

Menopausal asthma: a new biological phenotype?

M. P. Foschino Barbaro¹, V. R. Costa², O. Resta², R. Prato³, A. Spanevello⁴, G. P. Palladino¹, D. Martinelli², G. E. Carpagnano¹

¹Institute of Respiratory Disease, Department of Medical and Occupational Sciences, University of Foggia, Foggia, Italy; ²Institute of Respiratory Disease, University of Bari, Bari, Italy; ³Fondazione Salvatore Maugeri, IRCCS, Tradate, Italy; ⁴Department of Medical Sciences, Section of Hygiene, University of Foggia, Apulia Regional Epidemiological Observatory, Foggia, Italy

To cite this article: Foschino Barbaro MP, Costa VR, Resta O, Prato R, Spanevello A, Palladino GP, Martinelli D, Carpagnano GE. Menopausal asthma: a new biological phenotype? *Allergy* 2010; DOI: 10.1111/j.1398-9995.2009.02314.x.

Keywords

asthma breath condensate; eosinophils; hormones; menopause; neutrophils; nitric oxide; pH.

Correspondence

Dr Giovanna Elisiana Carpagnano, Institute of Respiratory Disease, Department of Medical and Occupational Sciences, University of Foggia, Foggia, Italy.
Tel.: 00390881733158
Fax: 00390881733040
E-mail: ge.carpagnano@unifg.it

Accepted for publication 28 November 2009

DOI:10.1111/j.1398-9995.2009.02314.x

Edited by: Thomas Bieber

Abstract

Background: Female hormones play an important role in women's lung health, especially in asthma pathophysiology. Although a growing interest has recently been aroused in asthma related to short-term reproductive states, menopausal asthma has been little studied in the past. The aim of the present study was to explore airway inflammation in menopausal asthmatic women in a noninvasive manner.

Methods: Forty consecutive women with menopausal asthma, 35 consecutive women with premenopausal asthma and 30 age-matched healthy controls were enrolled in the study. Urinary LTE-4, induced sputum inflammatory cells, and exhaled LTE-4, IL-6, pH, and NO levels were measured in all the subjects enrolled.

Results: Women with menopausal asthma showed decreased estradiol concentrations, high sputum neutrophils, and exhaled IL-6. Women with premenopausal asthma presented instead an essentially eosinophilic inflammatory pattern. Higher urine and breath condensate LTE-4 concentrations were found in premenopausal and menopausal asthma compared to controls.

Conclusion: Our results substantiate the existence of a new biological phenotype of menopausal asthma that is mainly characterized by neutrophilic airways inflammation and shares several characteristics of the severe asthma phenotype.

The increased prevalence of asthma registered in the last few years underlies the growing interest aroused by new and different phenotypes, including hormone-related ones (1, 2). Although female hormones have long been recognized as playing an important role in respiratory health (3–11) and particularly in asthma where the severity vary with short-term reproductive states, including the menstrual cycle and pregnancy (12–15), the literature on this topic remains confusing and contradictory.

Menopause, which generally occurs in women around the age of 50, can either coincide with the onset of asthma or be associated with a deterioration of a pre-existing asthma condition (16).

Abbreviations

BMI, body mass index; DHEAs, dihydro-epi-androsterone sulfate; FEV₁, forced expiratory volume in one second; FSH, follicle-stimulating hormone; FVC, forced vital capacity; HRT, hormone replacement therapy; IL-6, interleukin-6; LH, luteinizing hormone; LTE-4, leukotriene E-4; LTRAs, leukotriene receptor antagonists; NO, Nitric oxide.

Clinical observations indicate that menopausal-onset asthma frequently exhibits features which include absence of atopy, recurrent sinusitis, urticaria/angioedema, aspirin sensitivity and/or intolerance to angiotensin-converting-enzyme inhibitors, use of systemic steroids to control symptoms, frequent hospitalizations, and an altered perception of asthmatic symptoms ascribable to the psychological factors associated with menopause (17–19). Menopausal asthma, which occurs in 18% of the total number of female asthma patients, is generally quite severe, thus accounting for an important healthcare problem of today in terms of both management and follow up (2).

Although severe asthma is known to be characterized by an increased urine level of leukotriene E-4 (LTE-4) and to respond to antileukotriene treatment, to our knowledge there is currently no study available on the role either of systemic or of airway leukotrienes in menopausal asthmatics.

Menopause is accompanied by a new hormonal pattern that is characterized by high levels of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and low levels of estrogen and progesterone (20). Unlike physiological

hormonal status, in menopausal asthma, the hormonal pattern changes completely and resembles that of women at a fertile age (21). The concentrations of at least one hormone is outside the normal range in about 80% of asthmatic women, thus indicating that in childbearing women, asthma is frequently associated with alterations in production and metabolism of steroid hormones (22). Remarkably higher levels of 17-betaestradiol seem to play a key role in the onset and/or maintenance of menopausal asthma in predisposed women (21, 23).

