



Distinct profile of inflammatory and remodelling biomarkers in sputum of severe asthmatic patients with or without persistent airway obstruction

Silvana Cianchetti^{a*}, Cristina Cardini^a, Ilaria Puxeddu^b, Manuela Latorre^a, Maria Laura Bartoli^a, Matteo Bradicich^a, Federico Dente^a, Elena Bacci^a, Alessandro Celi^a and Pierluigi Paggiaro^a

ABSTRACT

Background: Both inflammatory and remodelling processes are associated with irreversible airway obstruction observed in severe asthma. Our aim was to characterize a group of severe asthmatic patients with or without persistent airway obstruction in relation to specific sputum inflammatory and remodelling biomarkers.

Methods: Forty-five patients under regular high-dose inhaled corticosteroid/ β -2agonist treatment were studied, after a follow-up period of at least 2 years, with a minimum of 4 visits. Periostin, TGF- β , RANTES, IL-8, GM-CSF, FGF-2, and cell counts were measured in induced sputum. Serum periostin was also measured.

Results: Sputum induction was successfully performed in all but 5 patients. There were no significant differences in demographic and clinical data between patients with non-persistent obstruction (NO: FEV₁/VC > 88%pred.) and those with persistent obstruction (O: a not completely reversible obstruction with FEV₁/VC < 88%pred. at each visit before the study visit). Patients with persistent obstruction had significantly higher sputum periostin and TGF- β concentrations than NO patients and a trend of higher serum periostin levels. GM-CSF and FGF-2 were significantly increased in NO compared to O patients. No differences between groups were found for RANTES, IL-8 and differential cell counts. Sputum periostin inversely correlated with functional parameters (prebronch. FEV₁: $\rho = -0.36$, $p < 0.05$; postbronch. FEV₁: $\rho = -0.33$, $p = 0.05$). Patients with high sputum periostin concentration (>103.3 pg/ml: median value) showed an absolute number of sputum eosinophils significantly higher than patients with low sputum periostin; this behavior was unobserved when serum periostin was considered.

Conclusions: Only periostin and TGF- β identified a subgroup of severe asthmatic patients with persistent airway obstruction. Sputum periostin was also inversely associated with FEV₁ and proved to be a more sensitive biomarker than serum periostin to identify severe asthmatics with higher sputum eosinophilia.

Keywords: Severe asthma, Remodelling, Airway inflammation, Biomarkers, Induced sputum

^aRespiratory Pathophysiology Unit, Department of Surgery, Medicine, Molecular Biology, and Critical Care, University of Pisa, Pisa, Italy
*Corresponding author Department of Surgery, Medicine, Molecular Biology, and Critical Care, University of Pisa Ospedale di Cisanello, Via Paradisa 2, 56124, Pisa, Italy E-mail: silvana.cianchetti@dict.unipi.it
Full list of author information is available at the end of the article

INTRODUCTION

Asthma is a chronic inflammatory disease also characterized by typical structural changes in the airways and in the lung parenchyma called remodelling. Smooth muscle alteration is known to be the main structural change in airway remodelling. It distinguishes severe from moderate asthmatics and may also contribute to the difficulty in obtaining a level of adequate disease control.¹ In particular, severe asthmatic patients with chronic persistent obstruction are reported to have increased airway smooth muscle area and a trend towards increased subepithelial fibrosis compared to patients with intermittent obstruction.² On the other hand, persistent eosinophilic airway inflammation is described as a key process for determining irreversible airway obstruction in severe asthma.³ Patients with asthma and irreversible severe airflow obstruction (post broncFEV₁<50%pred.) have shown a greater inflammatory process (expressed by exhaled nitric oxide and blood eosinophil count) and airway remodelling (assessed by bronchial wall thickening on high-resolution computed tomography scan) than patients with normal lung function, despite a similar pharmacological treatment.⁴ Therefore, persistent bronchial obstruction in severe asthmatic subjects seems to be associated with persistent inflammation and structural abnormalities in the airways.

Both the inflammatory and the remodelling processes are associated with high expression of chemokines, growth factors and matricellular proteins; all these molecules most likely contribute to bronchial hyperresponsiveness and to irreversible airway obstruction, which occurs especially in severe asthmatic patients, despite adequate corticosteroid treatment. Transforming growth factor- β -1 (TGF- β), produced by both inflammatory and airway structural cells, is a cytokine involved in airway inflammation and remodelling in severe asthma. Sputum levels of TGF- β have been found to be significantly higher in moderate persistent asthmatic patients compared to control subjects.⁵ This cytokine is a potent chemotactic factor and activator for different types of inflammatory cells,

