It should be noted that in all cases the sputum was recentrifuged at 10 900 rpm for 4 minutes prior to analysis, offering a possible explanation for some of the protein loss. Finally, the protein concentration in the sample sent for analysis after storage appears to depend upon the initial concentration (the coefficient of variation between samples for the method used to measure the concentration of α₂-macroglobulin is 9.0% for a concentration of 12 mg/L). Consequently, despite the heterogeneity of the results obtained, and given the low sample volume available in some cases, it was decided to always use fresh samples, process them as soon as possible after centrifugation, and keep them at 4°C prior to analysis.

Measurement of albumin concentration was performed using the standard method in our laboratory for use with low concentrations. In fact, this method is the same as that used routinely for the analysis of microalbuminuria in urine samples. This technique has been described previously in sputum and is known to show low variability.

TABLE 1
Anthropometric, Functional, and Clinical Characteristics of the 3 Groups of Patients (n=46) Included in the Study*

	Healthy Controls	Stable Asthmatics	Exacerbated Asthmatics
Number of patients	9	23	14
Sex, Men/Women	3/6	8/15	4/10
Age, years	37 (19)	31 (10)	30 (7)
FVC, % of reference	97.8 (7.7)	94.0 (8.7)	84.6 (22.3)
FEV ₁ , % of reference	101.1 (9.9)	93.6 (7.5)	74.3 (20.8)
PBD FEV ₁ , %	1.6 (3.9)	2.1 (4.7)	12.4 (10.0)
IC			
Number of patients	—	23	8
Mean dose (µg/mL)	800 (150)	1012 (582)	
LABA			
Number of patients	—	—	6
Mean dose (µg/dL)	—	—	25 (12)

*Data are shown as means (SD). FVC indicates forced vital capacity; FEV₁, forced expiratory volume in the first second; PBD FEV₁, change in FEV₁ after bronchodilator exposure; IC, inhaled corticosteroids (budesonide equivalents); LABA, long-acting β₂-adrenergics.

TABLE 2
Differential Cell Count in Induced Sputum and Relative Indices of Albumin and α₂-Macroglobulin Concentration*

	Healthy Controls	Stable Asthmatics	Exacerbated Asthmatics
Number of patients	9	23	14
FEV ₁ , % of reference	101.1 (9.9)	93.7 (8.1)	74.4 (20.9)
Total cell count, 10 ⁶ /g	8.0 (8.8)	4.2 (3.2)	10.4 (12.8)
Bronchial epithelial cells, %	2.4 (4.2)	3.8 (7.6)	1.3 (3.8)
Eosinophils, %	1.6 (2.3)	5.3 (9.4)	6.8 (10.5)
Neutrophils, %	26.8 (20.8)	32.8 (23.9)	59.8 (32.6)
Lymphocytes, %	2.7 (1.8)	3.0 (2.7)	2.1 (1.1)
Macrophages, %	66.5 (20.6)	55.1 (22.2)	29.8 (24.8)
RIAlb	1.2 (1.1)	2.9 (3.1)	6.0 (6.7)
	51.3 (51.8)	124.4 (136.2)	255.6 (289.9)
	/44.1 (3.3) [†]	/43.5 (2.2) [†]	/42.9 (2.3) [†]
RIα ₂ M	11.7 (10.9)	11.9 (14.7)	3.2 (3.8)
	26.9 (26.0)	21.9 (24.0)	7.0 (8.0)
	/2.3 (0.4) [‡]	/2.0 (0.5) [‡]	/2.2 (0.5) [‡]

*Data are shown as means (SD). FEV₁ indicates forced expiratory volume in the first second; RIAIb, relative index of albumin concentration; RIα₂M, relative index of α₂-macroglobulin concentration.

[†]Concentration of albumin in sputum/concentration of albumin in serum.

[‡]Concentration of α₂-macroglobulin in sputum/concentration of α₂-macroglobulin in serum

Statistical Analysis

Analysis was performed using the SPSS package version 10.0 (1999). Data are shown as means (SD). Comparisons of the means between the 3 groups were made using the Kruskal-Wallis nonparametric one-way analysis of variance for independent samples and pairwise comparisons were made using the Mann-Whitney U test. The Spearman correlation coefficient was used to assess correlations between variables.

