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ORIGINAL ARTICLE

Persistent airway inflammation and high exacerbation rate in asthma that starts at menopause

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ABSTRACT: ***Persistent airway inflammation and high exacerbation rate in asthma that starts at menopause. G. Balzano, S. Fuschillo, E. De Angelis, C. Gaudiosi, A. Mancini, M. Caputi.***

Background and Aim. **Asthma that begins around the time of menopause is frequently characterised by marked clinical severity and poor response to treatment. We sought to assess the clinical characteristics, bronchial responsiveness, perception of induced bronchoconstriction and airway inflammation in women with menopausal asthma, as compared to women of a similar age with pre-existing asthma.**

Methods. **Nine women with pre-existing asthma were selected for clinical severity (symptoms, lung function and medication requirements) similar to that in 11 women with menopausal asthma. Anti-asthmatic treatment in all of the study patients included high dose inhaled (with or without oral) corticosteroids.**

Results. **The women with menopausal asthma demonstrated less atopy, more chronic recurrent sinusitis, similar airway responsiveness, and similar perception of induced bronchoconstriction, but a significantly higher sputum eosinophil count (19.5 ± 10.8 versus 3.3 ± 4.3%; $p < 0.001$) and a higher severe exacerbation rate during the 1-year follow-up period (5.09 ± 4.85 versus 0.78 ± 0.97; $p < 0.05$). Sputum eosinophil count and severe asthma exacerbation rate correlated well in both groups considered as a whole ($r = 0.65$; $p < 0.005$).**

Conclusion. **The eosinophilic airway inflammation present in women with menopausal asthma is poorly responsive to anti-inflammatory treatment with corticosteroids and predisposes to frequent severe exacerbations. Airway inflammation should be monitored in women with menopausal asthma.**

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Keywords: Asthma, asthma severity, asthma perception, airway inflammation, menopause.

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Introduction

Considerable progress has been made in recent years in the understanding of asthma, and the inflammatory nature of the disease is well established [1]. Nevertheless, little is known about the determinants of severity, that is, those factors capable of influencing asthma severity, response to treatment, and prognosis. It has recently been proposed that sex hormones should be considered among the possible determinants of severity in asthma, as indicated by clinical and epidemiological data, experimental *in vitro* and *in vivo* studies, and, especially, by some natural models, such as menstrual cycle, pregnancy, and menopause [2].

Menopause, which generally occurs in women around the age of 50, is characterised, as compared to the fertile age, by a particular hormonal pattern, with higher levels of follicle-stimulating hormone and luteinising hormone and lower levels of estrogen and progesterone [3]. Menopause can coincide with the onset of asthma, a finding indirectly supported by epidemiological studies that recorded a peak in the frequency of asthma beginning in women around the age of 50 [4]. When asthma begins at menopause, it frequently presents with features such as absence of atopy, recurrent sinusitis, urticaria/angioedema, aspirin sensitivity and/or intolerance to angiotensin-converting-enzyme inhibitors, a need for systemic steroids for control of symptoms, and a need for frequent hospitalisation [5, 6]. Taken together, these observations suggest that asthma that coincides with menopause (menopausal asthma), as in other forms of late-onset asthma [7], is most often characterised by marked clinical severity and poor response to treatment. It is not known, however, if clinical severity is tied to the objective severity of asthma indices, or, alternatively, if there is an altered perception of asthmatic symptoms due to psychological factors associated with menopause [8].

The aim of this study was to assess the clinical characteristics, bronchial responsiveness, perception of induced bronchoconstriction, and airway inflammation in women with menopausal asthma (experimental subjects) as compared to women of

a similar age with pre-existing asthma (control subjects). Both groups of women had asthma of similar clinical severity, as based on symptoms, lung function, and medication requirements.

Methods

Study subjects

Eleven women with asthma starting at menopause, i.e., within 1 year before or after the last menstruation, were consecutively recruited. They all presented with moderate to severe asthma, as indicated by symptoms, lung function, and medication requirements [1]. Spirometry values at baseline are reported in table 1.

As a control group, we also studied nine menopausal women who had pre-existing asthma, i.e., asthma that had started at least 10 years before the last menstruation. These women were selected for having characteristics similar to those of the women with menopausal asthma: age, symptom severity, airway obstruction, and medication requirements for asthma control. Moreover, as indicated by clinical history, the pre-existing asthma had not worsened with menopause.

None of the subjects had ever smoked or received replacement estrogen treatment.

