Importance of concomitant local and systemic eosinophilia in uncontrolled asthma

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ABSTRACT Systemic and airway eosinophilia are recognised features of asthma. There are, however, patients who exhibit discordance between local and systemic eosinophilia. In this study, we sought to determine the prevalence and characteristics of patients with concordant and discordant systemic and bronchial eosinophilia.

We conducted a retrospective study on 508 asthmatics with successful sputum induction. We assessed the relationship between blood and sputum eosinophils by breaking down the population into four groups according to blood ($\geqslant$400 cells per mm^3) and sputum ($\geqslant$3%) eosinophils. Then, we prospectively reassessed the link between eosinophils and asthma control (Asthma Control Questionnaire (ACQ)) and exacerbation rate in a new cohort of 250 matched asthmatics.

In our retrospective cohort, asthmatics without eosinophilic inflammation were the largest group (49%). The group with isolated sputum eosinophilia (25%) was, compared with noneosinophilic asthma, associated with lower forced expiratory volume in 1 s (FEV_1) and FEV_1/forced vital capacity ratio and higher bronchial hyperresponsiveness and exhaled nitric oxide fraction (F_{ENO}). Asthmatics exhibiting isolated systemic eosinophilia (7%) had similar characteristics as noneosinophilic asthmatics. The group with concordant systemic and airway eosinophilia (19%) showed remarkable male predominance, and had the lowest airway calibre, asthma control and quality of life, and the highest bronchial hyperresponsiveness, F_{ENO} and exacerbation rate. The prospective cohort confirmed the different subgroup proportions and the higher ACQ and exacerbation rates in cases of diffuse eosinophilia compared with noneosinophilic asthmatics.

Concomitant systemic and bronchial eosinophilic inflammation contribute to poor asthma control.

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Introduction

Bronchial asthma is a complex airway inflammatory disease. The eosinophilic feature is recognised as a pivotal trait of the disease [1]. The technique of induced sputum has been instrumental in assessing the proportion of eosinophilic asthma. It is now accepted that eosinophilic asthma, defined as a sputum eosinophil count of 2–3% or higher, represents slightly less than half of the asthmatic population [2–5].

In asthma, sputum eosinophil count correlates with blood eosinophil count [4, 6–8] and blood eosinophil count is considered as a good surrogate marker for sputum eosinophil count (over 2–3% with a cut-off of 220 cells per mm^3 or 3%) [4, 9]. However, the sensitivity and specificity of blood eosinophils to predict a sputum eosinophil proportion of $\geqslant$3% are <80%, and there are patients who show discordance between local and systemic inflammation.

There is a huge controversy about the role of eosinophils as a key player in asthma severity [10–21]. Some studies have looked at airway eosinophils in bronchoalveolar lavage (BAL) [21], induced sputum [11, 12, 14, 16–18] and bronchial biopsies [19, 20], while others focused on systemic inflammation through blood eosinophil count measurement [20]. Discrepancies between studies may be linked to the different compartments sampled and, for some of the studies, to the limited number of subjects investigated.

While inhaled corticoids, the recommended mainstay treatment of asthma, have been consistently shown to reduce airway eosinophilic inflammation and improve asthma control [22], their effect on systemic eosinophilia was shown to be rather weak at usual doses [23]. By contrast, the new biologicals directed towards interleukin-5 were shown to dramatically decrease circulating blood eosinophils, an effect that was associated with the reduction of asthma exacerbation [24] and improvement in asthma control [25].

To the best of our knowledge, there has never been detailed investigation of the relationship between blood and sputum eosinophil in a large population of asthmatics. In this study, we sought to determine whether looking at blood and sputum eosinophilic inflammation might help in our understanding of asthma severity. We first retrospectively investigated the demographic, functional and symptomatic features of 508 asthmatics classified according to systemic and local eosinophilic inflammation, and then prospectively validated the relationship between asthma control and eosinophilic inflammation in a new cohort of 250 patients well matched to our retrospective cohort with respect to demographic, functional and treatment characteristics.

Material and methods

Subject characteristics

We conducted a retrospective study on a series of 508 patients with asthma recruited from the University Asthma Clinic of Liege, Liege, Belgium, between October 1, 2005 and June 27, 2011. The patients came from routine practice to University Hospital, Liege, and were recruited by two clinicians involved in asthma. Entry criteria were any patients with asthma aged $\geqslant$18 years who agreed to undergo detailed investigation at the asthma clinic. The visits were not parts of an asthma trial. All the patients who had a successful sputum induction were included in the study. Their demographic and functional characteristics are summarised in tables 1 and 2.