A new hypothesis on asthma does suggest a role for sex hormones in modulating the immunologic inflammation that characterizes this condition (24). Sex hormone reduction has, in fact, been found to be associated with a spontaneous synthesis, release, and action of several inflammatory cytokines (2, 25). Balzano et al. (26) were the first to explore airways inflammation in subjects with menopausal asthma by highlighting a biological pattern characterized by eosinophils associated to corticosteroid resistance as well as to predisposition to frequent severe exacerbations in these patients.

To the best of our knowledge, *notwithstanding* the recognized importance attached to the association between airways inflammation in asthma and sex hormones, no other study has been made available so far on the biological phenotype of menopausal asthma.

An increasing interest in the study of the airways inflammation is currently being aroused by two new techniques that allow for a noninvasive sampling from the airways: breath condensate and induced sputum (27, 28). These methods, which have been used widely for the biological study of several lung conditions, including asthma, are often suggested and resorted to in clinical settings (29).

Our aim was to explore the biological inflammatory phenotype of menopausal asthma. To this end, we investigated both systemic and airways inflammation in a group of patients with menopausal asthma, in a group of patients with premenopausal asthma and in a group of menopausal healthy controls by assessing urine LTE-4 levels, induced sputum inflammatory cells, and exhaled LTE-4, interleukin-6 (IL-6), pH, Nitric oxide (NO) levels in the three groups under study. The final objective of this study was to highlight potential correlations between and across sex hormones, the above inflammatory markers, and lung function.

Materials and methods

Subjects

Forty consecutive women with menopausal asthma (59.4 ± 1.6 year), 35 consecutive women with premenopausal asthma (52.1 ± 2.4 year) and 30 healthy menopausal women (58.8 ± 2.1 year) were recruited to the study from the outpatient facility of the Institute of Respiratory Disease of the University of Foggia, Italy. Written informed consent was obtained from all subjects, and the study was approved by the institutional ethics committee. All subjects were classified as premenopausal or menopausal on the basis of a questionnaire asking, among other things, whether or not they were still

menstruating or had entered menopause. They were considered to be menopausal asthmatics if their asthma had started at menopause, i.e. within 1 year before or after their last menstrual period.

Moreover, as indicated by their clinical history, the pre-existing asthma had not worsened at menopause. Non-Caucasian smokers or women on postmenopausal hormone replacement therapy (HRT) were excluded from the study. In the group of menopausal asthmatics, 21 women presented moderate persistent asthma, and 19 exhibited severe asthma. In the group of premenopausal asthmatics, 11 women presented moderate persistent asthma, and 9 exhibited severe asthma.

Asthmatics were treated according to the Global Initiative for Asthma (GINA) guidelines (30): Persistent moderate asthmatics were treated as baseline medications with medium doses of inhaled corticosteroids [equivalent to 200–500 μ g beclomethasone dipropionate (BDP)] and long acting beta agonist (LABA), while severe asthmatics with high doses of inhaled corticosteroids (equivalent 500–1000 μ g BDP) and LABA. At the first visit (visit 1), a complete baseline questionnaire requesting information on medical history (asthma quality-of-life questionnaire), menopausal status, hormone use, and other reproductive and lifestyle variables was administered to all subjects who were then given a physical examination, including body mass index (BMI) measurement, atopy assessment, exhaled NO measurement, spirometry with bronchial obstruction reversibility test, and methacholine bronchial challenge. Serum levels of hormones were measured after standardized rest, at the same time of day for all subjects as they are known to be extremely dependent on circadian rhythm, stress, treatments, etc.

After 3 days from first visit, women underwent breath condensate collection followed by sputum induction (visit 2).

Atopic status

A skin prick test (SPT) was performed for a panel of inhalant allergens as previously described (31) for common aeroallergens (Milan, Italy).

Lung function

Pulmonary function tests were performed within 1 day of the measurement of the breath condensate. Forced expiratory volume in one second (FEV1) and forced vital capacity (FVC) were measured using a spirometer (Sensormedics, Milan, Italy). The best value of three maneuvers was expressed as a percentage of the predicted normal value. After baseline evaluation, spirometry was repeated 15 min after the subjects had inhaled 400 mg of salbutamol as previously reported (32). Reversibility of airways obstruction was expressed in terms of the percentage changes from baseline of the FEV1.

Exhaled breath condensate

The oral exhaled breath condensate was collected using a condenser that allowed for the noninvasive collection of the

nongaseous components of the expired air (EcoScreen; Jaeger, Wurzburg, Germany). Subjects breathed through a mouthpiece and a two-way nonrebreathing valve, which also served as a saliva trap. They were asked to breathe at a normal frequency and at tidal volume, wearing a nose clip, for a period of 10 min. Subjects were instructed to swallow any saliva they felt in their mouth. Condensate, at least 1 ml, was collected as ice at -20°C , transferred to Eppendorf tubes, and stored at -80°C immediately. Samples were stored for not more than 2 months before analysis. Amylase concentration was measured in all samples to exclude any salivary contamination.

