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Original Article

Inflammatory Response of Rapid Onset Asthma Exacerbation☆

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ABSTRACT

The association between onset of asthma exacerbations and inflammatory response has not been sufficiently studied.
Objective: To determine the inflammatory mechanisms of rapid onset (RO) asthma exacerbations.
Method: We designed a prospective, multicentre study that included 34 patients from accident and emergency departments who suffered from asthma exacerbations. They were distributed into three groups, depending on the asthma onset speed: fast (<24h), intermediate (25-144h) and slow (>145h). Clinical data was collected from sputum, blood and urine samples when first treated and after 24h, so as to determine the inflammatory cell counts and soluble markers.
Results: The asthmatics who suffered a RO exacerbation showed higher elastase [1.028 (1.140); 310 (364); 401 (390) ng/ml] (P<.05) and albumin [46.2 (4.3); 42 (3.4); 39.9 (4.8 g/l)] (P<.05) concentrations in the blood sample. Neutrophils, eosinophils (blood or sputum), eosinophil cationic protein (ECP) (blood), interleukin 8 (IL8) (blood) and leukotriene E4 (LTE4) (urine) were high in the three groups (P>.05). An association was shown between the onset of exacerbation and the severity of obstruction (FEV_1) (r=−0.360; P=.037), eosinophils in sputum (r=−0.399; P=.029), albumin (r=−0.442; P=.013), and IL8 in sputum (r=0.357; P=.038).
Conclusions: The results suggest early activation of neutrophilic and eosinophilic responses in asthma exacerbations. However, bronchial swelling may play an important role in the initial inflammatory response in the exacerbations depending on the speed of the onset.

Respuesta inflamatoria de la exacerbación asmática de instauración rápida

Palabras clave:
Exacerbación grave de asma
Inflamación
Eosinófilos
Neutrófilos
Edema

RESUMEN

No se ha estudiado suficientemente la asociación entre la rapidez de instauración de la crisis de asma y la respuesta inflamatoria desencadenada.
Objetivo: Determinar los mecanismos inflamatorios que caracterizan la exacerbación asmática de instauración rápida.
Método: Se diseñó un estudio prospectivo y multicéntrico en los servicios de urgencias hospitalarias, que evaluó a 34 pacientes que se distribuyeron en tres grupos en función de las horas de instauración de la exacerbación asmática: (menos de 24 h), instauración intermedia (25-144 h), e instauración lenta (145 o

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más horas). Se recogieron datos clínicos, de esputo, sangre y orina en el momento de la primera atención y pasadas 24 h, determinándose celularidad inflamatoria y marcadores solubles.
Resultados: Los pacientes con exacerbación rápida presentaron una significativa mayor concentración de elastasa (1.028 ± 1.140; 310 ± 364; 401 ± 390 ng/ml) y albúmina (46,2 ± 4,3; 42 ± 3,4; 39,9 ± 4,8 g/l) en sangre. El número de neutrófilos, eosinófilos (tanto en sangre como en esputo), los niveles de proteína catiónica del eosinófilo (PCE) (sangre), interleuquina 8 (IL8) (sangre) y leucotrieno E4 (LTE4) (orina) estaban elevados en los tres grupos (p > 0,05). Se constataron asociaciones lineales entre el tiempo de instauración de la exacerbación y la intensidad de la obstrucción (FEV_1) (r = –0,360; p = 0,037), los eosinófilos en esputo (r = –0,399; p = 0,029), la albúmina (r = –0,442; p = 0,013); y con la IL8 (r = 0,357; p = 0,038).
Conclusiones: Los resultados sugieren una activación precoz de la respuesta neutrofílica y eosinofílica en la exacerbación asmática. No obstante, es posible que el edema bronquial juegue un papel importante en la respuesta inicial inflamatoria de las exacerbaciones dependiendo del tiempo de instauración.