The study was approved by the local ethics committee, and all subjects gave their informed written consent.

Study design

The study was an open study (fig. 1). At entry, clinical history, objective examination, spirometry

Table 1. - Functional characteristics of the study subjects at baseline and 1-year follow-up*

	Menopausal asthma (*N* = 11)†		**Pre-existing asthma (*N* = 9)‡**	
	Baseline	**1-year follow-up**	**Baseline**	**1-year follow-up**
FEV_1: ml	1,545 ± 540	1,294 ± 516	1,403 ± 317	1,155 ± 368
% of predicted	75.2 ± 19.9	64.9 ± 21.5	68.11 ± 17.5	58 ± 20.6
FVC: ml	2,340 ± 527	2,137 ± 568	2,224 ± 409	1,975 ± 521
% of predicted	96.3 ± 15	90.3 ± 20.3	90.11 ± 16.3	72.7 ± 21.8
FEV_1/FVC %	65 ± 10.81	59.4 ± 9.4	63.1 ± 9.93	58 ± 6
Post-bronchodilator				
FEV_1: ml	1,763 ± 495	1,480 ± 476	1,570 ± 344	1,322 ± 321
% of baseline	117.5 ± 16	117 ± 10.82	113 ± 7.8	118.83 ± 11.44
Exacerbation rate		5.9 ± 4.85		0.78 ± 0.97

Definition of abbreviations:
FEV_1 = forced expiratory volume in 1 s; FVC = forced vital capacity.
* Data is expressed as mean ± SD.
† No. of observations: 7.
‡ No. of observations: 6.

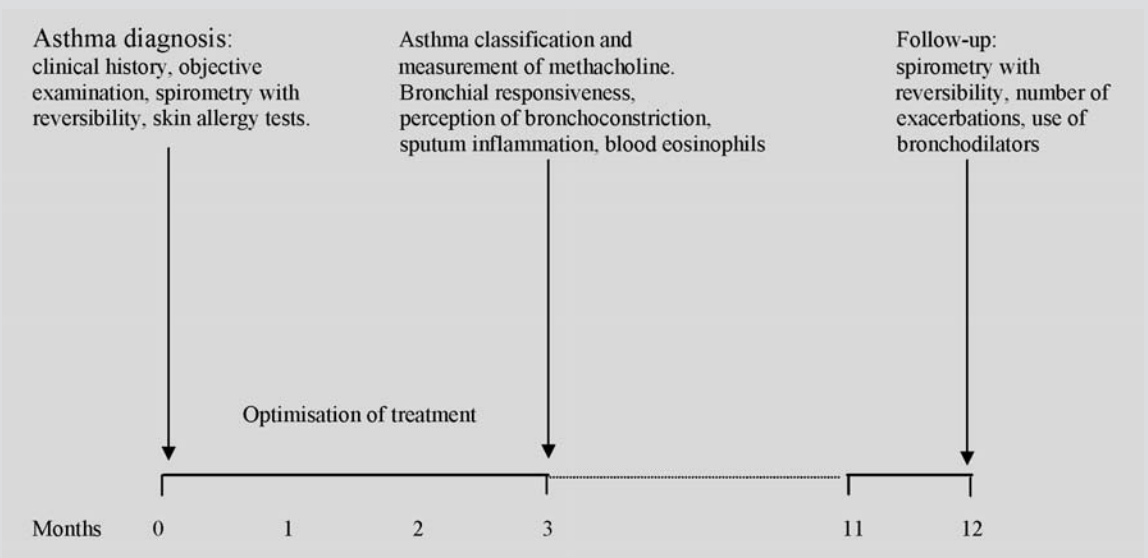

Fig. 1. - Study design.

with reversibility of bronchial obstruction, and skin prick tests for common allergens were performed to establish a diagnosis of asthma according to widely accepted criteria [1].

To avoid influences on asthma classification and measurements by either undertreatment or overtreatment, we established a 3-month run-in period for all study subjects in which the anti-asthmatic treatment was optimised, i.e., the best possible clinical and functional control of asthma with the smallest possible dose of drugs was obtained, by means of monthly visits.

After optimisation of treatment, asthma severity was classified according to guidelines of the Global Initiative for Asthma (GINA) on the basis of symptoms, spirometry, and medication requirements [1]. Then, airway responsiveness as determined by a methacholine inhalation test, perception of the methacholine-induced bronchoconstriction by a Borg dyspnea scale, airway inflammation as determined by a cellular analysis of the induced sputum, and peripheral blood eosinophils were measured. All laboratory measurements were carried out by personnel who were not aware of the group to which each study subject belonged.