Measurement of IL-6 and LTE-4

A specific enzyme immunoassay (EIA) kit (Cayman Chemical, Ann Arbor, MI, USA) was used to measure IL-6 and LTE-4 concentrations in breath condensate. The assays were validated directly by gas chromatography/mass spectrometry. The intraassay and interassay variability was 10%. The detection limits of the assays were 1.5 and 25 pg/ml. The reproducibility of the repeated measurements of IL-6 and LTE-4 was confirmed by the Bland and Altman test and by the variation coefficient (33, 34).

Exhaled pH measurement

A stable pH was achieved in all cases after deaeration/decarbonation of breath condensate specimens by bubbling with argon (350 ml/min) for 10 min, as previously reported (35). pH was then measured within 5 min of condensate collection by means of a pH meter (Jenway-350, Ltd. Gransmore Green, England) with a 0.00–14.00 pH range and a resolution/accuracy on the order of 0.01 ± 0.02 pH. The reproducibility of the repeated measurements of pH was confirmed by the Bland and Altman test and by the variation coefficient (33, 36).

Measurement of exhaled NO

A rapid-response chemiluminescence NO analyzer (NIOX) was used to quantify oxides of nitrogen (NOs). Two-point calibrations were performed daily using 5.2-parts-per-million calibration gas.

Exhaled NO (FENO) was measured using a previously described restricted breath technique, which employed expiratory resistance and positive-mouth pressure to close the velum and exclude nasal NO, and a constant expiratory flow of 45 ml/s. Subjects inhaled to total lung capacity and exhaled while targeting a constant pressure of 20 mm Hg. Exhalations proceeded until a clear NO plateau of at least 3 s duration was achieved. Repeated exhalations were performed until three plateaus agreed within 5% (37, 38).

Sputum induction and analysis

According to the method described by Spanevello et al., (39) sputum was induced through the inhalation of hypertonic saline solution (4.5%) with an ultrasonic nebulizer (DeVilbiss

65; DeVilbiss Corporation, Somerset, PA, USA) and analyzed after selection of plug. Twelve menopausal, 10 premenopausal asthmatics, and seven controls were not able to produce adequate sputum samples (defined as containing at least 500 nonsquamous cells), and their expectorates were discharged.

Statistical analysis

Data were expressed as the mean $\pm$ SD. One-way ANOVA test and Kruskal–Wallis rank tests were used to compare groups. A Bartlett's test for equal variances was performed to find out whether the ANOVA test was applicable. Correlation between variables was measured using the Spearman rank correlation test. Correlations were performed in the group of menopausal and premenopausal asthma.

Significance was defined as a *P*-value of <0.05 . Stata 10.1tm MP software for MAC OS 10.5.6 was used to store and analyze the data.

Results

The anthropometric, hormonal, and functional data of menopausal asthmatics, premenopausal asthmatics, and healthy controls are reported in Table 1.

There was significant difference among the groups (Kruskal–Wallis $P < 0.05$) for the concentrations of FSH, LH, dihydro-epi-androsterone sulfate (DHEAs), and estradiol. Significantly decreased concentrations of FSH, LH, and DHEAs were found in menopausal asthmatics compared to premenopausal asthmatics and healthy controls (39.8 ± 6 vs 56.9 ± 5.5 and 56.8 ± 2.8 mUI/ml, $P < 0.001$; 17 ± 4.2 vs 29.6 ± 4.6 and 25.2 ± 1.6 mUI/ml, $P < 0.001$; 0.45 ± 0.1 vs 0.9 ± 0.2 and 0.7 ± 0.2 ng/ml, $P < 0.001$). Higher concentration of estradiol levels was observed in menopausal and premenopausal asthmatics than in healthy controls (27.9 ± 4.8 and 24.1 ± 5.2 vs 7.8 ± 1.3 pg/ml, $P < 0.001$).

Significantly lower percentages of induced sputum eosinophils were found in menopausal asthmatics compared to premenopausal asthmatics (17.5 ± 5 vs $32.2 \pm 7.5\%$, $P < 0.001$). The same was true for exhaled NO (26.2 ± 5.1 vs 34.96 ± 6.8 ppb), exhaled pH (7.5 ± 0.1 vs 7.8 ± 0.1 , $P < 0.001$), exhaled LTE-4 (124.9 ± 53.4 vs 515.7 ± 152.4 pg/ml, $P < 0.001$), and urinary LTE-4 (594.4 ± 214 vs 712.72 ± 388.4 pg/ml, $P < 0.05$). Higher percentages of neutrophils were instead observed in menopausal asthmatics compared to premenopausal asthma (50.8 ± 6.3 vs $40.6 \pm 4.6\%$, $P < 0.001$). A trend toward the increase was also found for exhaled IL-6 (31.5 ± 6.6 vs 28.8 ± 7.3 pg/ml, $P > 0.05$).

The percentages of induced sputum eosinophils showed a positive correlation with exhaled NO ($\rho = 0.83$, $P < 0.001$; Panel A, Fig. 1) and with exhaled LTE-4 ($\rho = 0.87$, $P < 0.001$; Panel B, Fig. 1).