Asthma was diagnosed based on the presence of chronic respiratory symptoms such as cough, breathlessness or dyspnoea together with the demonstration of airflow variability. The latter was defined by airway hyperresponsiveness shown by one or more of the following: increase in forced expiratory volume in 1 s (FEV_1) of >12% and 200 mL following inhalation of 400 μg salbutamol; or inhaled concentration of methacholine provoking a 20% fall in FEV_1 of <16 $mg \cdot mL^{-1}$. Methacholine challenge was performed according to a standardised methodology as previously described [26]. Subjects were characterised as atopic if they had at least one positive specific IgE test (>0.35 $kU \cdot L^{-1}$; Phadia, Groot-Bijgaarden, Belgium) for at least one common aeroallergen (cat, dog, house dust mites, grass pollen, tree pollen and a mixture of moulds).

Exacerbation in the previous year was defined by a course of oral corticoids for $\geqslant$3 days for a case of asthma worsening. Nasal polyps and sinusitis was diagnosed by an ear, nose and throat physician either by endoscopy or sinus computed tomography. Gastro-oesophageal reflux was diagnosed either by symptoms of pyrosis at history taking or the presence of oesophagitis demonstrated by gastroscopy.

Study design

Patients underwent exhaled nitric oxide fraction (F_{ENO}) measurement at a flow rate of 50 $mL \cdot s^{-1}$ according to the European Respiratory Society (ERS)/American Thoracic Society (ATS) recommendations (NIOX; Aerocrine, Solna, Sweden). F_{ENO} was first measured and followed by spirometry with bronchodilation, sputum induction and blood sampling. All tests were performed on the same day.

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TABLE 1 Demographic, control and treatment characteristics for the whole population

Characteristics	Retrospective cohort	Prospective cohort
Subjects n	508	250
Males/females n	201/307	99/151
Age years	52 (19–88)	50 (16–85)
Age of onset		
<12 years	24	22
12–40 years	37	36
⩾40 years	39	42
Height cm	167±9	168±9
Weight kg	74±16	73±17
Atopy yes/no (%)	296/212 (58)	148/102 (59)
Current smokers	101 (20)	55 (22)
Exposure pack-years	22 (0.5–60)	25 (2–60)
Ex-smokers	99 (19)	38 (15)
Exposure pack-years	15 (0.5–90)	17 (0.5–63)
Bronchiectasis	19[f]	17
Gastro-oesophageal reflux	79	71*
Nasal polyposis	22[##]	27
Sinusitis	42[##]	41
Rhinitis	58	56
Exacerbations per patient per year[#]	0.68±1.50[¶¶]	0.86±2.02[++]
LABA	319 (63)	141 (56)
LTRA	93 (18)	50 (20)
Theophylline	16 (3)	11 (4)
ICS		
Steroid naïve	153 (30)	82 (33)
Low dose[¶]	73 (14)	35 (14)
Moderate dose[+]	138 (27)	63 (25)
High dose[§]	144 (28)	70 (28)
Oral corticosteroids	32 (6)	25 (10)

Data are presented as median (range), %, mean±SD or n (%), unless otherwise stated. Exacerbations were evaluated during the year prior to the visit. LABA: long-acting β_2-agonist; LTRA: leukotriene receptor antagonist; ICS: Inhaled corticosteroids. High dose: >1000 μg·day^{-1} beclomethasone. [#]: during the year prior to the visit; [¶]: ⩽500 μg per day beclomethasone; [+]: 500–1000 μg per day beclomethasone; [§]: >1000 μg per day beclomethasone; [f]: n=174; [##]: n=273; [¶¶]: n=428; [++]: n=237. *: p<0.05.

Quality of life was assessed using the self-administered Asthma Quality of Life Questionnaire [27] and asthma control by the Asthma Control Questionnaire (ACQ) of JUNIPER *et al.* [28].

Sputum was induced and processed as previously reported [29], and was successful in 78% of the patients encountered in our asthma clinic, which is similar to previous reports [30, 31]. Cell count were estimated on samples centrifuged (cytospin) and stained with Diff-Quick after counting 500 cells (Dade, Brussels, Belgium).

Prospective study validation

To validate the results of the retrospective analysis of the link between asthma control and eosinophilic inflammation, we conducted a prospective study.

A new cohort of 250 consecutive patients was recruited from routine practice between June 30, 2011 and January 12, 2013. None of these patients had been included in the retrospective cohort. Their demographic, functional and treatment characteristics were similar to the retrospective population (tables 1 and 2).

Exacerbation rate for the prospective population was measured through a telephone call over a period of 12 months following the visit to the asthma clinic, during which treatment was initiated or adjusted according to asthma control, lung function and inflammatory markers at the discretion of the clinician. 13 patients were lost to follow-up and in the 17 patients in whom the observation period was <1 year, we calculated the annualised exacerbation rate.