Finally, at the follow-up visit at one year, spirometry with reversibility of bronchial obstruction was performed, and the number of severe exacerbations and as-required use of bronchodilator medications in the preceding year were recorded.

Measurements

Spirometry was performed according to the American Thoracic Society recommendations [9] by using a computerised pneumotachograph (Vmax 22; Sensor Medics Italia, Milano, Italy). After baseline evaluation, spirometry was repeated 15 minutes after the subject had inhaled 400 µg (4 puffs) of salbutamol, delivered by a metered dose inhaler attached to a large-volume spacer device. Reversibility of airway obstruction was expressed in terms of the percent change from baseline of the forced expiratory volume in 1 s (FEV_1).

Skin prick tests were done with 12 commonly inhaled allergens, including house dust mites, pollens, molds, animal danders, and positive and negative control extracts (Lofarma Allergeni, Milano, Italy).

A methacholine inhalation test was carried out in accordance with the method currently used in our laboratory [10] by using a dosimeter Mefar MB3 (Markos-Mefar, Monza, Italy). The results were expressed in terms of PD_{20}, i.e., the dose, in micrograms of methacholine, that produces a 20% decrease in FEV_1. In our laboratory, a PD_{20} of ≤ 50, between 51 and 400, and between 401 and 1,600 indicates a severe, moderate, or mild increase in bronchial responsiveness, respectively, whereas normal bronchial responsiveness is reflected by a PD_{20} of > 1,600.

Before both spirometry and the methacholine inhalation test, bronchodilator drugs were withheld for a sufficient time, in relation to their duration of action, to avoid interference with the measurements [9, 10]. Inhaled short-acting $beta_2$-agonists, inhaled long-acting $beta_2$-agonists, inhaled muscarinic receptor antagonists, and oral slow-release theophilline were withheld for 8, 24, 12, or 48 hours respectively before measurements. The other drugs – oral and inhaled corticosteroids and oral leukotriene receptor antagonists – were continued at the same dose [9, 10].

Perception of induced bronchoconstriction was measured during the methacholine inhalation test by recording the subjective assessment of dyspnea rated on a modified Borg scale [11] for each methacholine dose-induced decrease in FEV_1. A regression of the FEV_1 percent decrease from baseline and the corresponding Borg score were obtained for each subject, and the results were expressed in terms of the intercept and slope values of the regression [12].

Airway inflammation was assessed by cellular analysis of the ultrasonically nebulised hypertonic saline-induced sputum, according to the method currently used in our laboratory [13]. The results were expressed in terms of total cell count (number of nonsquamous cells per milliliter of sputum) and differential cell count (eosinophils, neutrophils, macrophages, lymphocytes, and epithelial cells as a percentage of nonsquamous cells).

Peripheral blood eosinophils were counted by flow cytometry (Sismex SF 3000, TOA Medical Electronic Co. LMT, Kobe, Japan) and expressed as number of cells per µl of blood.

After completion of the laboratory tests, subjects were asked to record, for the subsequent 12 months, the number of asthmatic exacerbations (defined as an increase in asthma symptoms, airway obstruction, and as-needed use of bronchodilator medications) requiring the use of an oral corticosteroid cycle. They were also asked to record, for the first week of each month, the mean daily number of puffs of inhaled bronchodilators used on an as-required basis for relief of asthmatic symptoms.

At the one-year follow-up visit, spirometry with reversibility of bronchial obstruction was performed, and the total number of severe exacerbations and mean number of days of bronchodilator use reported by subjects were recorded.

Treatment of chronic recurrent sinusitis

In subjects presenting symptoms and signs of chronic, recurrent sinusitis, an oral antibiotic treatment (amoxicillin, 1 g twice daily, for 2 weeks) was given at the beginning of the run-in period. In addition, a nasally inhaled corticosteroid (fluticasone propionate, 100 µg for each nostril, once daily) was continued for the entire study period.

Statistical analysis

Data was reported as mean ± standard deviation (SD). Differences between groups were analysed by the Student *t*-test. A p value of < .05 was considered significant. Comparison of categorical data was performed by the Fisher exact test. Correlations were analysed by linear regression.