Introduction

The magnitude and pattern of systemic and bronchial inflammation which trigger asthma exacerbations are responsible for their intensity and short- or long-term severity.[1,2] Several studies carried out on patients who died of asthma or suffered a near-fatal asthma exacerbation suggest that onset time may be related to the type of underlying bronchial inflammatory response,[3,4] the trigger agent,[5] and the speed of the clinical response to the treatment given.[6] However, the inflammatory response of the bronchial lumen or submucosa differs and depends on whether it is triggered by tobacco, atopy, or a virus.[7] Furthermore, it depends on the type of inflammation pattern in the airway which corresponds to the patient's asthma phenotypes[8-11] and, therefore, affects the physiology of the airway, so conditioning both the physiological changes and the type of response produced. One particular aspect that has hardly been studied is the relationship between the inflammatory profile and the onset time from when respiratory symptoms begin, i.e. the speed of the crisis. There are few data collected from bronchial samples obtained using non-invasive techniques, and only a few aspects linked to onset time have been studied in part.[12,13] Therefore, it is plausible to pose the hypothesis that the speed of the onset of asthma exacerbations is related to the type of bronchial and systemic inflammatory response, and that this could differ depending on the profile of the inflammatory biomarkers present, so conditioning the severity and magnitude of the exacerbation. The aim of this work, is therefore to determine the type of systemic inflammatory response and that of the bronchial lumen by analysing indirect or specific inflammation markers from the eosinophilic and neutrophilic inflammatory cell lines present in asthma exacerbations, and their relationship with the crisis onset time.

Patients and Methods

Patients

We performed a prospective, multi-centre study, recruiting patients with an asthma exacerbation who had been treated in the accident and emergency department of the participating hospitals (Clínic Hospital, Mataró Hospital, the Hospital de la Santa Creu i Sant Pau in Barcelona, La Fe Hospital in Valencia) between January 2007 and June 2009. All the patients had symptoms of exacerbation of the airway and pulmonary obstruction, following the criteria described for exacerbations of the Spanish Guidelines for the Management of Asthma (GEMA).[14] The research protocol was approved by the hospitals' Ethics Committees. All the patients were informed and agreed to participate voluntarily in the research study; their informed consent was collected, they were included in the study and the detailed data in the protocol were collected.

Variables

The main variable in the study was the speed of onset of the crisis, which was defined as the time (in hours) from the start of clinical deterioration perceived by the patients themselves (determined by an increase in usual asthma symptoms during both day or night, and/or an increase in the need to use bronchodilator rescue medication) until health care was given, at which time diagnosis of an exacerbation was made and specific treatment begun.[14]

Other variables were: demography (age, sex); clinical variables regarding the patients' asthma characteristics prior to the exacerbation, such as baseline severity, number of previous admissions due to asthma (and severity), daily maintenance dose of inhaled corticosteroids taken in the previous month, lung function, known atopy, and tobacco consumption. Exacerbation variables included the suspected trigger being an infection (with axillary body temperature over 37°C),severity of exacerbation, parenteral or oral anti-inflammatory medication administered, and especially, the number of hours passed since the onset of respiratory symptoms (daytime, night time or both, the need to use bronchodilator rescue medication).

Biological sputum, blood and urine samples were taken on 2 occasions as parameters of the inflammation in the exacerbation, first at the time of receiving medical attention, and 24h later, during clinical testing in the short-term follow-up. The tests determined both indirect bronchial oedema inflammatory markers (albumin) and neutrophilic inflammatory response markers (neutrophils, neutrophil elastase, and IL-8) as well as eosinophilic inflammatory response (eosinophils, cationic proteins, and leukotriene E4). All samples were sent en bloc for analysis to the Biochemistry Department of the Hospital de la Santa Creu i Sant Pau in Barcelona, where all the analyses of soluble markers were carried out centrally. Blood and sputum cell counts were carried out in each hospital, except those from the Mataró Hospital which were centralised at the Hospital de la Santa Creu I Sant Pau. The sputum cell counts were performed by trained, experienced staff, and the cellularity obtained was confirmed in subsequent tests.

At 30 days of follow-up after the exacerbation, the clinical data were collected and patient variables completed. The patient variables were mainly atopy (prick test) and pulmonary function (forced expiratory volume in the first second (FEV_1).