This study was conducted with the approval of the ethics committee of CHU Liege.

TABLE 2 Functional and inflammatory characteristics for the whole population

Characteristics	Retrospective cohort	Prospective cohort
FEV_1 % predicted	84±19	82±21
FEV_1/FVC %	73±11	71±15*
PC_{20} mg·mL^{-1} geometric mean (range)	3.20 (0.025–16)	2.92 (0.05–16)
Reversibility %	11±14	9±10
ACQ score	2.01±1.38	2.00±1.25
AQLQ score	4.61±1.35	4.46±1.39
Blood eosinophil count cells per mm^3	230 (0–3220)	188 (0–1133)*
***F*$_{ENO}$ ppb**	27 (0–247)	25 (4–348)
Sputum eosinophils %	2 (0–94)	2.8 (0–90)
Sputum neutrophils %	45 (0–100)	49 (0–100)

Data are presented as mean±SD or median (range), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; *F*$_{ENO}$: exhaled nitric oxide fraction. *: $p<0.05$.

Statistical analyses

We used blood eosinophil count and sputum eosinophil percentage to subdivide our asthmatic population into four groups. The chosen blood ($\geqslant$400 cells per mm^3) and sputum ($\geqslant$3%) threshold values were considered as the limit of abnormality by our routine laboratory. The results were expressed as mean±SD or mean±SEM for continuous variables; median (range) was preferred for skewed distributions. For categorical variables, the number of observations and percentages are given in each category. Comparisons between different subgroups were performed with a Kruskal–Wallis test. The Spearman correlation coefficient was used to measure the association between clinical parameters.

Power calculations indicated a required total sample size of 250 subjects to confirm a change in ACQ score $\geqslant$0.5 between noneosinophilic asthmatics and patients exhibiting diffuse eosinophilic inflammation, with a power of 80%. The results were considered to be significant at the 5% critical level ($p<0.05$).

Results

The demographic, functional and inflammatory characteristics of the retrospective cohort of asthmatics (n=508), classified according to their blood (threshold 400 cells per mm^3) [25] and sputum eosinophil counts (threshold 3%), are described in tables 3–5. The patients without evidence of eosinophilic inflammation represented the largest group, accounting for 49% of the cohort. Those patients with selective airway eosinophilic inflammation came second, accounting for 25% of the patients, whereas those exhibiting the reverse picture were much less numerous, only representing 7%. Patients combining systemic and airway eosinophilic inflammation account for 19% of the patients.

The inhaled corticoids treatment regimen was similar between subgroups (table 3).

Compared with noneosinophilic asthmatics, the characteristics of patients exhibiting isolated sputum eosinophilia were a higher proportion of males and atopic subjects, higher total serum IgE levels, lower FEV_1 and FEV_1/forced vital capacity ratio, and higher bronchial hyperresponsiveness and reversibility to β_2-agonists (fig. 1, and tables 4 and 5). As for patients with high levels of blood eosinophils without sputum eosinophilia, they had a higher total serum IgE compared with noneosinophilic asthmatics (fig. 1 and table 5). The presence of both local and systemic eosinophilic inflammation was strikingly more frequent in males, and associated with the greatest lung function impairment, and the lowest asthma control (mean±SEM ACQ score 2.54±1.45 *versus* 1.88±1.39; mean difference 0.66, 95% CI -0.99– -0.32 ($p=0.0001$)) (figs 1 and 2) and quality of life (table 4). They also had the highest *F*$_{ENO}$ while those with eosinophilia in only one compartment had similar, intermediate *F*$_{ENO}$. Noneosinophilic asthmatics had the lowest *F*$_{ENO}$, comparable to that found in healthy subjects (fig. 3 and table 5).

Patients with increased sputum eosinophilia reported greater numbers of severe exacerbations in the previous year, and this was particularly the case for those displaying concordant systemic and airway eosinophilia (table 3). Likewise, the proportion of sinusitis and nasal polyps was clearly raised in eosinophilic asthmatics while there was no difference regarding the gastro-oesophageal reflux (table 3).