Results

According to the selection criteria, the two groups were of similar age (58.4 ± 8.1 years for the menopausal asthma group versus 58.7 ± 5.0 years for the control group), and the control subjects' asthma was of significantly longer duration than that of the menopausal asthma subjects (33.3 ± 7.9 years versus 11.7 ± 9.9 years). The occurrence of atopy was higher in the control group (6/9, or 66%) than in the menopausal asthma group (3/11, or 27%), although the difference was not significant. Sinusitis was much more frequent in the menopausal asthma group than in the control group (8/11, or 72%, versus 1/9, or 11%; $p < 0.05$). No one in either group reported a history of urticaria/angioedema, aspirin sensitivity, or intolerance to angiotensin-converting-enzyme inhibitors.

The anti-asthmatic treatment after optimisation and the asthma severity level established at the end of the run-in period are summarised in table 2. A similar number and dose of medications, in particular, oral and inhaled corticosteroids, were needed for the best possible control of asthma in the two groups. According to the selection criteria, all subjects presented with moderate or severe asthma, and no significant difference was found between groups in the mean (± SD) GINA level of asthma severity.

The results of the methacholine inhalation test and the Borg test for perception of bronchoconstriction, obtained at the end of the run-in period, are reported in table 3. Airway responsiveness to methacholine was increased in all the study subjects, with no difference between the groups. Perception of induced bronchoconstriction did not differ significantly between the groups.

The results of cellular analysis of the induced sputum and peripheral blood eosinophils are reported in table 4. A highly significant increase in sputum eosinophils in menopausal asthma subjects, as compared to control subjects, was demonstrated (19.5 ± 10.8% versus 3.3 ± 4.3%; $p < 0.001$), whereas no difference between groups was found in the total cell count or in the percentage of neutrophils, lymphocytes, macrophages, or epithelial

Table 2. - Ant-iasthmatic treatment after optimization, and asthma severity level

	Menopausal asthma (N = 11)	**Pre-existing asthma (N = 9)**
Oral corticosteroids		
No. of patients (%)	5 (45)	4 (44)
Mean (± SD) mg/day of prednisolone	10.5 (± 3.7)	10 (± 4.1)
Inhaled corticosteroids		
No. of patients (%)	11 (100)	9 (100)
Mean (± SD) µg/day of beclomethasone or equivalent	2,273 (± 467)	2,444 (± 176)
Inhaled anticholinergics		
No. of patients (%)	11 (100)	9 (100)
Oral slow-release theophylline		
No. of patients (%)	2 (18)	1 (11)
Oral leukotriene receptor antagonists		
No. of patients (%)	9 (81)	6 (66)
GINA level of asthma severity (mean ± SD)	3.3 ± 0.8	3.4 ± 0.5

Table 3. - Results of methacholine-inhlalation test, and perception of induced bronchocostriction*

	Menopausal asthma (N = 11)	**Pre-existing asthma (N = 9)**
PD_{20} FEV_1: (µg of methacholine)	64.3 ± 106.4	44.3 ± 183.7†
Borg/FEV_1 regression:		
Intercept (a)	0.4065 ± 0.624	0.986 ± 0.81
Slope (b)	0.1389 ± 0.057	0.1360 ± 0.067

Definition of abbreviations: PD_{20} FEV_1 = dose of methacholine producing a 20% decrease in FEV_1.
* Values are expressed as mean ± SD.
† Geometric mean ± SD.

Table 4. - Cellular analysis of induced sputum and peripheral blood eosinophils*

	Menopausal asthma (*N* = 11)	**Pre-existing asthma (*N* = 9)**
Total cell count per ml (10^5)	13.4 ± 10.1	12.2 ± 5.1
Eosinophils (% of nsc)	19.5 ± 10.8	3.3 ± 4.3†
Neutrophils (% of nsc)	48.5 ± 13.2	51.6 ± 15.4
Macrophages (% of nsc)	26.7 ± 8.9	37.5 ± 15
Lymphocytes (% of nsc)	0.8 ± 1.2	1.5 ± 1.5
Epithelial cells (% of nsc)	5.1 ± 7.7	5.8 ± 4.4
Blood eosinophils (n°/µl of blood)	384 ± 239	232 ± 154

* Values are expressed as mean ± SD.
Significance of difference between groups: † = $p < .001$.

cells. The absolute number of peripheral blood eosinophils was higher in the menopausal asthma group than in the control group, but the difference was not significant.