Techniques

- *Bronchial secretions*. Sputum was obtained after a saline solution (3ml) containing 5mg of salbutamol was inhaled through a nebuliser, the operation being carried out under positive pressure with a high oxygen flow for 20 min. All the patients followed the same protocol for giving the bronchial sample, and all the sputum was processed in the same way. Initial chemical treatment with dithiotheitrol (DTT) was performed at a 1:10 dilution (Sputolysisn, Calbiochem Corp., San Diego, CA). The total volume of the processed sputum sample was the sum of the volume corresponding to 4 times the weight in mg of the buffers treated with DTT, plus the volume of the PBS itself. The differential inflammatory cell count from the cell precipitate determined the percentage of neutrophils and eosinophils, following the procedure described by Pizzichini et al.15 IL-8 in the frozen sputum supernatant (frozen at –80°C before analysis) was determined by chemiluminescence (IL-8 - Siemens®, ref. LK8-P1) in an IMMULITE analyser, following the manufacturer's instructions and the reference values provided, and after previously standardising the technique in the biochemistry laboratory.
- *Blood samples*. EDTA blood samples and blood in a tube with agar were obtained daily. They determined, respectively, the leukocyte formula, and the levels of neutrophil elastase and cationic eosinophil protein in saline using ELISA techniques. The manufacturer's instructions were followed for using the assay kit for detecting both inflammatory markers (Human PMN-Elastase ELISA - SG Servicios Hospitalarios SL, ref. BLK-4-269 and ECP - Siemens®, ref. LKEOZ, respectively). Albumin in plasma concentrations were only determined on the second day of emergency care. Measurements were performed by colourimetry (ALB plus, ref. 1929640, Roche Diagnostics® GmbH, Mannheim, Germany). The extraction of venous blood at 30 days determined the total IgE for each patient, which served as a quantitative biomarker of atopy, and Alternaria alternata-specific IgE as a risk factor of near-fatal asthma. This was performed following the usual procedure of the central biochemistry laboratory in each of the participating hospitals.
- *Urine samples*. Urine samples were used to determine levels of leukotriene E4 using the ELISA technique (LTE-4, GE HealthCare®, ref. RPN-224) following the kit manufacturer's recommendations and standardisation of the detection technique.
- *Pulmonary function*. Both spirometry and maximum expiratory flow (MEF) were measured with a Datospir 500 spirometer (Sibelmed S.A., Barcelona) and an officially approved measuring device, respectively, following the recommendations of the Spanish Society of Pneumology and Thoracic Surgery (SEPAR) and the recommendations for their use for treating asthma described in the GEMA guidelines.[14]

Statistical Analysis

Mean values and standard deviation were used to describe the variables analysed in the sample. Pearson's correlation coefficient was used to quantify, first, the linear correlation between the main variable (hours elapsed from the start of clinical deterioration until emergency healthcare was given) and the secondary variables, and second, the correlations between the different inflammatory markers, and from these the severity of the obstruction (MEF). Regarding the study's main variable, the sample was divided into 3 groups taking into account 2 criteria when establishing the cut-off points. These were maintaining homogeneity in the same sample size (n) in each group (frequency distribution of the main variable), and the study hypothesis (rapid onset, progressive short-term clinical deterioration and prolonged deterioration). To compare the means of the groups, the Kruskal-Wallis non-parametric test was used to analyse the differences between independent samples, and the χ^2 test was used to compare categorical variables, with Fisher's exact test being used when appropriate. A multiple regression model was performed to estimate the value of the main variable, the following being includrd in the model: the variables that showed statistical significance in the univariate analysis and those for which it is biologically plausible that they could act as confounding or modifying variables of the estimated effect (speed of onset of the asthma crisis). $P<.05$ was considered statistically significant. The analysis of the data was performed using version 15.0 of the SPSS program.