In patients treated with high doses of inhaled corticosteroids (n=144), the proportions of the different subgroups were similar to those found in the whole cohort, and the subgroup exhibiting both bronchial and

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TABLE 3 Retrospective cohort: demographic and treatment characteristics of asthmatics (n=508) according to blood and sputum eosinophil count

	Blood eosinophils <400 cells per mm^3, sputum eosinophils <3%[#]	Blood eosinophils <400 cells per mm^3, sputum eosinophils ⩾3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils <3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils ⩾3%
Subjects	249 (49)	128 (25)	34 (7)	97 (19)
Males/females n	78/171	54/74*	16/18	53/44***
Age years	52 (21–86)	53 (21–88)	51 (21–85)	51 (19–86)
Age of onset				
<12 years	22.6	27.6	26.5	23.3
12–40 years	34.5	36.2	32.3	45.6
⩾40 years	42.9	36.2	41.2	31.1
Height cm	166±9	168±9	169±9	169±9
Weight kg	73±16	74±15	76±17	75±17
BMI kg·m^{-2}	26.3±5	26.4±5	26.3±4.8	26.4±5.3
Atopy yes/no (%)	126/123 (51)	82/46 (64)*	22/12 (65)	66/31 (68)**
Current smokers	54 (22)	29 (23)	6 (18)	12 (12)*
Bronchiectasis[¶,+]	19	13	17	26
Gastro-oesophageal reflux	77	81	86	77
Nasal polyposis[§]	9	25***	37***	43***
Sinusitis[§,ƒ]	34	38	42	61***
Rhinitis	53	59	72	65
Exacerbations per patient per year[##]	0.42±0.9	0.93±2.72*	0.59±0.98	1.5±2.5***
ICS				
Steroid naïve	82 (33)	31 (24)	9 (26)	31 (32)
Low dose[¶¶]	30 (12)	20 (16)	5 (15)	18 (19)
Moderate[++]	70 (28)	37 (29)	11 (32)	20 (21)
High dose[§§]	67 (27)	40 (31)*	9 (26)	28 (29)

Data are presented as n (%), median (range), % or mean±SD, unless otherwise stated. BMI: body mass index; ICS: inhaled corticosteroids. [#]: comparator group; [¶]: n=174; [+]: based on chest computed tomography (CT); [§]: based on sinus CT and nasal endoscopy; [ƒ]: n=273; [##]: during the year prior to the visit; [¶¶]: ⩽500 µg per day beclomethasone; [++]: 500–1000 µg per day beclomethasone; [§§]: >1000 µg per day beclomethasone. *: p<0.05; **: p<0.01; ***: p<0.001.

systemic eosinophilic inflammation had the highest exacerbation rate and the poorest lung function (table 6).

In order to validate the relationship between poor asthma control and comprehensive eosinophilic inflammation, we prospectively recruited a population of 250 asthmatics whose demographic, functional and inflammatory characteristics were similar to our retrospective cohort (table 1 and 2). In this population, we found similar proportions of patients in the different subgroups (47% were patients without evidence of eosinophilic inflammation, 32% exhibited isolated sputum eosinophilia, 4% had isolated systemic eosinophilia and 17% combined systemic and airway eosinophilia) and confirmed that patients with both systemic and airway eosinophilic inflammation had poorer asthma control compared with noneosinophilic asthma (mean±SEM ACQ score 2.23±1.40 *versus* 1.79±1.12; mean difference 0.44, 95% CI 0.007–0.89 (p<0.05)) (fig 2).

In the prospective cohort, patients exhibiting diffuse eosinophilic inflammation had a higher number of exacerbations in the year prior to the visit to the asthma clinic (mean±SD 1.11±1.65, n=41) than noneosinophilic patients (mean±SD 0.77±2.55, n=111) (p<0.05). Interestingly, for the whole cohort, the exacerbation rate over the 12 months following the asthma clinic visit decreased compared with that occurring in the year before (mean 0.86±2.02 (95% CI 0.61–1.12) *versus* mean 0.50±1.18 (95% CI 0.35–0.65) after) (p=0.015) (see the online supplementary material for all details on ACQ score and exacerbation rate in the prospective cohort). Moreover, in the year following the visit to the asthma clinic, the number of exacerbations was significantly higher in patients advised to take high-dose ICS (mean±SD 1.06±1.63, n=93) than in those advised to take low (mean±SD 0.17±0.62, n=58) or moderate (mean±SD 0.15±0.40, n=55) doses of ICS or not to take ICS at all (0±0, n=31) (p<0.0001).

TABLE 4 Retrospective cohort: functional characteristics, asthma control and quality of life of asthmatics (n=508) according to blood and sputum eosinophil count

	Blood eosinophils <400 cells per mm^3, sputum eosinophils <3%$^{\#}$	Blood eosinophils <400 cells per mm^3, sputum eosinophils ⩾3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils <3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils ⩾3%
FEV_1 % predicted	87±19	83±20*	84±23	75±19***
FEV_1/FVC %	75±10	72±9*	77±10	71±10***
TLC % predicted	101±16	101±20	92±21	103±15
FRC % predicted	109±29	108±20	97±30	101±18
K_{CO} % predicted	89±19	92±20	99±14**	94±26*
PC_{20} mg·mL^{-1} geometric mean (range)	3.99 (0.05–16)	2.32 (0.025–16)*	4.53 (0.05–16)	1.49 (0.05–16)**
Reversibility %	8±9	13±14*	9±12	17±16***
ACQ score	1.88±1.39	1.87±1.19	1.98±1.5	2.54±1.45***
<0.75	58 (23)	26 (20)	7 (21)	10 (10)**
0.75–1.5	45 (18)	30 (23)	10 (29)	15 (15)
>1.5	146 (59)	72 (56)	17 (50)	72 (74)*
AQLQ score	4.67±1.36	4.84±1.3	4.47±1.26	4.3±1.4*