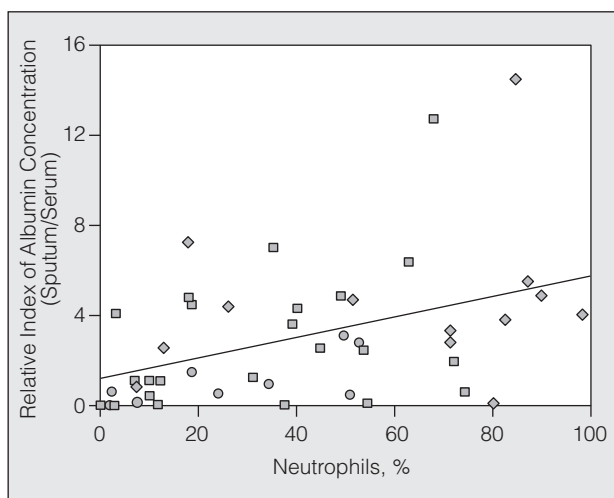


Figure 1. Relationship between the percentage of neutrophils in sputum and the relative index of albumin concentration ($\mu\text{g/mL}$) in the 3 groups. Circles indicate healthy subjects; squares, stable asthmatics; diamonds, exacerbated asthmatics. The regression line is shown.

Results

Of the 49 individuals initially included in the study, 3 were excluded: 2 asthmatic patients, 1 with stable asthma and 1 with exacerbated asthma, because the sample obtained displayed very low viability that was not cytologically valid ($<40\%$), and a third patient with exacerbated asthma because a processable sample could not be obtained. In the remaining 46 individuals (9 control subjects, 23 patients with stable asthma, and 14 patients with exacerbated asthma) the RIAIb and RI α_2 M was determined. The anthropometric and functional characteristics, sputum cytology, and concentrations of albumin and α_2 -macroglobulin in blood and sputum in the 3 groups are shown in Tables 1 and 2.

The mean value for RIAIb was 1.2 (1.1) in the control group, 2.9 (3.1) in the stable asthma group, and 6.0 (6.7) in the exacerbated asthma group. The RI α_2 M values were 11.7 (10.9), 11.9 (14.7), and 3.2 (3.8) in the control, stable asthma, and exacerbated asthma groups, respectively. Comparison of the exacerbated asthma, stable asthma, and control groups using the Kruskal-Wallis test revealed a statistically significant difference ($P=.034$). When pairwise comparisons were made using the Mann-Whitney U test, significant differences were found between the exacerbated asthma group and the other 2 groups, but not between the stable asthma and control groups. The RIAIb value, which reflects the extent to which vascular permeability is altered, was lowest in the control group, higher in the stable asthma group, and highest in the exacerbated asthma group. The difference in the RI α_2 M values between the stable asthma and control groups was not statistically significant ($P=.06$) in the nonparametric analysis (Mann-Whitney U test), while the difference between the control and exacerbated asthma groups was significant. The direction of the trend in this index was

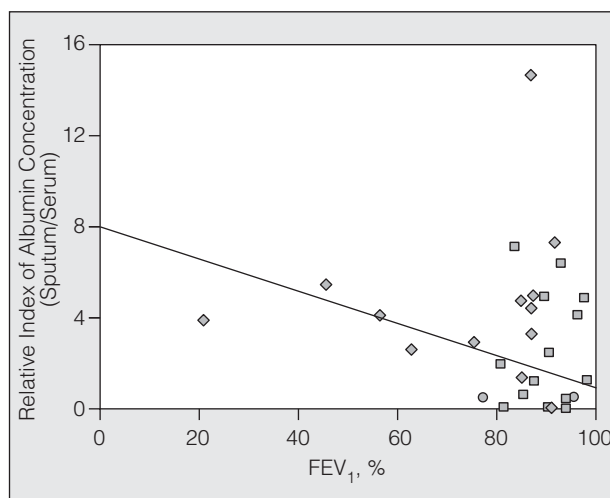


Figure 2. Relationship between forced expiratory volume in the first second (FEV_1 , as percentage of reference value) and the relative index of albumin concentration ($\mu\text{g/mL}$) in patients with exacerbated asthma.

unexpected since the exacerbated asthma group presented the lowest RI α_2 M.