Estradiol levels were found to correlate positively with urinary LTE-4 ($\rho = 0.78$, $P < 0.001$; Panel A, Fig. 2), percentages of neutrophils in the induced sputum ($\rho = 0.6$, $P < 0.001$; Panel B, Fig. 2), and exhaled IL-6 ($\rho = 0.37$, $P < 0.001$; Panel C, Fig. 2).

Table 1 Anthropometric, hormonal, and functional data of patients with menopausal asthma with premenopausal asthma and of healthy controls

	Menopausal asthma	Premenopausal asthma	Healthy controls
Age (year)	59.4 ± 1.6	52.1 ± 2.4	58.8 ± 2.1
Atopy	0/40	26/35	0/30
Smoking habit	0/40	0/35	0/30
AQLQ (score)	12.4 ± 1.9**	8.6 ± 1.8	0**
FEV ₁ (%)	65.8 ± 8.5	68.8 ± 7.4	92.5 ± 4.5*
FVC (%)	94.8 ± 7.8	91.6 ± 6.6	102.5 ± 4.7
FEV ₁ /FVC (%)	61.1 ± 6.5	63.8 ± 5.6	89.0 ± 3.2**
BMI (Kg/m ²)	31.5 ± 1.3	27.6 ± 0.9	30.4 ± 1.5
FSH (mUI/ml)	39.8 ± 6**	56.9 ± 5.5	56.8 ± 2.7
LH (mUI/ml)	17 ± 4.2**	29.6 ± 4.6	25.2 ± 1.6
Estradiol (pg/ml)	27.9 ± 4.8	24.1 ± 5.2	7.8 ± 1.3
Prolactin (ng/ml)	13.9 ± 3.9	13.9 ± 3.2	12.2 ± 1.9
Progesteron (ng/ml)	0.5 ± 0.1	0.6 ± 0.1	0.6 ± 0.2
DHEAs (ng/ml)	0.45 ± 0.1*	0.9 ± 0.2	0.7 ± 0.2
Sputum macrophages (%)	31.9 ± 2.6	26.9 ± 3.4	67.4 ± 1.9*
Sputum eosinophils (%)	17.5 ± 5**	32.2 ± 7.5	0.9 ± 0.2**
Sputum neutrophils (%)	50.8 ± 6.3*	40.6 ± 4.6	37.1 ± 5.7**
Sputum total cells count × 10 ⁶ /ml	4.0 ± 1.5	4.6 ± 0.9	4.4 ± 1.2
Exhaled NO (ppb)	26.2 ± 5.1*	34.96 ± 6.8	6.7 ± 2**
Exhaled pH	7.5 ± 0.1	7.8 ± 0.1	7.9 ± 0.1
Exhaled IL-6 (pg/ml)	31.5 ± 6.6	28.8 ± 7.3	27.5 ± 2.3**
Exhaled 12 LTE-4 (pg/ml) (p(pg/ml)	124.9 ± 53.4**	515.7 ± 152.4	55.3 ± 10.1***
Urinary LTE-4 (pg/ml)	594.4 ± 214**	712.72 ± 388.4	103.4 ± 15.3***

BMI, body mass index; DHEAs, dihydro-epi-androsterone sulfate; FSH, follicle-stimulating hormone; FVC, forced vital capacity; IL-6, interleukin-6; LH, luteinizing hormone; LTE-4, leukotriene E-4; NO, nitric oxide.

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$.

Discussion

The main result of this study was the identification of a biological inflammatory phenotype of menopausal asthma other than that of premenopausal asthma, the former being mainly characterized by a neutrophilic pattern of airway inflammation (high percentages of neutrophils in both induced sputum and exhaled IL-6 levels) and the latter mainly by an eosinophilic pattern (high percentages of eosinophils in both induced sputum and exhaled LTE-4 levels). Menopausal asthmatics exhibited higher values of urinary and exhaled LTE-4 compared to controls. Moreover, estradiol concentrations were found to be lower in menopausal asthmatics and to correlate positively with inflammatory markers.

Although the relationship between sexual hormones and inflammation has widely been recognized, only one study on airway inflammation in menopausal asthma to date has focused on such a relationship (26). Contrary to the Balzano study, (26) which showed increased eosinophil levels in the induced sputum of menopausal asthmatics, we have been able to describe for the first time a prevailing increase in neutrophil levels in the sputum of menopausal asthmatics compared to that of women of fertile age. As a matter of fact, the population enrolled in the present study included menopausal women with moderate-to-severe asthma with more severe and not-well-controlled asthma symptoms compared

to the population of the Balzano study (26). Furthermore, as discussed by the same Balzano et al., (26) the lack of neutrophils in the sputum observed in menopausal asthmatics could be ascribed either to the small size of the sample or to the possibility that the subjects in question were all representative of a single, eosinophil-mediated asthma subtype. The significant decrease in exhaled pH and the increase in exhaled NOs observed both in menopausal and premenopausal asthmatics are perfectly in line with previous data and provides additional proof of the severity of asthma in the subjects selected in our study (40, 41).