Results

Only the patients who complied with all the protocol were analysed in the study. 8 were finally excluded; of these, 5 did not commit themselves to the programmed visits and 3 had samples which were unsuitable for analysis. Thirty-four patients were included with an asthma exacerbation, none near-fatal, but 18% needed oxygen therapy for acute respiratory failure ($P02\leq55$ mmHg).16 The means of days and nights passed with exacerbation symptoms were 5.5 (3.5) and 4.9 (3.5), respectively. Nine patients were considered non-atopic; these were those who tested negative in the prick test for environmental pneumoallergens and had IgE<160 UI/ml (lower limit adopted by the reference laboratory). The clinical characteristics of the entire sample are detailed in table 1.

Depending on the hours passed from the onset of clinical deterioration, the patients were divided into 3 groups. Table 1 shows the clinical and asthma exacerbation characteristics of the groups analysed in accordance with the hours passed from the start of the exacerbation. Statistically significant differences were found between the groups regarding the variables: number of visits to the accident and emergency department in the previous year ($P=.03$), previous admissions to the ICU for severe exacerbations ($P=.05$), and result of MEF on the 2nd day of healthcare after intensifying the treatment ($P=.008$); the first of these variables was the most frequent, and the last the least, among the group with the longer evolution time.

All of the biological markers analysed were shown to be high (table 2). No statistically significant differences were found in the neutrophil and eosinophil counts in sputum and blood. Only the group with the shortest evolution time had higher elastase levels on the first day ($P=.05$) and higher serum albumin levels measured on the 2nd day ($P=.008$), compared with the other groups. Table 2 shows the values obtained in the biological tests performed on each time group.

The analysis of the correlation of quantitative variables shows a statistically significant negative linear relationship between the crisis onset time and the severity of the obstruction (MEF) ($r=-0.360$; $P=.037$), sputum eosinophils ($r=-0.399$; $P=.029$) and serum albumin levels ($r=-0.442$; $P=.013$); there was a positive linear relationship with sputum IL8 levels ($r=0.357$; $P=.038$).

The multiple regression model was constructed taking into account both the clinical and biological variables considered to be of clinical interest (dependent variable: onset time), and the independent variables. Blood eosinophil count on the 1st day, MEF of the exacerbation, serum elastase on 1st day, serum albumin on 2nd day, leukotriene count on 2nd day, tobacco consumption, and the taking of oral or parenteral corticosteroids immediately before

Table 1
Clinical characteristics of patients and asthma exacerbation depending on onset time

	Evolution time (hours)			
	All (n=34)	Up to 24 (n=10)	From 25 to 144 (n=14)	145 or more (n=10)
Demographic and asthma characteristics prior to asthma exacerbation				
Age (years)	43 (20)	35 (18)	47 (15)	43 (17)
Female (%)	62	80	50	60
Active smoker (%)	36	40	42	22
Severity of asthma (I/M/M/S) (%)	(18/18/32/32)	(40/10/40/10)	(7/29/29/35)	(10/10/30/50)
Years of evolution	16 (15)	10 (9)	22 (9)	16 (12)
Doses/day inhaled steroids (Ìg)	446 (473)	198 (356)	534 (455)	577 (541)
Positive prick-test (%) (none to Alternaria alternata)	63	67	69	50
IgE (UI/ml)	256 (316)	326 (375)	209 (341)	244 (219)
Hospital admissions (asthma)	2.7 (3.3)	2.3 (4.1)	2.1 (2)	4.1 (4)
Admissions in last year (asthma)	0.5 (1)	0.4 (0.9)	0.3 (0.6)	1 (1.4)
Visits to A&E in previous year (asthma)	2 (2.8)	2.1 (3.1)	0.7 (1.9)	3.7 (3.4)*
Admissions to ICU (asthma) (%)	18	0	14.3	40**
Characteristics of the asthma exacerbation				
Suspected infection (%)	62	40	64	80
Patients with oral steroids prior to visit (%)	42	60	25	44
MEF exacerbation 1st (%)	49 (20)	57 (18)	46 (17)	46 (22)
MEF exacerbation 2nd (%)	59 (40)	75 (14)	54 (17)	50 (21)*
FEV_1 (%)	85 (03)	91 (12)	77 (38)	90 (19)

A&E: Accident and Emergency; FEV_1: forced expiratory volume in first second; I/M/M/S: Intermittent/mild/moderate/severe; MEF: maximum expiratory flow; 1st: results on first day; 2nd: results on second day. Data are expressed in percentages or as means (standard deviation).