Data are presented as mean±SD or n (%), unless otherwise stated. FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; TLC: total lung capacity; FRC: functional residual capacity; *K*CO: transfer coefficient of the lung for carbon monoxide; PC_{20}: provocative concentration of methacholine causing a 20% fall in FEV_1; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire. $^{\#}$: comparator group. *: p<0.05; **: p<0.01; ***: p<0.001.

When pooling both the retrospective and the prospective cohort (n=758), there was a weak but significant relationship between ACQ and sputum eosinophil count (r=0.16, p<0.0001) but not between blood eosinophils and ACQ (r=0.02, p=0.57) (fig .4).

Discussion

The original finding of this study is that patients exhibiting both local and systemic eosinophilic inflammation had more severe asthma reflected by lower baseline lung function, higher bronchial responsiveness to methacholine, poorer asthma control and quality of life, and a greater number of

TABLE 5 Retrospective cohort: inflammatory characteristics of asthmatics (n=508) according to blood and sputum eosinophil count

	Blood eosinophils <400 cells per mm^3, sputum eosinophils <3%$^{\#}$	Blood eosinophils <400 cells per mm^3, sputum eosinophils ⩾3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils <3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils ⩾3%
IgE kU·L^{-1}	87 (1–7338)	211 (3–6785)***	180 (13–2329)*	225 (1–17183)***
Blood eosinophils %	1.7 (0–5.4)	3.2 (0–7)***	6 (0.3–15)***	8 (0.4–30)***
Blood eosinophils per mm^3	140 (0–380)	250 (0–390)***	490 (400–1220)***	590 (400–3220)***
Blood neutrophils %	59 (27–82)	57 (34–91)	57 (41–76)	52 (32–67)***
Blood neutrophils per mm^3	4180 (76–11 080)	4370 (2290–15 410)	5040 (1760–10 010)	3965 (1820–8670)
Sputum eosinophils %	0.3 (0–2.9)	9 (3–79)***	0.6 (0–2.8)	26 (3.2–94)***
Sputum eosinophils per mm^3	2.7 (0–1020)	70 (6–5226)***	4.8 (0–1796)	287 (5–33 375)***
Sputum neutrophils %	58 (0–100)	39 (0–90)***	57 (0.2–99)	30 (0.2–91)***
Sputum neutrophils per mm^3	422 (0–73 440)	334 (1–9588)	560 (14–160 974)	259 (1–15 441)
Fibrinogen g·L^{-1}	3.2 (1.9–10)	3 (2–6)	3.3 (2.6–5)	3.4 (2.2–7)
CRP mg·L^{-1}	1.7 (0.2–10)	2 (0.2–14)	1.4 (0.5–4)	1.6 (0.2–13)
F_{ENO} ppb	17 (0–192)	37 (2–222)***	32 (5–93)**	77 (11–247)***

Data are presented as median (range). CRP: C-reactive protein; F_{ENO}: exhaled nitric oxide. $^{\#}$: comparator group. *: p<0.05; **: p<0.01; ***: p<0.001.

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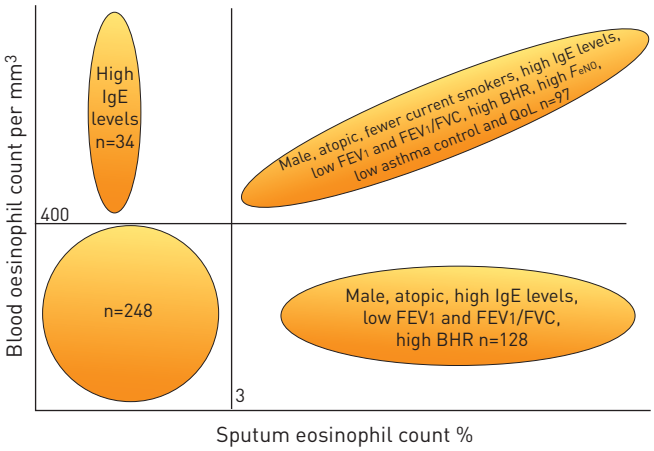

FIGURE 1 Demographic, functional and inflammatory characteristics of patients according to their local and systemic eosinophil count. FEV1: forced expiratory volume in 1 s; FVC; forced vital capacity; BHR: bronchial hyperresponsiveness; F_{eNO}: exhaled nitric oxide fraction; QoL: quality of life.

exacerbations in the previous year. This suggests that the global magnitude of eosinophilic inflammation is a significant factor in disease severity.