To get new insights into menopausal asthma airway inflammation in a noninvasive manner, we investigated for the first time the expression of soluble markers, neutrophils, and eosinophils in the breath condensate (IL-6, LTE-4) as well as in the exhaled pH and NOs of the women enrolled in the present study. In the group of premenopausal asthma, higher levels of exhaled LTE-4, exhaled pH, and exhaled NOs were observed that are in line with the results of previous studies on eosinophilic inflammation in nonmenopausal age asthma (42–44). Instead the increased exhaled IL-6 levels observed in menopausal asthma seem to substantiate our assumption that we are dealing with a new biological phenotype of menopausal asthma mainly characterized by a neutrophilic airway inflammation. Our hypothesis is that this new phenotype shares characteristics similar to those of the

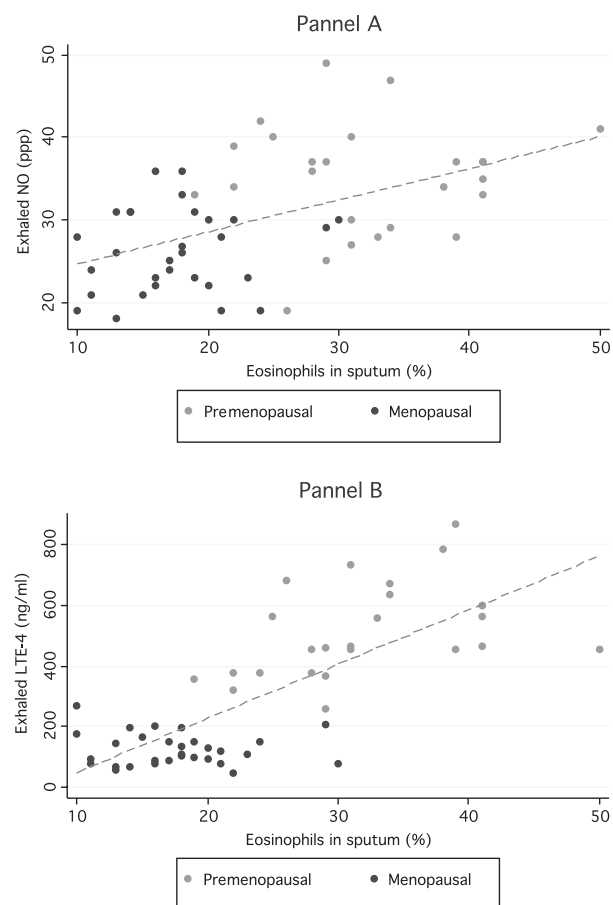


Figure 1 Panel A: Positive correlation between the percentages of induced sputum eosinophils and exhaled NO. Panel B: Positive correlation between the percentages of induced sputum eosinophils and with exhaled LTE.

phenotype described by the ENFUMOSA study group: severe asthma showing a typical female predilection (4.4 : 1), reduced association with atopy, higher percentages of neutrophils in induced sputum (45), and poor response to corticosteroid therapy.

The measurement of urinary leukotriene-E4 concentration is a sensitive and noninvasive method of assaying total-body cysteinyl leukotriene production and changes in cysteinyl leukotriene levels in specific microenvironment, including airways. This measurement has been used for monitoring exposure to asthma triggers and as a marker of susceptibility to treatment with leukotriene receptor antagonists (46, 47). LTE-4 has been largely studied in asthma where higher concentrations of it have been found in urine, sputum, and breath condensate (29, 46, 47). Elevated levels of LTE-4 have also been reported in the severe asthmatics of the ENFUMOSA study (45). To our knowledge, no study has so far been carried out on the measurement of this leukotriene in menopausal asthma. For the first time, we have been able to describe the higher levels of LTE-4 both in the urine and in