and is also involved in epithelial transformation, sub-epithelial fibrosis and airway smooth muscle alteration.⁶ In this respect, Ferreira et al.⁷ reported that the decreased periostin-TGF- β pathway in the muscle cells of severe asthmatic biopsies was associated with a more proliferative phenotype of airway smooth muscle cells, suggesting a prominent role of both periostin and TGF- β in the structural changes in the airways of severe asthmatic patients with persistent bronchial obstruction. Periostin is a matricellular protein that increases during eosinophilic inflammation,⁸ but it is also directly involved in airway remodelling. In this context, Bobolea et al.⁹ reported increased sputum periostin levels in severe asthmatic subjects with fixed, compared to variable, airway obstruction. Periostin has also demonstrated to have a weak inverse relationship with the degree of airflow obstruction, as measured by the FEV₁% predicted,¹⁰ and has been described as an independent risk factor for FEV₁ decline in severe asthmatic patients on long-term ICS treatment.¹¹ A profibrotic role has been reported for fibroblast growth factor-2 (FGF-2) inversely correlating with the pulmonary function in mild to severe asthmatics, either in sputum or in bronchial biopsies.¹² Conversely, recent studies have highlighted a protective role of FGF-2 in pulmonary diseases for its ability to enhance epithelial cell regeneration^{13,14} and to antagonize TGF- β proremodelling effects,¹⁵ thus suggesting a potential therapeutic use of FGF-2 in asthma treatment.

Among the inflammatory chemokines, the expression of interleukin-8 (IL-8), but not of the regulated on activation normal T cell expressed and secreted (RANTES), increased in the airway smooth muscle cells of endobronchial biopsies obtained from subjects with severe asthma.¹ In particular, IL-8 was found to stimulate the proliferation and migration of bronchial smooth muscle cells,¹⁶ suggesting a role in the remodelling process in severe asthma. Moreover, a proinflammatory action has been described for this chemokine in the sputum of severe asthmatics, where IL-8 levels correlated with total neutrophils but not with lung function.¹⁷ This finding was confirmed by another

study reporting that IL-8 and other chemokines and growth factors, including RANTES and the granulocyte-macrophage colony-stimulating factor (GM-CSF) in sputum samples of severe asthmatics, did not distinguish between patients with or without chronic persistent obstruction.²

According to this background, we tried to characterize a group of severe asthmatic patients with or without persistent airway obstruction in relation to specific inflammatory/remodelling biomarkers. In particular, Periostin, TGF- β , RANTES, IL-8, GM-CSF, FGF-2, and cell counts were measured in induced sputum. We also included non-invasive or minimally invasive measures of inflammation by using exhaled nitric oxide (NO), blood eosinophil counts, and serum periostin.

To the best of our knowledge, no previous studies have analyzed this pattern of inflammatory/remodelling mediators in severe asthmatic subjects with different airway obstruction.

PATIENTS AND METHODS

Asthmatic patients

We enrolled 45 severe asthmatic patients; 42 were non-smokers; the 3 ex-smoker patients had quit smoking at least 3 years before and had a smoking history of less than 10 pack-years. These patients were selected from our database of severe asthmatics, according to the presence, or not, of persistent bronchial obstruction. Severe asthma was defined according to the International European Respiratory Society/American Thoracic Society Guidelines on Severe Asthma (ERS/ATS):¹⁸ all patients were either uncontrolled or partly controlled despite regular treatment with high-dose inhaled corticosteroids plus long-acting β -₂ agonists (LABA) often associated with other drugs, or they remained controlled only with this high level of treatment. All recruited patients satisfied this definition during a follow-up period (at least 2 years, with a minimum of 4 visits) in which symptoms and pulmonary function were regularly assessed and asthma treatment was appropriately managed, according to the international recommendations:¹⁹ typical symptoms with or without risk

factors, associated with the demonstration of a large variability over time in FEV₁ (in repeated spirometric measurements) or in the presence of a positive reversibility test or methacholine test. We included in this study only patients followed for a long period of time in our clinic, who had previously demonstrated these functional abnormalities. All patients had been evaluated for allergic sensitization and for the presence of comorbidities, which were treated appropriately. We excluded patients with other pulmonary diseases, including COPD and asthma-COPD overlap syndrome, according to clinical history and the presence of risk factors for COPD.

Each patient was evaluated in a stable phase of the disease and outside the recent exacerbation period. During the study visit all patients underwent lung function tests, blood sampling, exhaled nitric oxide measurement and induced sputum collection.

The patients were divided into two groups according to the post-bronchodilator FEV₁/VC (% of the predicted value). The former group included patients reporting FEV₁/VC < 88-89% of the predicted value both before and after bronchodilator administration, at each visit before the study visit: they were classified as persistent bronchial obstruction group. All the other patients had a FEV₁/VC \geq 88-89% of the predicted value in most of the visits before the study visit: they were assigned to the non-persistent airway obstruction group.