* *P*<.05.

** (Fisher exact test) *P*=.065.

Table 2
Biological results of the asthma exacerbation depending on onset time

	Evolution time (hours)			
	All (n=34)	Up to 24 (n=10)	From 25 to 144 (n=14)	145 or more (n=10)
Sputum				
Eosinophils 1st (%)	11.7 (15)	18 (21)	13 (13)	3.4 (2.5)
Eosinophils 2nd (%)	7.7 (13)	8.7 (19)	9.9 (13)	2.7 (2.9)
Neutrophils 1st (%)	49 (22)	45.8 (19)	46.4 (22)	56.4 (26)
Neutrophils 2nd (%)	52 (22)	46.3 (26)	53.8 (20)	57.2 (21)
IL-8 1st (pg/ml)	10,994 (11,207)	9,189 (6,309)	10,576 (8,681)	13,204 (17,128)
IL-8 2nd (pg/ml)	12,822 (15,913)	9,942 (6,678)	8,602 (7,648)	21,610 (26,012)
Blood				
Eosinophils 1st ($\times 10^6$/l)	472 (492)	590 (519)	395 (432)	454 (566)
Eosinophils 2nd ($\times 10^6$/l)	73 (2)	14 (2)	84 (2)	115 (3)
Neutrophils 1st ($\times 10^6$/l)	7,587 (3,602)	7,948 (4,119)	7,566 (4,262)	7,252 (2,164)
Neutrophils 2nd ($\times 10^6$/l)	8,726 (3,701)	8,109 (3,980)	8,634 (3,530)	9,471 (3,918)
Eo cationic p. 1st (ng/ml)	13.8 (11)	15.3 (11)	14.8 (13)	11.9 (5,8)
Eo cationic p. 2nd (ng/ml)	12.6 (10)	11.0 (7.7)	15.2 (14)	10.3 (6)
Elastase 1st (ng/ml)	555 (748)	1,028 (1,140)	310 (364)	401 (390)*
Elastase 2nd (ng/ml)	586 (720)	578 (606)	678 (961)	465 (392)
Serum albumin 2nd (g/l)	42.7 (4.8)	46.2 (4.3)	42 (3.4)	39.9 (4.8)*
Urine				
LTE4 1st (pg/ml)	521 (242)	440 (202)	520 (244)	602 (271)
LTE4 2nd (pg/ml)	506 (198)	465 (203)	561 (170)	471 (228)

LTE4: leukotriene E4; 1st: result on first day; 2nd: result on second day.
Data are expressed in percentages or as means (standard deviation).

* *P*<.05.

emergency healthcare, did not reveal statistical significance. However, blood eosinophils (*P*=.105) and serum albumin (*P*=.197) were of statistical significance.

On the other hand, a negative linear relationship was confirmed between the obstruction of the air flow (MEF) and the blood leukocyte count on the first day (r=–0.353; *P*=.04), and urine LTE4 on the first day (r=–0.342; *P*=.048). A positive association was found between MEF and serum albumin on the 2nd day (r=0.414; *P*=.02)

Other correlations of biological interest between soluble and cellular markers of systemic inflammation are shown in table 3.

Discussion

The results suggest an early activation of both the neutrophilic and eosinophilic responses in asthma exacerbations. However, it was not possible to identify a typical pattern of inflammation which depended on the onset time of the crisis. Bronchial swelling plays an important role in the pathogenesis of rapid onset exacerbations, having a greater effect on plasma proteins the longer it lasts, and immediately causing an obstruction in the air flow. Regarding clinical characteristics, what stands out, despite our not finding statistically

Table 3
Most relevant relationships between markers of systemic inflammation

Blood Albumin (g/l)	Elastase 1st day (ng/ml) r=0.433; *P*=.015	Elastase 2nd day (ng/ml) r=0.454; *P*=.012	Cationic p. 1st day (ng/ml) r=0.434; *P*=.015	Cationic p. 2nd day (ng/ml) r=0.439; *P*=.015
Urine LTE_4 (pg/ml) 2nd day	Blood leukocytes 1st day r=0.302; *P*=.087	Blood leukocytes 2nd day r=0.513; *P*=.025		