Another new finding of this study is that it provides figures on the proportion of asthmatics classified according to the site of eosinophilic inflammation. Overall, asthmatics without any sign of eosinophilic inflammation account for almost half of the patients while one-quarter to one-third had selective airway eosinophilia. Patients with systemic and airway eosinophilic inflammation represent one-fifth of the patients those with isolated systemic eosinophilic inflammation are rather rare. These proportions are found irrespective of asthma treatment received including high doses of inhaled corticoids. We believe our new classification based on eosinophilic inflammation is pertinent to the clinician and goes in line with the need to phenotype severe asthmatics as advocated by the recent ERS/ATS guidelines [32].

As mentioned, the link between eosinophils and asthma severity has been extensively debated. The first attempts to investigate this relationship were based on sampling BAL or biopsies during bronchoscopy. The invasive nature of the procedure has obviously limited the number of subjects studied, which may have led to contrasting results because of the interindividual patient variability. Being less invasive, the technique of induced sputum has considerably widened the series of patients investigated and has been key to the emergence of recognition of several inflammatory asthma phenotypes. Previous studies found higher levels

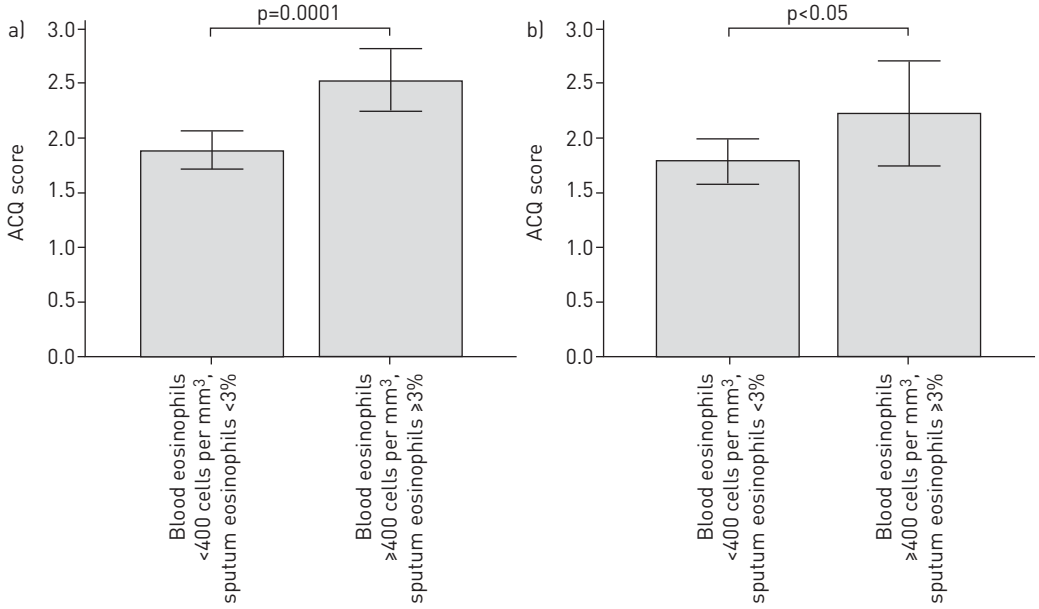

FIGURE 2 Asthma Control Questionnaire (ACQ) score according blood and sputum eosinophil count. The presence of both local and systemic eosinophilic inflammation was associated with lower asthma control in the a) retrospective (n=508) and b) prospective cohort (n=250). Data are presented as mean±SEM.