The study was undertaken in accordance with the Helsinki Declaration and was approved by the Local Ethical Committee, within the context of an observational multicenter project on severe asthma in Italy (n. 1245/2016). Informed consent was obtained from each patient for the use of personal data.

Methods

Spirometry

Pulmonary function tests were performed with the same equipment (Elite Series plethysmography Medical Graphics, St Paul, Minnesota, USA)²⁰; the predicted values were obtained from the reference equations of the ERS.²¹ A bronchodilator reversibility test was conducted by measuring

No.	45
Sex (male/female)	13/32
Age (yrs)	60 ± 13
BMI	28 ± 5.9
Atopy (yes/no)	30/15
Age of onset, yrs:	39 ± 19.5
- Early, n (%)	9 (20)
- Late, n (%)	36 (80)
Disease duration (yrs)	21.3 ± 13.7
Asthma control (GINA):	
- controlled, n (%)	19 (42)
- partially controlled, n (%)	22 (49)
- uncontrolled, n (%)	4 (9)
Treatment step (GINA):	
- step 4, n (%)	28 (62)
- step 5, n (%)	17 (38)

Table 1. Characteristics of the asthmatic subjects. Values are expressed as means ± SDs unless otherwise specified

FEV₁ before and 15–20 min after 400 mcg of salbutamol, and the response was evaluated on the basis of the ERS/ATS recommendations.²²

Sputum induction and processing

Sputum was induced according to the ERS Task Force recommendations.²³ After measurement of baseline FEV₁ and pre-treatment with 200 µg of inhaled salbutamol, a hypertonic saline solution (NaCl 4.5%) was nebulized by an ultrasonic nebulizer (Devilbiss Ultraneb 2000; Devilbiss Healthcare, Somerset, PA, USA) with 2.8 mL/min output and was inhaled for 3 5-minute periods. Nebulization was stopped after 15 minutes or when FEV₁ fell by ≥ 20% from baseline value. Saline-induced bronchoconstriction was promptly relieved by short-acting β₂-agonist inhalation.

Sputum samples were processed within 1 hour after collection. All dense portions were selected by using an inverted microscope and were then processed as previously described.²⁴ Differential

cell count on at least 300 non-squamous cells was performed on cytospin slides, and leukocytes were then expressed as percentage of total inflammatory cells, excluding squamous cells. Samples with cell viability <50% and cytospin slides with an amount of squamous cells such that 300 inflammatory cells could not be counted were considered inadequate and therefore discarded. The inflammatory phenotypes were defined according to previous data.^{9,25}

The concentrations of periostin in sputum supernatants were measured by a high sensitivity ELISA kit according to the manufacturer's protocols with minor modification (Human Periostin ELISA kit, sensitivity: <10 pg/ml; Abcam, Cambridge, UK).

RANTES, IL-8, FGF-2, GM-CSF and TGF-β concentrations were measured using commercially available immunoassays (Quantikine ELISA Kit, sensitivity: 6.6 pg/ml, 3.5 pg/ml, 3 pg/ml, 3 pg/ml

	O	NO	p value
Sex (male/female)	9/11	5/15	n.s.
Age (yrs)	63 ± 13.9	58 ± 13.1	n.s.
BMI	27 ± 3.5	29 ± 7.4	n.s.
Atopy, n (%)	14 (70)	16 (80)	n.s.
Age of onset, yrs:	41 ± 19	37 ± 20	n.s.
- Early, n (%)	4 (20)	5 (25)	
- Late, n (%)	16 (80)	15 (75)	
Disease duration (yrs)	21.3 ± 12	21.2 ± 14	n.s.
Asthma control (GINA):			n.s.
- controlled, n (%)	8 (40)	9 (45)	
- partially controlled, n (%)	10 (50)	9 (45)	
- uncontrolled, n (%)	2 (10)	2 (10)	
Treatment step (GINA):			n.s.
- step 4, n (%)	14 (70)	11 (55)	
- step 5, n (%)	6 (30)	9 (45)	
Comorbidities:			n.s.
- rhinosinusitis/nasal polyposis	15 (75)	17 (85)	
- gastroesophageal reflux, n (%)	3 (15)	2 (10)	
Prebronch. FEV ₁ (% pred.)	63.9 ± 11.6	96.9 ± 12.5	<0.001
Postbronch. FEV ₁ (% pred.)	73.5 ± 13.4	103 ± 15.5	<0.001
Postbronch. FEV ₁ /VC (% pred.)	71.9 ± 6.1	92.0 ± 3.8	<0.001
Sputum inflammatory cells (x10 ⁶ /g)	2.7 (0.44-17.6)	2.8 (0.08-27.8)	n.s.
Sputum neutrophils (x10 ⁶ /g)	1.1 (0.03-15.8)	1.3 (0-11.1)	n.s.
Sputum neutrophils %	36.7 (6.2-89.8)	31.3 (0-82.3)	n.s.
Sputum eosinophils (x10 ⁶ /g)	0.6 (0.01-9.3)	0.5 (0-5.8)	n.s.
Sputum eosinophils %	27 (1-86.9)	22 (0-90.4)	n.s.
FeNO (ppb)	20 (6-83)	24 (8-87)	n.s.
Blood eosinophils (eos/mm ³)	260 (50-910)	295 (70-1020)	n.s.
Serum periostin (ng/ml)	51.7 (29.8-151.7)	39.8 (16.6-106.4)	= 0.06