The results of spirometry with reversibility of bronchial obstruction at the one-year follow-up visit, as well as the number of severe asthmatic exacerbations and the mean daily use of inhaled bronchodilators reported by study subjects at the end of the one-year follow-up period, are summarised in table 1. Spirometry did not differ significantly between the two groups. Moreover, no difference was found in either group between spirometry at one year and initial spirometry (i.e., FEV_1, forced vital capacity [FVC], and FEV_1/FVC% absolute values and post-bronchodilator FEV_1 as percentage of baseline). The number of asthma exacerbations requiring the use of oral corticosteroids was significantly higher in the menopausal asthma group than in the control group. Similarly, the mean number of daily puffs of inhaled bronchodilators used on an as-required basis for the control of symptoms was higher in the menopausal asthma group, but the difference was not significant.

Finally, in the two groups considered as a whole, the number of severe asthmatic exacerbations during the one-year follow-up period correlated well with the percentage of eosinophils in the induced sputum ($r = 0.65$; $p < 0.005$) (fig. 2).

Discussion

The prevalence of asthma is high worldwide and appears to be increasing in many countries [14]. Fortunately, most patients present with mild or moderate disease; that is, it is easy to control with proper education and effective medications [1]. In contrast, a minority of patients are affected by severe asthma that (a) is difficult to control; (b) requires regular treatment with potentially toxic drugs, such as oral and high-dose-inhaled corticosteroids; and (c) is associated with frequent hospitalisations and poor quality of life [1, 15]. Severe asthma absorbs considerable clinical resources [16] and is the object of intensive medical research [17-20]. In fact, although remarkable progress has been made in past decades on the pathogenesis of asthma, little is known about factors capable of influencing the severity of the disease [21, 22].

Asthma starting around the time of menopause may be a useful human model of severe asthma, in that it appears to be frequently characterised by pronounced severity and poor response to treatment, as indicated by clinical and epidemiological observations [4-6]. In addition, some well-known changes in the levels of sex hormones occur with menopause [3], and sex hormones are thought to be potential determinants of severity in asthma [2]. In this regard, it has recently been demonstrated that (a) post-menopausal women, who have lower serum levels of estrogen than those of a fertile age, show a lower risk of developing asthma than pre-menopausal women of a similar age; and (b) replacement therapy with estrogen increases, in a dose-dependent manner, the risk of asthma [23] and can cause bronchospasm in some individuals [24]. Taken together, these observations suggest that, during menopause, physiologically low levels

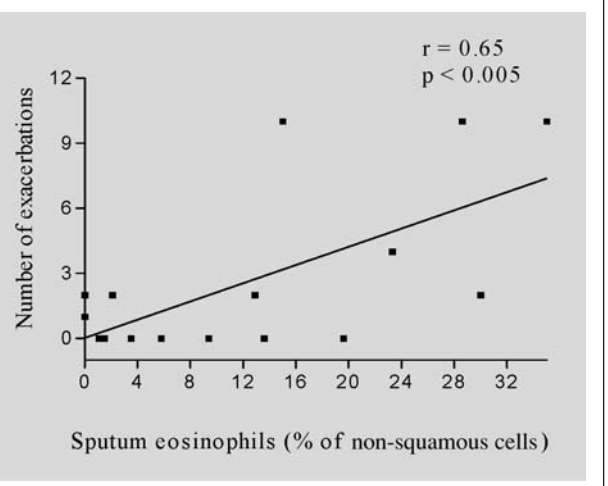

Fig. 2. - Correlation between sputum eosinophils and number of asthmatic exacerbations during 1- year follow-up period.

of estrogen may have protective effects against asthma, whereas abnormally high levels of this sex hormone, either naturally occurring or iatrogenically induced, may increase the risk of asthma [2].

In our study, menopausal asthma was not associated with atopy but was often associated with chronic, recurrent sinusitis. Because sinusitis is considered a potential contributing factor to difficult asthma [17], our study subjects presenting with chronic sinusitis were treated with a standardized, widely accepted therapy, including an oral antibiotic for 2 weeks and a nasally inhaled corticosteroid for the duration of the study. Therefore, any influence of chronic upper airway inflammation on the asthma measurements and response to anti-asthmatic treatment in our study can be considered unlikely.

We adopted other measures to overcome, at least in part, the possible limitations deriving from the open nature of our study. Any influences of undertreatment or overtreatment on the asthma classification and measurements were excluded by preventive optimisation of the anti-asthmatic treatment. Moreover, subjective influences on the measurements were avoided by blinding the study personnel to the group to which each subject belonged.