No significant relationship was found between the percentage of eosinophils and either the RIAIb or the RI α_2 M. However, a significant relationship was observed between the percentage of neutrophils and the RIAIb ($r=0.39$; $P=.008$) that was not seen with the RI α_2 M ($r=0.035$; $P=.82$) (Figure 1). The inverse relationship observed between the FEV_1 —in absolute values (L), but also to a lesser degree as a percentage of the reference value—and the RIAIb ($r=-0.43$; $P=.009$) was not seen with the RI α_2 M ($r=-0.206$; $P=.24$). The relationship between FEV_1 and RIAIb appeared to be maximal in the exacerbated asthma group (Figure 2). There was no relationship between oxygen saturation determined by pulse oximetry and either RIAIb ($r=-0.33$; $P=.19$) or RI α_2 M ($r=-0.12$; $P=.84$).

Discussion

The results obtained in this study confirm that it is possible to measure serum protein exudate, a marker of vascular permeability, during the acute phase of spontaneous exacerbations in patients with asthma. The level of this protein exudate increases considerably in the acute phase compared with the stable phase, during which the patient remains asymptomatic. As expected, stable patients appear to present a higher degree of bronchial protein exudate than healthy control subjects; however, the difference did not achieve statistical significance in this study. It is noteworthy that altered vascular permeability did not appear to be related to oxygen saturation (assessed by pulse oximetry) but was related to the degree of obstruction. This would imply that extravasation of serum proteins in the airways does not affect gas exchange but does increase respiratory resistance.

The results of this study on the acute phase of asthma are consistent with those described by other authors.⁷⁻¹⁰ A group from Hamilton, Canada,^{7,8} described similar levels of protein extravasation in exacerbated asthma and control subjects to those found in this study; these results are, however, somewhat different from those described by other groups using sputum. This discrepancy may be explained by the types of patient selected, the techniques used to process the sputum, or the analysis of albumin concentration using methods that can introduce errors in the measurements.

In this study, albumin has proven to be the best marker of altered vascular permeability. Although albumin was initially used as an indicator of the dilution in the sampling method (principally bronchoalveolar lavage), it was soon realized that its usefulness was inferior to that of other markers such as urea. In addition, values were found to vary widely with age, smoking habit, and the degree of underlying inflammation, thereby further limiting its usefulness.¹⁷ Nevertheless, the relationship with inflammation confirmed its effectiveness as a marker of increased vascular permeability induced by inflammation¹⁸ and, therefore, its capacity to respond to antiinflammatory treatment.¹⁹ In parallel, recent advances in biochemical and immunologic techniques such as radioimmunoassay and nephelometry have allowed measurements of substances found at very low levels in body fluids to be made accurately and with extraordinary specificity. Albumin concentration is easily measured using these techniques and represents a paradigm for a low molecular weight protein (49 kilodaltons) that is thought to exhibit passive intracellular transport. In contrast, α_2 -macroglobulin is an example of a high molecular weight plasma protein (714 kilodaltons) that is believed to undergo active intracellular transport, requiring intercellular gaps in the blood vessels for its extravasation. Thus, the relative coefficient of excretion (concentration in extravascular fluid/concentration in serum of a low molecular weight protein such as albumin divided by the concentration in extravascular fluid/concentration in serum of a high molecular weight protein such as α_2 -macroglobulin) has been described as an acceptable indicator of vascular permeability in nasal and bronchial samples.²⁰ However, in the present study, this coefficient did not prove useful when applied to α_2 -macroglobulin, since its concentration was lower in patients with exacerbated asthma than in those with stable asthma. Although the reason for this observation remains to be determined, it is of interest to note that in acute patients the sputum was induced by salbutamol nebulization at a relatively high dose—1 mL of salbutamol (5 mg) with 3 mL of isotonic saline. This may be important because it has been described that salbutamol would have the capacity to reintroduce α_2 -macroglobulin into epithelial cells and reduce edema, thereby providing a possible explanation for the low concentrations obtained.²¹