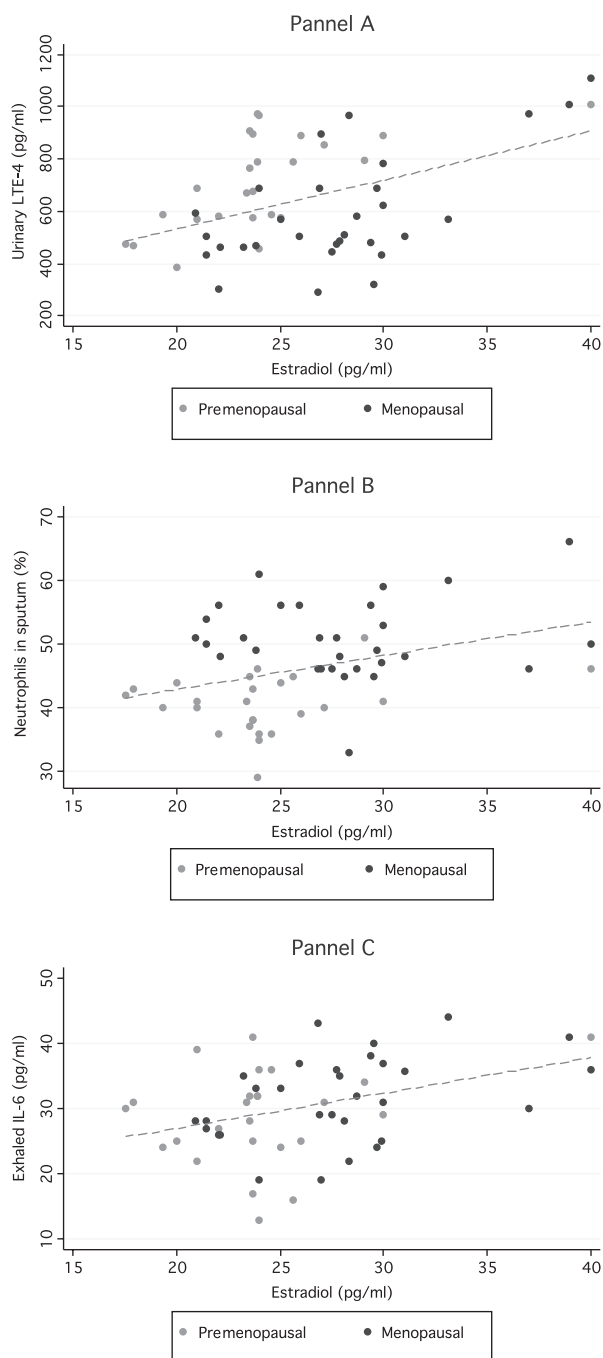


Figure 2 Panel A: Positive correlation between estradiol levels and urinary LTE-4. Panel B: Positive correlation between estradiol levels and percentages of neutrophils in the induced sputum. Panel C: Positive correlation between estradiol levels and exhaled IL-6.

the exhaled breath of menopausal asthmatics compared to healthy controls. This seems to suggest the possibility of implementing a new antileukotriene therapeutic approach also in difficult-to-treat menopausal asthma. The high concentrations of LTE-4 found in the urine of menopausal

asthmatics on high-dose inhaled steroids already reported in literature do confirm that leukotriene synthesis is not sensitive to the steroid regulation (48, 49). As a matter of fact, the measurement of urinary and exhaled LTE-4 concentration has already been proposed to help identify those patients with asthma most likely to benefit from leukotriene receptor antagonists (LTRAs) (47, 50).

The healthy subjects enrolled in this study showed some biological signs of an underlying airway inflammation in that they exhibited higher percentages of neutrophils and exhaled IL-6 compared to the levels previously reported in controls (39, 51). These results are in line with our previous reports (GE Carpagnano, MP Foschino Barbaro, unpublished data) and could be traced back to the fact that the healthy women enrolled were age-matched with the asthmatics, and therefore more above the average age of the controls of other studies.

To avoid any potential bias in terms of results, smokers were excluded from the population of the present study as smoking is a possible source of error related as it may be to (i) earlier menopause (likely to be ascribed to its anti-estrogenic effects), as well as to and (ii) worsened respiratory health.

As expected, a correlation was found between the eosinophil concentrations observed in the sputum and the exhaled NOs of our subjects and also in the urinary and exhaled LTE-4 levels already reported by other authors (29). Exhaled NOs, urinary LTE-4, and sputum eosinophils have recently

emerged as potential tools to assess and treat asthma airway inflammation, especially in combination with the conventional methods used to control airway conditions (29).

On the other hand, consistently with other authors, no correlation was found between these biological markers and lung function, thus not substantiating the hypothesis that decreased lung function and airway inflammation account for two different steps of asthma development (52).

For the first time, we have been able to describe a correlation between estradiol, whose concentrations were found to follow a pattern similar to that of fertile age, and inflammatory markers. This correlation provides additional proof that sex hormones are involved in asthma airway inflammation.

In conclusion, we described a new phenotype of menopausal asthma, which is mainly characterized by a neutrophilic airway inflammation, sharing some of the features of the asthma phenotype reported by the ENFUMOSA study group, including severity, the nonsensitivity to steroids, poor symptom control, and higher levels of LTE-4.

Now, in consideration of the fact that menopausal asthma accounts for a difficult-to-treat condition and that this difficulty is thought to be partially ascribable to several still-obscure interferences between sexual hormones and airway inflammation, we suggest that menopausal asthma might be considered a new clinical-biological phenotype that strongly deserves to be additionally elucidated as it accounts for 18% of women's asthma.