Table 2. Characteristics of severe asthmatic patients with or without persistent airway obstruction (O and NO group, respectively), at the time of the study visit. Values are expressed as means ± SDs or median (range) unless otherwise specified

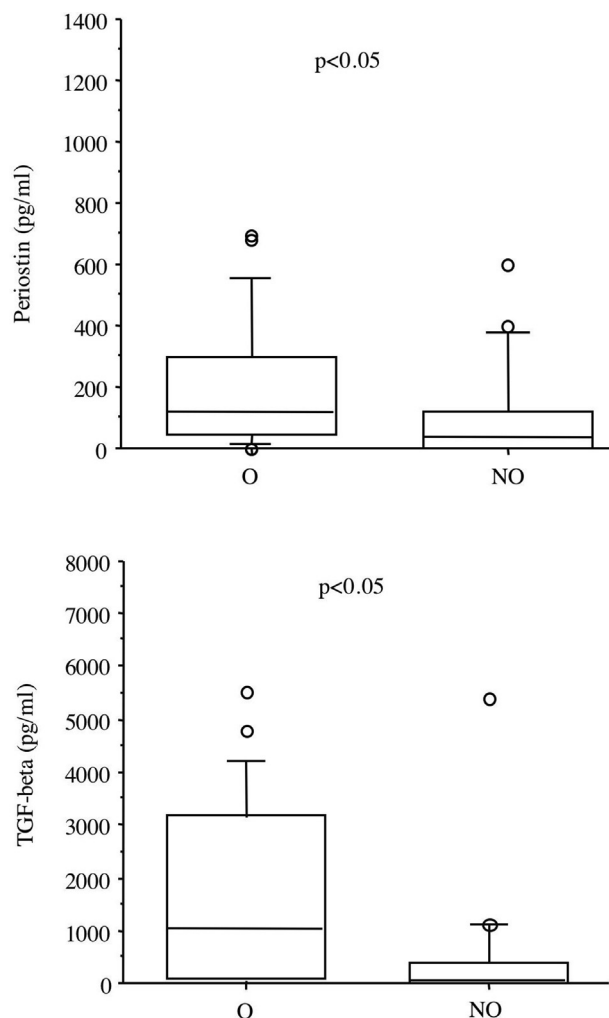


Fig. 1 Sputum periostin and TGF- β in patients without or with persistent bronchial obstruction (NO or O).

and 4.6 pg/ml, respectively for each component; R&D Systems Inc., Minneapolis, USA) All measurements were made in duplicate.

The biological samples were analyzed by 2 investigators who were blinded to the clinical characteristics of the patients.

Blood processing

Blood samples were examined for eosinophil counts; the remaining blood was centrifuged at $1,000\times g$ for 10 min after 60 ± 10 min of rest at room temperature to allow clotting. The supernatant was then centrifuged again to ensure complete cell removal. The serum was then collected and stored at -80°C for periostin analysis.

Exhaled nitric oxide

Fractional exhaled nitric oxide (FeNO) was measured at a respiratory flow rate of 50 ml/s using a chemiluminescent analyzer (HypAir FeNO, Medisoft, Belgium), according to the current guidelines.²⁶

Data processing and statistical analysis

Data normally distributed were expressed as means and standard deviations (SD), and they were analyzed by the unpaired Student's *t*-test while data without normal distribution were presented as median (range) and examined by the Mann-Whitney U test. Categorical data were compared by contingency table analysis.

The correlations among variables were assessed by Spearman's correlation test. Significance was accepted at the 95% level. Statistical analysis was performed using GraphPad Prism 5 (GraphPad Software Inc., San Diego, CA, USA).