The increased vascular permeability in the acute phase of inflammation appears to be a rapidly initiated

process designed to facilitate the arrival of new inflammatory cells in the inflamed area. Although the mechanism associated with the chronic phase is not fully understood, it is assumed that inflammatory cells located in the tissue release preformed substances, such as histamine and leukotrienes, in response to certain stimuli. These substances would have immediate effects on endothelial cells, causing them to alter their expression profiles of surface proteins and thereby leading to altered vascular permeability that would permit protein extravasation. This would facilitate cell migration from the blood vessels to the tissue.³ In the present study, a significant relationship was found between the absolute neutrophil count and the concentrations of albumin and α_2 -macroglobulin, a relationship that was not apparent with other cells such as eosinophils. While the mechanistic basis of this relationship remains to be determined, we suggest 2 possibilities. On the one hand, the stimulus that induces cell extravasation would also induce the parallel migration of neutrophils (this would be true, for instance, of cysteinyl leukotrienes). Another possibility would be that protein exudation is induced by a mediator released by the neutrophils themselves, in such a way that the greater the number of neutrophils, the greater the amount of the substance that would be released and the greater the exudate produced. In support of this hypothesis, in addition to the association between neutrophils and albumin extravasation in this study we also found a relationship between neutrophils and obstruction, and between obstruction and extravasation of albumin. This 3-way association makes it highly probable that leukotrienes represent the common mediators that can be produced by neutrophils and are able to induce extravasation and bronchoconstriction.²² It would be possible to confirm this hypothesis in future studies using specific leukotriene antagonists to inhibit the process.

Very little is known about the role of altered vascular permeability in the severity of asthma attack. Although its involvement has been deduced from models of allergen provocation, in which increased vascular permeability appears in the immediate phase, its true contribution to the severity of the attack remains to be elucidated. Some authors, such as Gómez et al,²³ have described altered ventilation-perfusion ratios in exacerbated asthma that, in association with bronchoconstriction, cause worsening of gas exchange; these authors suggest that the vascular changes would be mediated by arachidonic acid derivatives. This group also found that the vascular changes are dependent on inflammation, since they appear to be corrected by antiinflammatory treatment.²⁴

Although it was not a specific aim of the study, the stability of α_2 -macroglobulin measurements was analyzed and was found to be highly unstable. Consequently, measurements should be made as soon as possible in fresh samples (samples can be stored for up to 24 hours at 4°C). Although albumin is much more

stable, when analyzed in parallel, similar processing conditions should be used.

This study has certain limitations worth considering. Firstly, the concentration of α_2 -macroglobulin in a significant number of cases was below the detection limit of the technique used (2.6 mg/mL). Similarly, a small number of albumin concentrations fell below the detection limit of the technique (2 μ g/mL). Although these values appear in the lab report, the fact that they are below the detection threshold means that their accuracy cannot be ensured. Consequently, we assigned a concentration of 2 mg/mL to all cases reported with α_2 -macroglobulin concentrations below 2.6 mg/mL, and 2 μ g/mL for all those albumin concentrations below 2.2 μ g/mL. Although this could introduce errors, their impact is considered minimal. In future studies, this problem can be easily resolved by using a lower dilution in the processing of the sputum (eg, 1:4). In addition, although patients with stable asthma presented a higher level of bronchial protein exudate, the results did not achieve statistical significance, since the size of the sample was calculated in order to identify differences between stable and exacerbated asthma rather than between controls and stable asthma. Finally, it is possible that sputum induction with hypertonic saline affects the results obtained when determining the concentration of proteins in induced sputum.^{6,25} Since this would introduce a systematic error in our results that would affect all groups similarly, we believe that it would not invalidate the results obtained.

From the results of this study, we can conclude that it is possible to use induced sputum for the noninvasive measurement of altered pulmonary vascular permeability in acute asthma through extravasation of serum proteins such as albumin; however, the validity of α_2 -macroglobulin has not been confirmed for this purpose. Likewise, we have confirmed that there is an extravasation of protein in the acute phase of asthma that is significantly higher than in the stable phase. In addition, our results indicate that this extravasation could be clinically relevant since it is associated with obstruction via mechanisms that should be confirmed in future studies.

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