References

- Lange P, Parner J, Prescott E, Ulrik CS, Vestbo J. Exogenous female sex steroid hormones and risk of asthma and asthma-like symptoms: a cross sectional study of the general population. *Thorax* 2001;**56**:613–662.
- Foschino Barbaro MP, De Tullio R, Cagnazzo MG, Depalo A, Carpagnano GE, Spanevello A et al. Asthma and the menopause. *Ageing Lung* 2006;**1**:13–19.
- Real FG, Svanes C, Omenaas ER, Antó JM, Plana E, Jarvis D et al. Lung function, respiratory symptoms, and the menopausal transition. *J Allergy Clin Immunol* 2008;**121**:72–80.e3. Epub 2007 Oct 29.
- Becklake MR, Kauffmann F. Gender differences in airway behaviour over the human life span. *Thorax* 1999;**54**:1119–1138.
- Real FG, Svanes C, Omenaas ER, Antó JM, Plana E, Janson C et al. Menstrual irregularity and asthma and lung function. *J Allergy Clin Immunol* 2007;**120**:557–564.
- Salam MT, Wenten M, Gilliland FD. Endogenous and exogenous sex steroid hormones and asthma and wheeze in young women. *J Allergy Clin Immunol* 2006;**117**:1001–1007.
- Gómez Real F, Svanes C, Björnsson EH, Franklin KA, Gislason D, Gislason T et al. Hormone replacement therapy, body mass index, and asthma in perimenopausal women: a cross sectional survey. *Thorax* 2006;**61**:34–40.
- Svanes C, Real FG, Gislason T, Jansson C, Jögi R, Norrman E et al. Association of asthma and hay fever with irregular menstruation. *Thorax* 2005;**60**:445–450.
- Troisi RJ, Speizer FE, Willett WC, Trichopoulos D, Rosner B. Menopause, postmenopausal estrogen preparations, and the risk of adult-onset asthma. A prospective cohort study. *Am J Respir Crit Care Med* 1995;**152**(4 Pt 1):1183–1188.
- Skobeloff EM, Spivey WH, St Clair SS, Schoffstall JM. The influence of age and sex on asthma admissions. *JAMA* 1992;**268**:3437–3440.
- Eliasson O. The male-female ratio of hospital admissions for asthma. *Am Rev Respir Dis* 1985;**131**(Pt 2):A110.
- Rees L. An aetiological study of premenstrual asthma. *J Psychosom Res* 1963;**52**:191–197.
- Hanley SP. Asthma variation with menstruation. *Br J Dis Chest* 1981;**75**:306–308.
- Gibbs CJ, Coutts II, Lock R, Finnegan OC, White RJ. Premenstrual exacerbation of asthma. *Thorax* 1984;**39**:833–836.
- Eliasson O, Scherzer HH, DeGraff AC Jr. Morbidity in asthma in relation to the menstrual cycle. *J Allergy Clin Immunol* 1986;**77**(1 Pt 1):87–94.
- Bonner JR. The epidemiology and natural history of asthma. *Clin Chest Med* 1984;**5**:557–565. Review.
- Durwood BJ. Concomitant problems with asthma. *Clin Chest Med* 1983;**5**:557–565.
- Myers JR, Sherman CB. Should supplemental estrogens be MSDN as steroid-sparing agents in asthmatic women? *Chest* 1994;**106**:318–319.
- Ten Brinke A, Ouwkerck ME, Zwindermann AH, Spiuhooven P, Bel EH. Psychopathology in patients with severe asthma is associated with increased health care utilization. *Am J Respir Crit Care Med* 2001;**163**:1093–1096.
- Kohler PO. Clinical endocrinology. New York: John Wiley, 1986.
- Della Torre F, Cassani L, Segale M et al. Asma ed orticaria nell'anziano: ruolo degli ormoni ipofiso-gonadici in menopausa. *Rassegna Geriatria* 1988;**24**:165–171.
- Rubio Ravelo L, Gago Rodríguez B, Almirall Collazo JJ, Bell Heredia L, Fernández Fernández L. Comparative study of progesterone, estradiol and cortisol concentrations in asthmatic and nonasthmatic women. *Allergol Immunopathol (Madr)* 1988;**16**:263–266.