RESULTS

The clinical and demographic characteristics of the 45 asthmatic patients examined are shown in Table 1. The majority of these patients were atopic with late onset asthma (>12 yrs), and received a step 4 level of treatment according to GINA¹⁹ with medium dose of ICS/LABA combinations (250-500 mcg/daily fluticasone or equivalent according to the equivalence table reported by GINA) and other additional controllers (LTRA in 18 and tiotropium in 8).

Sputum induction was successfully performed in all but 5 patients who provided inadequate sputum samples (high salivary contamination); these 5 patients were therefore excluded from the analysis. According to the post-bronchodilator FEV₁/VC ratio, patients were divided into 2 groups: 20 with persistent airway obstruction (O) and 20 without persistent airway obstruction (NO) (Table 2). The best FEV₁ (the highest FEV₁ value obtained in all previous spirometric measurements), post-bronchodilator FEV₁ and VC (obtained on the day of the study visit) were significantly lower in O than in NO. There were no significant differences in the other demographic/clinical data or sputum inflammatory cells, nor in the distribution of additional pharmacological

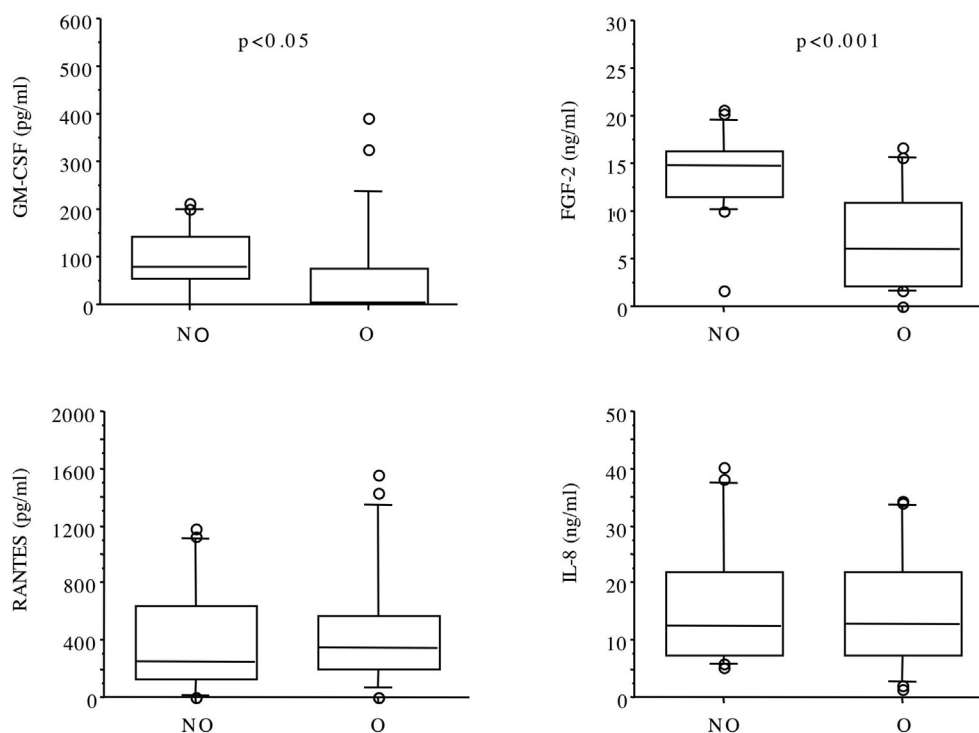


Fig. 2 Sputum biomarkers in patients without or with persistent bronchial obstruction (NO or O).

treatment (tiotropium: 4 in NO and 4 in O; LTRA: 10 in NO and 8 in O) between O and NO patients. As regards non-invasive biomarkers, we observed only a trend for higher serum periostin levels in the O compared to the NO group.

In relation to sputum soluble mediators, NO asthmatic patients had lower levels of sputum periostin and TGF- β than O patients (Fig. 1). GM-CSF and FGF-2 increased significantly in NO compared with O patients; no differences between groups were found for RANTES and IL-8 (Fig. 2).

When the patients were considered together, a positive correlation was observed between absolute number of sputum eosinophils and GM-CSF as well as between sputum neutrophils and IL-8 (Table 3). Sputum eosinophils also correlated with FeNO and blood eosinophils. Among the sputum mediators, only sputum periostin was inversely associated with functional parameters (prebronch. FEV₁: $\rho = -0.36$, $p < 0.05$; postbronch. FEV₁: $\rho = -0.33$, $p = 0.05$), whereas a positive correlation was observed between sputum FGF-2 and prebronch. FEV₁ ($\rho = 0.41$, $p < 0.05$) and postbronch. FEV₁ ($\rho = 0.45$, $p < 0.01$).

The predominant inflammatory pattern in the induced sputum was eosinophilic (45% of patients) or mixed granulocytic (32.5% of patients).