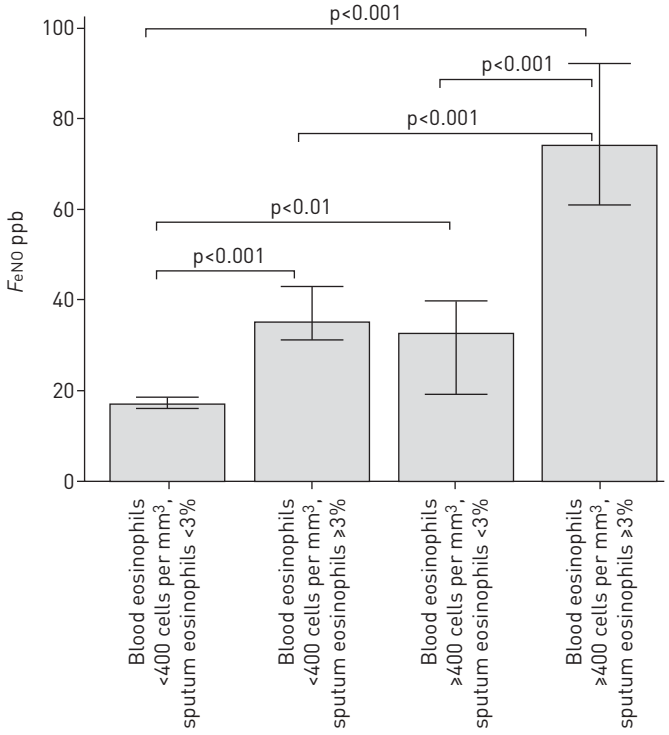

FIGURE 3 Exhaled nitric oxide fraction (*Fe*NO) levels in patients classified according to the presence and/or absence of blood and sputum eosinophilia. Patients exhibiting both local and systemic inflammation had the highest level of *Fe*NO (n=508). The presence of uncontrolled eosinophilic inflammation either at local or systemic level was associated with intermediate levels of *Fe*NO. The group of asthmatics showing no increase in eosinophilic inflammation had the lowest level of *Fe*NO. Data are presented as mean±SEM.

of sputum eosinophil counts in uncontrolled asthmatics [17, 33]. Another study showed that patients with high blood eosinophilia (>250 cells per mm^3) had lower FEV1 values and worse asthma control than those with normal blood eosinophil counts [34]. Recently, VOLBEDA *et al.* [20] have shown that patients with uncontrolled asthma exhibit higher eosinophil numbers in peripheral blood and a trend for a higher eosinophil count in induced sputum, but they did not provide detailed analysis on the discordance between blood and airway eosinophilia. Here, in our retrospective cohort, we found that patients exhibiting eosinophilic inflammation both in blood and sputum had more severe asthma and poorer asthma control than noneosinophilic asthmatics, a finding which was validated in our prospective cohort. Our data show that isolated sputum eosinophilic inflammation is associated with impaired airway calibre and increased airway hyperresponsiveness compared with noneosinophilic asthmatics, while those combining both systemic and eosinophilic had further functional impairment, which is likely to partly contribute to worse asthma control and quality of life. Three-quarters of this group had an ACQ score >1.5 while only 10% had well-controlled asthma with an ACQ score <0.75. However, having an eosinophilic trait, whichever the compartment, is associated with a slightly raised proportion of atopy and higher serum IgE levels.

It is important to note that patients in the group with blood and sputum eosinophilia had much higher sputum eosinophil counts compared with those with sputum eosinophilia alone. Beyond the categorical analysis, the extent of eosinophilic airway infiltration has to be taken into consideration. Our finding reinforces the role of airway eosinophilia in the loss of asthma control previously suggested on smaller sample of patients [13]. The intensity of blood eosinophilic inflammation in patients exhibiting diffuse eosinophilic inflammation was comparable to that of asthmatics with isolated blood eosinophilia, a group that was less severe. Moreover, there was no correlation between ACQ and blood eosinophil counts. This suggests that circulating eosinophils by themselves are not direct actors of asthma severity but are important in providing a pool of cells that can be attracted in the airways.

Approximately 70% of patients exhibiting diffuse eosinophilic inflammation were receiving maintenance treatment with inhaled corticoids. While we could speculate that raising the dose of ICS in those receiving low to moderate doses would have resulted in a reduction of eosinophilic inflammation, it is noteworthy that 30% of the ICS treated patients were already receiving high doses. This highlights the relative resistance to corticoids in some patients and the need for complementary treatment to target eosinophils. Among the patients receiving high doses of inhaled corticoids, 53% exhibited either blood or sputum eosinophilia that could make them potential candidates for treatment with anti-interleukin-5 [25, 35]. A recent study has shown that omalizumab is more efficient in reducing exacerbation when patients express a Th2 profile including high blood eosinophil counts or *Fe*NO levels [36]. Of course, as our study was a field study, we

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TABLE 6 Retrospective cohort: demographic, functional and inflammatory characteristics of patients receiving high doses of corticosteroids (n=144)