LTE_4: leukotriene E4; P: value of statistical significance reached; r: Pearson's correlation coefficient; 1st day: result on first day; 2nd: result on second day.

significant differences between the groups, is that the patients with the fastest crisis onset time are usually women. They have fewer crises triggered by infections, have a lower mean intake of inhaled corticosteroids, take a higher proportion of oral corticotherapy, and show a greater reversal of bronchial obstruction at 24h. This suggests that these patients have a different profile in terms of the impact of exacerbations with a more rapid onset. The results for the pattern of inflammation obtained in this study have to be interpreted with caution, but they do not differ, in general, from those found in studies carried out on acute asthma in children and adults, particularly when the main trigger is a virus acting alone or combined with an allergen in the environment.[1,13] In many exacerbations the activation of neutrophilic and eosinophilic inflammation of the airway is dual and mixed, as other authors have reported.[17] In our study the levels of eosinophils in sputum on the first day were most marked in those groups with the most rapid onset crises This was probably related to a predominance of eosinophils in the bronchial mucosa due to inadequate basal treatment, which increased after the exacerbation was triggered. The dramatic drop in eosinophil levels on the 2nd day also stands out, this being more intense in crises with a more rapid onset and responding better to corticosteroid treatment. On the other hand, the presence of neutrophils was also significant, although, biologically, the response to treatment differed from that in the presence of eosinophils. Neutrophils can be found in sputum samples of children with asthma exacerbations caused mainly by viral infections,[17] and they can mask predominant basal levels of eosinophils during the exacerbation in some patients.[18] Furthermore, it has been reported that the pattern of inflammation can be different in future exacerbations, depending on what triggers them.[19] In our study, the association between both inflammation and biomarker patterns and the time passed from the onset of the exacerbation was shown not to be statistically significant, except for neutrophil elastase and blood albumin. Most exacerbations were triggered by a viral infection. These patients' symptoms started in the upper airway, they had a temperature above 37.5 °C, and did not require antibiotics to make short-term clinical progress. The inflammatory cell pattern found does not appear to differ from that of children with asthma exacerbations; of these, the ones with neutrophilic inflammation did not have exacerbations with a faster onset.[20] This study confirms that bronchial eosinophilic infiltration is associated with the evolution time of the crisis, but this is probably conditional on prior treatment with corticosteroids. Bronchial swelling produced by plasma extravasations (soluble mediators and multimeric proteins such as albumin and alpha 2 macroglobulin) to the airway varies, but is significant, and it is associated with the time passed since onset, loss of control and with prolonged alteration of vascular permeability.[12-21]

Physiopathologically, it is possible that the activation of cells and soluble mediators in plasma causes an initial systemic mobilisation of neutrophils, with immediate bronchial infiltration, which in turn stimulates simultaneous vasodilatation and extravasations of the swelling at the level of the bronchi,[21] and constitutes a determining factor in the subsequent migration of eosinophils to the bronchial submucosa.[22,23] The inflammatory cell patterns described are, in some ways, the same as those found in obstructive and inflammatory airway diseases (mainly asthma and COPD),[24] although the concentrations of certain cellular markers and cytokines/chemokines,[25-27] both from neutrophilic (neutrophils, IL-8 or elastase) and eosinophilic (eosinophils, IL-5, ECP) cell lines, show different values in some exacerbations depending on the degree of control,[8,28] severity,[9,20,29] the bronchial obstruction,[30] the biological sample selected,[24] the spectrum of disease or inflammatory phenotype of the airway,[2,31-33] and the natural history of the evolution of the inflammatory disease.[24,31,34,35] Furthermore, the high concentrations found of the metabolite of the cysteinyl leukotriene (LT4) are the expression of the great activation of both granulocytic cell lines, and constitutes a non-invasive systemic marker of inflammation which can be used for the global measurement of the inflammatory cascade.[36] For these reasons, we believe that the overall inflammatory response of both the cells and mediators (eosinophilic and neutrophilic) in blood and the airway is established either at the same time or one very soon after the other, and only in sudden, near-fatal cases of asthma (under 1h) is it possible to determine the predominance of a very concrete response, for example, neutrophilic infiltration of the bronchial submucosa as a defined pattern of rapid onset asthma exacerbations.[4]