Finally, according to the median value of sputum and serum periostin of the 40 patients (cut-off value), we subdivided the patients into different groups: patients with high or low periostin levels in sputum (\geq or $<$ 103.3 pg/ml: median value), and patients with high or low serum periostin levels (\geq or $<$ 46.4 ng/ml: median value) (Fig. 3). Patients with high periostin levels in sputum showed an absolute number of sputum eosinophils significantly higher than that of patients with low sputum periostin. Conversely, patients with high periostin levels in serum did not show a higher number of eosinophils in sputum compared to patients with low serum periostin. There were no significant differences in the other clinical parameters and in sputum/blood biomarkers between the high/low periostin groups. Of note, the first group (high periostin levels in sputum) had a percentage of patients with persistent obstruction (67%) and uncontrolled asthma (22%) higher than the one showed by the low sputum periostin group (42% and 0, respectively), although this difference was not statistically significant. Similar results were observed when

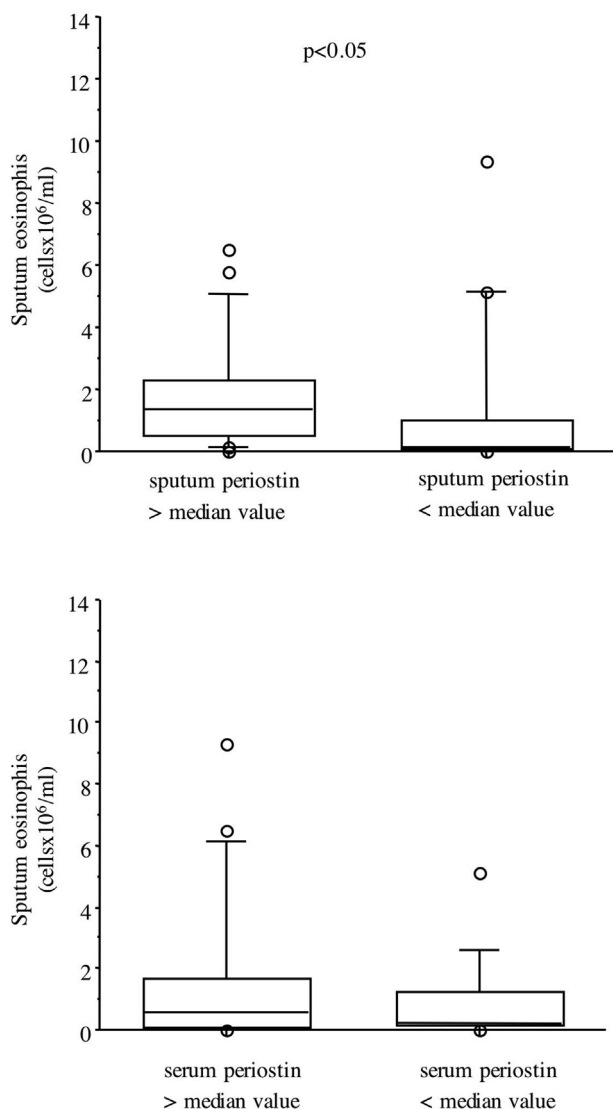


Fig. 3 Sputum eosinophils in patients with high/low sputum (>/<103.3 pg/ml) or serum (>/< 46.4 ng/ml) periostin levels.

comparing the high serum periostin group (persistent obstruction: 67% and uncontrolled asthma: 11%) with the low serum periostin group (33% and 5%, respectively).

DISCUSSION

In this study we identified a group of severe asthmatic patients with or without persistent airway obstruction on the basis of a cut-off value of post-bronchodilator FEV₁/VC ratio ≤88%pred. to characterize them in relation to specific inflammatory/remodelling biomarkers. Among the soluble mediators evaluated, we demonstrated higher levels of sputum periostin and TGF-β and a trend towards increased serum periostin in the patients with persistent obstruction; conversely, sputum GM-CSF and FGF-2 were higher in the patients without obstruction. No statistically significant differences were found between the two groups in terms of blood biomarkers, sputum inflammatory cells and demographic/clinical data.