23. Farina F, Colombi S, Cantore R, Pastore N, Centanni F, Galimberti M. Study of hypo-physal and gonadal hormones and cases of postmenopausal occurrence of bronchial asthma. *Minerva Med* 1986;**77**:243–247.
24. Balzano G, Fuschillo S, Melillo G et al. Asthma and sex hormones. *Allergy* 2001;**56**:13–20.
25. Pfeilschifter J, Kodiz R, Pfohl M et al. Changes in proinflammatory cytokine activity after menopause. *Endocr Rev* 2002;**23**:90–119.
26. Balzano G, Fuschillo S, De Angelis E, Gaudiosi C, Mancini A, Caputi M. Persistent airway inflammation and high exacerbation rate in asthma that starts at menopause. *Monaldi Arch Chest Dis* 2007;**67**:135–141.
27. Horváth I, Hunt J, Barnes PJ, Alving K, Antczak A, Baraldi E et al. Exhaled breath condensate: methodological recommendations and unresolved questions. *Eur Respir J* 2005;**26**:523–548.
28. Carpagnano GE, Foschino-Barbaro MP, Spanevello A, Resta O, Carpagnano F, Mulé G et al. 3p microsatellite signature in exhaled breath condensate and tumor tissue of patients with lung cancer. *Am J Respir Crit Care Med* 2008;**177**:337–341.
29. Gogate S, Katial R. Pediatric biomarkers in asthma: exhaled nitric oxide, sputum eosinophils and leukotriene E4. *Curr Opin Allergy Clin Immunol* 2008;**8**:154–157.
30. Guidelines from the Global Strategy for Asthma Management and Prevention, Global Initiative for Asthma (GINA). 2007. Available from: <http://www.ginasthma.org>.
31. Dreborg S. Skin testing. The safety of skin tests and the information obtained from using different methods and concentrations of allergen. *Allergy* 1993;**48**:473–475.
32. Pellegrino R, Viegi G, Brusasco V, Crapo RO, Burgos F, Casaburi R et al. Interpretative strategies for lung function tests. *Eur Respir J* 2005;**26**:948–968.
33. Bland JM, Altman DG. Measuring agreement in method comparison studies. *Stat Methods Med Res* 1999;**8**:135–160.
34. Montuschi P, Barnes PJ. Exhaled leukotrienes and prostaglandins in asthma. *J Allergy Clin Immunol* 2002;**109**:615–620.
35. Profita M, La Grutta S, Carpagnano E, Riccobono L, Di Giorgi R, Bonanno A et al. Noninvasive methods for the detection of upper and lower airway inflammation in atopic children. *J Allergy Clin Immunol* 2006;**118**:1068–1074. Epub 2006 Sep 12.
36. Borrill Z, Starkey C, Vestbo J, Singh D. Reproducibility of exhaled breath condensate pH in chronic obstructive pulmonary disease. *Eur Respir J* 2005;**25**:269–274.
37. American Thoracic Society; European Respiratory Society. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;**171**:912–930.
38. Kharitonov SA, Gonio F, Kelly C, Meah S, Barnes PJ. Reproducibility of exhaled nitric oxide measurements in healthy and asthmatic adults and children. *Eur Respir J* 2003;**21**:433–438.
39. Spanevello A, Gonfalonieri M, Sulotto F et al. Induced sputum cellularity. *Am J Respir Crit Care Med* 2000;**162**:1172–1174.
40. Carpagnano GE, Barnes PJ, Francis J, Wilson N, Bush A, Kharitonov SA. Breath condensate pH in children with cystic fibrosis and asthma: a new noninvasive marker of airway inflammation? *Chest* 2004;**125**:2005–2010.
41. van Veen IH, Sterk PJ, Schot R, Gauw SA, Rabe KF, Bel EH. Alveolar nitric oxide versus measures of peripheral airway dysfunction in severe asthma. *Eur Respir J* 2006;**27**:951–956. Epub 2006 Jan 30.
42. Shibata A, Katsunuma T, Tomikawa M, Tan A, Yuki K, Akashi K et al. Increased leukotriene E4 in the exhaled breath condensate of children with mild asthma. *Chest* 2006;**130**:1718–1722.
43. Brunetti L, Francavilla R, Tesse R, Strippoli A, Polimeno L, Loforese A et al. Exhaled breath condensate pH measurement in children with asthma, allergic rhinitis and atopic dermatitis. *Pediatr Allergy Immunol* 2006;**17**:422–427.
44. Pavord ID, Shaw D. The use of exhaled nitric oxide in the management of asthma. *J Asthma* 2008;**45**:523–531. Review.
45. The ENFUMOSA Study Group. The ENFUMOSA cross-sectional European multicentre study of the clinical phenotype of chronic severe asthma. *Eur Respir J* 2003;**22**:470–477.
46. Rabinovitch N. Urinary leukotriene E4. *Immunol Allergy Clin North Am* 2007;**27**:651–664. vii
47. Cai C, Yang J, Hu S, Zhou M, Guo W. Relationship between urinary cysteinyl leukotriene E4 levels and clinical response to antileukotriene treatment in patients with asthma. *Lung* 2007;**185**:105–112. Epub 2007 Mar 28.
48. Sampson AP, Castling DP, Green CP, Price JF. Persistent increase in plasma and urinary leukotrienes after acute asthma. *Arch Dis Child* 1995;**73**:221–225.
49. Laitinen LA, Laitinen A, Haahtela T, Vikka V, Spur BW, Lee TH. Leukotriene E-4 and granulocytic infiltration into asthmatic airways. *Lancet* 1993;**341**:989–990.
50. Montuschi P, Mondino C, Koch P, Barnes PJ, Ciabattini G. Effects of a leukotriene receptor antagonist on exhaled leukotriene E4 and prostanoids in children with asthma. *J Allergy Clin Immunol* 2006;**118**:347–353. Epub 2006 Jul 3.
51. Carpagnano GE, Foschino Barbaro MP, Resta O, Gramiccioni E, Valerio NV, Bracciale P et al. Exhaled markers in the monitoring of airways inflammation and its response to steroid's treatment in mild persistent asthma. *Eur J Pharmacol* 2005;**519**:175–181.
52. Strunk RC, Szefer SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. *J Allergy Clin Immunol* 2003;**112**:883–892.