The severity of the bronchial obstruction in the exacerbation was related to factors derived from the granulocyte component and albumin in blood, and the metabolite of cysteinyl leukotriene in urine, as well as the expression of the predominantly eosinophilic activation, which has been associated with the constant intensity of the bronchial obstruction and with the most severe instability of the airway.[30,36,37] Although it is likely that the direction of the correlation between MEF and time may change in the first few hours, depending on what triggers the inflammatory response, the association mentioned with markers is relevant in terms of the immediate progress of the obstruction, above all when there are moderate correlations between them (table 3). The initial role of vascular permeability, determined by extravasated albumin in the bronchoalveolar lavage and the bronchial mucosa at onset and the degree of asthma deterioration has been recently demonstrated.[21] The methodology followed in the study design may have conditioned in some way final patient recruitment. For example, the sputum technique was not available on 2 consecutive days if the patient was recruited on a Friday, and also, some candidate subjects refused to repeat the same tests at 24h, as established in the protocol. Therefore, the main limitation of the study is the lack of patients with a very rapid onset of the exacerbation, which has conditioned the patient sample, giving rise to big differences in the onset time of the crisis. However, this heterogeneity has made it possible to obtain 3 groups of symptom onset times which are representative of what actually happens to patients when they go to the accident and emergency department with an exacerbation. The groups of asthmatics considered were represented in all the groups and there were hardly any clinical differences between them, although if there had been more subjects in each group, statistical significance may have been

reached for some variables. This would depend on the type of asthmatics recruited and the condition of the prior inflammatory activation in each patient, although a study design recruiting patients who were very different in these ways could mask the differences. It is worth noting that the group with the slowest onset time had more severe asthma data with worse control, despite the moderate or high doses of inhaled corticosteroids, while the group with the fastest onset had fewer doses, and characteristics pointing to a bigger allergic component. It is possible that a larger sample size would have affected the patients' short-term bronchial and systemic inflammatory profiles at the onset of a crisis, and in the multiple regression analysis proposed statistical significance could have been reached for the above mentioned variables. However, intervening factors which are prior to the crisis or which are just in the early stages can condition the analysis of the design and so make the analysis of biologically interesting events quite unviable unless they are designed experimentally. For example, clinical factors such as smoking or parenteral or oral corticotherapy may have had a greater effect on the pattern of inflammation present and the speed of onset of the exacerbation, especially if we bear in mind the relatively low percentage of prior oral steroid consumption among the patients with longer onset crises. However, our study was not able to determine its influence as there was no strong predominance in any group. Similar studies with children who had previously been given corticotherapy showed that it did not modify the initial inflammatory pattern,[20] while only peripheral eosinophilia and albumin showed a tendency towards statistical significance in the multiple regression analysis used to analyse onset times, thus showing them to be relevant biomarkers of short-term clinical prognosis and0 response to treatment. On this matter, authors such as Norzila et al have established that prior oral corticotherapy does not have a dramatic effect on the inflammatory pattern present (eosinophilia and improvements in bronchial obstruction) until 24h later,[13,17] probably due to the prolonged significant effect of the inflammatory reaction triggered and the corticosensitivity of neutrophilia in the biological and clinical contexts in which it predominates, such as in patients who smoke or have a viral infection.[38-41]

To summarise, the results of our study suggest an early activation of both the neutrophilic and eosinophilic response in asthmatic exacerbations. Broncial swelling may play an important role in the diagnosis of very fast-onset exacerbations and, furthermore, in slow-onset exacerbations as a direct expression of significant cellular and molecular activation in the airway.

Conflict of Interest

The authors affirm that they have no conflict of interest.

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