There is evidence that severe asthmatic patients with chronic persistent obstruction have an increased airway smooth muscle area and a trend towards increased subepithelial fibrosis compared to those with intermittent obstruction.^{1,2} Periostin expression is known to be associated with airway remodelling by contributing to airway fibrosis,^{27,28} TGF-β activation and collagen production.²⁹ Sputum TGF-β concentrations have shown to correlate positively with airway wall thickening in moderate-to-severe asthmatics.⁵ Our study is the first to evaluate simultaneously the levels of TGF-β and of periostin in the sputum of severe asthmatics with a different bronchial obstruction; in this respect, the increased levels of TGF-β and periostin observed in patients with persistent obstruction suggest a greater degree of airway remodelling in this group. In contrast to our results, severe asthmatics with persistent airflow obstruction displayed a decreased number of periostin and

	rho	p
Sputum eosinophils (x10 ⁶ /g)		
- sputum GM-CSF (pg/ml)	0.42	<0.05
- FeNO (ppb)	0.37	<0.05
- Blood eosinophils (eos/mm ³)	0.47	<0.01
Sputum neutrophils (x10 ⁶ /g)		
- sputum IL-8 (ng/ml)	0.68	<0.0001

Table 3. Correlations between sputum biomarkers and other inflammatory parameters.

TGF- β positive muscle cells.⁷ The discrepancy with our findings is not surprising if we consider that we measured the secretion rather than the intracellular store of periostin and TGF- β , and that the concentrations of soluble mediators in sputum samples are influenced by all the inflammatory and resident cells, and not only by the muscle cells.

Both airway remodelling and persistent eosinophilic inflammation can accelerate the decline in respiratory function,^{3,30} which in some cases may evolve into irreversible airway obstruction in severe asthmatics. An inverse correlation has been reported between the percentage of the smooth muscle area and FEV₁ (%pred.) in subjects with severe asthma.² In our study both periostin and TGF- β levels increased in patients with persistent airway obstruction, but only periostin inversely correlated with FEV₁. In keeping with these results, periostin levels in sputum have shown to inversely correlate with postbronchodilator FEV₁/VC in severe asthma⁹ and to be associated with FEV₁ decline.^{10,11} Sputum concentrations of TGF- β also have been reported to correlate negatively with FEV₁ (% pred.) but, differently from our study, the correlation included moderate asthmatics and controls.⁵

Several growth factors other than TGF- β have been associated with asthma severity. In this respect, an increased expression of FGF-2 by infiltrating inflammatory cells, especially eosinophils, has been observed in the submucosa of asthmatic patients.³¹ Bissonnette et al. reported that FGF-2 levels were higher in the sputum and bronchial biopsies of severe asthmatics compared to patients with mild-to-moderate asthma and to controls.¹² However, in contrast to our findings, they found an inverse correlation with the pulmonary function. The different clinical and inflammatory characteristics of the subjects examined could explain the conflicting results.

However, recent studies have called into question the *in vivo* profibrotic action of FGF-2. In a mouse model of bleomycin-induced pulmonary fibrosis, a protective role was described for FGF-2.¹³ These data support a model in which FGF-2 acted as protective growth factor after lung epithelial injury for its ability to induce epithelial

cell regeneration; in the same study the authors demonstrated that the FGF-2 expression increased in inflammatory cells, facilitating its delivery to injury areas, and suggested the possibility of an autocrine FGF-2 feedback signalling mechanism contributing to the resolution of inflammation as a result of sufficient epithelial recovery. According to these results, other studies reported that recombinant FGF2 *in vivo* protects from IFN- γ -induced lung emphysema¹⁴ and airway hyperreactivity in response to allergen exposure.³² Furthermore, Schuliga et al. showed that FGF-2 *in vitro* could antagonize the TGF- β proremodelling effects on airway smooth muscle cells,¹⁵ and suggested the possibility of using FGF-2 as therapy for the treatment of asthma. In our study, the highest levels of FGF-2 in patients without airway obstruction and the positive correlation with pulmonary function are in line with these findings, suggesting a protective effect rather than a proinflammatory/profibrotic role *in vivo*.

Kaminska et al. reported that chronic persistent obstruction in severe asthmatics was associated with a higher number of inflammatory cells in sputum (percentage of combined eosinophils and neutrophils), with earlier age of onset and longer disease duration. However, they found no differences between patients with or without bronchial obstruction for any of the sputum biomarkers examined.² The low number of patients included in the biomarker measurements and the use of a different quantification technique might explain the discrepancy from our results.

Despite regular treatment with high-dose inhaled corticosteroid plus LABA often associated with other drugs, the predominant inflammatory pattern of the patients in our study was eosinophilic (or mixed granulocytic); sputum eosinophils correlated with FeNO, blood eosinophils, and sputum GM-CSF (a well-known pro-inflammatory mediator), confirming the strong relationship between these biomarkers and airway eosinophils.