The most relevant result of our study is the presence of an intense asthmatic inflammation of the airways in subjects with menopausal asthma, as demonstrated by elevated numbers of eosinophils in the induced sputum [25]. Conversely, small numbers of eosinophils, often within the normal range [26], were found in the airways of subjects with pre-existing asthma; this finding is consistent with well-controlled asthma and an optimal response to anti-inflammatory treatment [25, 27, 28]. Although no difference between groups was found in peripheral blood eosinophils, the number of these cells was higher than normal in three subjects in the menopausal asthma group but none in the control group.

Our results are quite similar to those obtained in a recently published large, multicenter, observational study comparing 163 patients with severe asthma with 158 patients whose asthma was controlled by low doses of inhaled corticosteroids [18]. That study showed less atopy and, despite intensive anti-inflammatory treatment, ongoing airway inflammation in the severe asthma group, although the inflammation, in contrast to our results, was predominantly neutrophilic [18]. In addition, the study demonstrated a female/male ratio of 4.4:1 in the severe asthma group, as compared to 1.6:1 in the control asthma group [18]. Thus, the patients with severe asthma in the previous study [18] were very similar to those in our study.

The possible reasons for persistence of significant eosinophilic inflammation in the airways of patients with menopausal asthma, despite a shorter duration of the disease and similar anti-inflammatory treatment, are not understood. However, we can speculate, also on the basis of previous studies [2, 23, 24], that the airway eosinophilia in our subjects might be poorly responsive to corticosteroid therapy because of unknown metabolic alterations, possibly related to abnormal changes in hormone levels.

According to the selection criteria, all of our study, subjects presented with moderate or severe asthma. Neutrophilia of the airways has frequently been reported in patients with severe persistent asthma [18, 29], but we did not find an increased number of sputum neutrophils in our subjects; this may be due to chance, to the small sample size in our study, or to the possibility that our subjects, according to a recent hypothesis [30], were all representative of a single, eosinophilic-mediated asthma subtype.

Apart from an increased severity of objective indices of disease, namely, airway inflammation, responsiveness, and obstruction, the pronounced severity of menopausal asthma could alternatively be due to a blunted perception of dyspnea, possibly related to psychological factors [8]. In fact, because of reduced perception of asthma symptoms, patients might not perceive a developing exacerbation and thus not seek medical care; this might contribute to deterioration of the disease. Moreover, impaired perception of dyspnea has been related to the degree of sputum eosinophilia [31], and, as described above, our subjects with menopausal asthma presented with persistently increased sputum eosinophilia. Theoretically, a higher than normal perception of dyspnea could also be responsible for an increased severity of asthma, in that it could lead to more requests for medical care [8] and, plausibly, a reduced quality of life. However, our data indicates that perception of induced bronchoconstriction was similar in the two study groups, so differences in asthma severity cannot be attributed to differences in this subjective factor.

The asthma exacerbation rate is considered to be a reliable marker of clinical severity of asthma, as indicated by recent, carefully conducted studies [15, 32], and an increased exacerbation rate in our subjects seems to testify to the clinical relevance of the persistence of eosinophilic airway inflammation, despite treatment with high-dose-inhaled (and, in some cases, oral) corticosteroids.

The current study presents at least two weaknesses. First, the sample size is small, but this is due to the objective difficulties encountered in finding women with menopausal asthma. Second, as generally occurs with severe asthma, it can be difficult to distinguish, on clinical grounds, patients with asthma from patients with chronic obstructive pulmonary disease. However, our study subjects had been followed at our hospital for many years and they presented with one or more clear asthmatic features, such as reversibility of airway obstruction, severe bronchial hyperresponsiveness, and eosinophilic airway inflammation.

However, our data is in line with clinical observations and indirect evidence coming from epidemiological studies that asthma that starts at menopause is generally severe, frequently nonatopic, and accompanied by chronic, recurrent sinusitis. Moreover, we have demonstrated that, in women with menopausal asthma, a significant in-

crease in airway eosinophilic inflammation persists despite treatment with corticosteroids.

In conclusion menopausal asthma seems to be frequently characterised by marked severity and by some factors of difficult asthma, in particular the absence of atopy and a chronic sinusitis, although airway responsiveness and perception of induced bronchoconstriction are similar to those characteristics in patients with pre-existing asthma. Importantly, the eosinophilic airway inflammation present in menopausal asthma seems to be poorly responsive to anti-inflammatory treatment with corticosteroids and to predispose to frequent severe exacerbations.

Our findings should be kept in mind when patients with menopausal asthma are included in experimental studies regarding asthma severity and eosinophilic airway inflammation.

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