A recent study explored the relationship between airway eosinophils and periostin levels in the serum and the induced sputum of asthmatic subjects.³³ Serum periostin levels were significantly higher than those measured in sputum, a result that was consistent with our findings. The same authors reported that serum

and sputum periostin were predictive of eosinophilic inflammation ($\geq 3\%$ sputum eosinophils), although the strength of the association was relatively modest. In a group of 62 patients with severe asthma and persistent eosinophilic inflammation, periostin was higher in the sputum of patients with eosinophilic inflammation compared to subjects with mixed granulocytic patterns.⁹ This result is in line with our findings demonstrating an increased number of eosinophils in the high sputum periostin group. These data support the hypothesis that airway inflammation and remodelling are two different events that may be simultaneously present in asthmatic patients. Moreover, in our study only patients with high sputum but not serum periostin levels had a higher absolute number of sputum eosinophils, suggesting that sputum periostin is a more sensitive biomarker than serum periostin not only to identify patients with persistent obstruction but also those with a higher number of eosinophils in sputum.

Airway remodelling has usually been assessed by histologic evaluation of bronchial wall abnormalities (sub-epithelial fibrosis, smooth muscle cells and mucous gland hypertrophy/hyperplasia), but these measurements can only be obtained with invasive procedures and are only related to large airways.^{1,7} As an alternative, some authors assessed airway thickness by high-resolution computed tomography scanning.² We used persistent airway obstruction as a marker of functional remodelling. This approach, associated with structural abnormalities in airway smooth muscle and/or submucosal fibrosis in bronchial biopsies,^{2,4,7} has been previously used by other authors to select patients with or without airway remodelling.⁹

A limitation of our study is the wide variability in the amount of the eosinophilic inflammation, a characteristic usually seen in Type 2 severe asthma. In this respect, when we considered the presence of blood eosinophils $>150/\mu\text{l}$ and/or FeNO >25 ppb (these criteria are now considered as expression of Type 2 inflammation), we found that all but 6 of our patients satisfied this definition and that the distribution range of NO, blood eosinophil and serum periostin levels in the 2 subgroups was high. In this context, it has been reported that serum periostin, FeNO and blood eosinophils were subjected to significant intrasubject variability;⁸ therefore, it is

not surprising that the variability associated with these parameters may increase when these inflammatory mediators are measured in different subjects. Consistent with this observation, our results are in line with those reported by previous studies:^{2,9,11} in particular, by using, in analogy to these studies, the standard deviation or interquartile range as dispersion measures of the FeNO, blood eosinophils and serum periostin levels we have obtained a variability of similar extent (data not shown), suggesting that the high distribution range associated to those biomarkers is in line with the characteristics of severe asthmatics.

Another limitation of our study is represented by the relatively low number of asthmatics examined, which was also due to the difficulty to obtain an adequate sputum volume for performing all biomarker measurements. However, our sample size is in line with that reported by other studies evaluating sputum soluble mediators in severe asthmatics.^{2,5,9} Our results provide original and interesting information, although additional studies are needed to confirm these findings in a larger sample.

In conclusion, we identified periostin and TGF- β as two sputum biomarkers associated with persistent bronchial obstruction in severe asthmatic patients. Considering the role of these two molecules in experimental remodelling models, our results suggest their possible contribution to the development of not completely reversible airway obstruction in severe asthma. However, sputum periostin seems to be more sensitive than serum periostin to detect patients with persistent airway obstruction.

Abbreviations

BMI: body mass index; FeNO: fraction of exhaled nitric oxide; FEV₁: forced expiratory volume in 1 s; FGF-2: fibroblast growth factor-2; GM-CSF: granulocyte-macrophage colony-stimulating factor; ICS: inhaled corticosteroids; IL-8: interleukin-8; IFN: interferon; LABA: long-acting β -2agonist; LTRA: leukotriene receptor antagonist; RANTES: regulated on activation, normal T-cells expressed and secreted; TGF- β : transforming growth factor- β -1; VC: vital capacity

Ethic approval

The study was performed in accordance with the Helsinki Declaration and approved by the Local Ethical Committee, within the context of an observational multicentre study on severe asthma in Italy (n. 1245/2016). Informed consent was obtained by each patient for the use of personal data.

Author contributions

SC contributed to the study design, analyzed and interpreted the results, and wrote the manuscript. CC and IP contributed to the study design and to the processing and collection of the data. ML, MLB, MB and FD were involved in the data collection. EB and AC contributed to the study design and the data acquisition. PP was responsible for the study design, supervised research and performed a critical revision of the manuscript. All authors read and approved the final version of the manuscript.

Consent for publication

Not applicable.

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Data availability

The data used or analyzed during this study are available on reasonable request.

Declaration of competing interest

None of the authors have any competing interests in relation to the submitted work.

Author details

^aRespiratory Pathophysiology Unit, Department of Surgery, Medicine, Molecular Biology, and Critical Care, University of Pisa, Pisa, Italy. ^bImmunology and Allergology Unit, Department of Clinical and Experimental Medicine, University of Pisa, Pisa, Italy.

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