	Blood eosinophils <400 cells per mm^3, sputum eosinophils <3%[#]	Blood eosinophils <400 cells per mm^3, sputum eosinophils ⩾3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils <3%	Blood eosinophils ⩾400 cells per mm^3, sputum eosinophils ⩾3%
Subject n (%)	67 (47)	40 (28)	9 (6)	28 (19)
Males/females n	20/47	16/24	5/4	15/13
Age years	52 (21–86)	59 (26–88)	62 (44–85)	52 (29–86)
Age of onset %				
<12 years	25	35	0	30
12–40 years	33	30	50	40
⩾40 years	42	35	50	30
Exacerbations per patient per year[¶]	0.76±1.06	0.95±1.29	1.25±1.17	3.52±5.00***
FEV1 % predicted	77±17	77±20	71±28	65±18**
FEV1/FVC %	73±11	68±13	70±11	64±9**
ACQ score	2.57±1.96	1.97±1.25	3.19±1.79	3.18±1.38
AQLQ score	4.11±1.39	4.52±1.40	3.59±1.47	3.50±1.31
***F*ENO ppb**	14 (4–114)	30 (8–119)***	10 (5–58)	55 (11–139)***
Blood eosinophils per mm^3	160 (10–350)	225 (0–390)***	465 (400–1030)***	615 (400–3220)***
Sputum eosinophils %	0.25 (0–2.6)	8.4 (3–52.2)***	0.4 (0–2.7)	22.3 (3.6–94.4)***

Data are presented as median (range) or mean±SD, unless otherwise stated. FEV1: forced expiratory volume in 1 s; FVC: forced vital capacity; ACQ: Asthma Control Questionnaire; AQLQ: Asthma Quality of Life Questionnaire; *F*ENO: exhaled nitric oxide fraction. [#]: comparator group; [¶]: during the year prior to the visit. *: p<0.05; **: p<0.01; ***: p<0.001.

cannot rule out poor compliance in some patients, making the interpretation of corticoid resistance in these asthmatics difficult.

Here, we deliberately chose to classify our patients according to pre-specified criteria, which were blood and sputum eosinophils. Our approach was therefore different from that used in hierarchical unsupervised cluster analysis. We do not deny the great interest of this type of cluster analysis in the emergence of clinical asthma phenotypes, but we believe that classifying asthmatics according to their eosinophilic profile is useful because sputum and blood eosinophils are good biomarkers to target treatment and predict response to corticoids or biologicals directed towards Th2 cytokines [37, 38]. Furthermore, it has recently been advocated that sputum eosinophils may be a valuable outcome in asthma drug trials [37]. How our asthma groups may fit with those found by others after cluster analysis remains to be determined [39–41]. There are some similarities coming out of both approaches regarding the link between eosinophilia and sex. While females are the dominant sex among adult asthmatics, our data show that this is mainly accounted for by the noneosinophilic group even if females remained the predominant sex in those with isolated airway

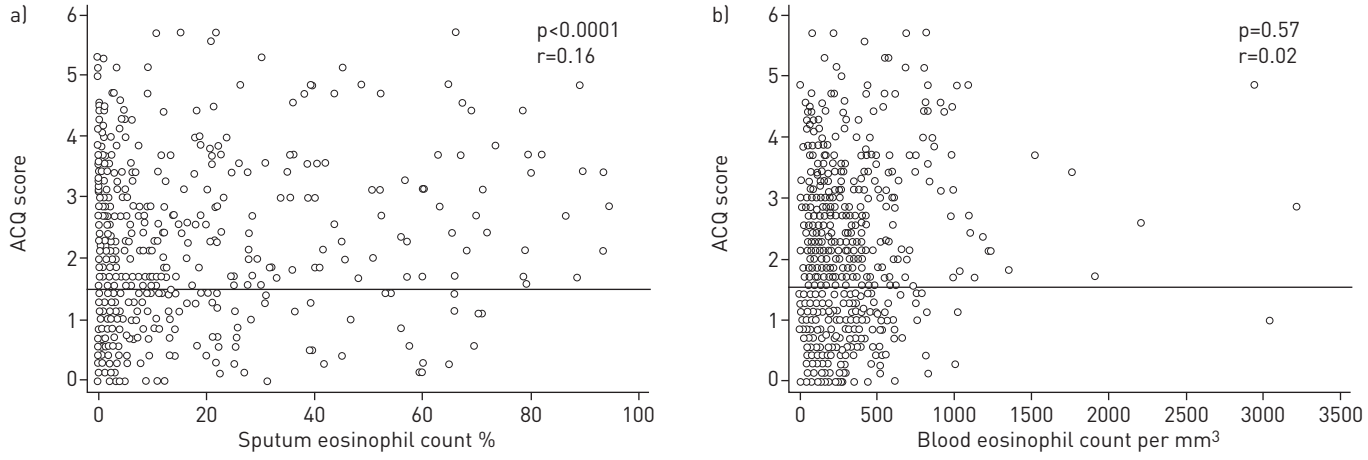

FIGURE 4 Correlation between Asthma Control Questionnaire (ACQ) score and a) sputum and b) blood eosinophil count when combining the prospective and retrospective cohorts (n=758). Asthma is considered uncontrolled if the ACQ score is >1.5.

eosinophilic asthma. It is noteworthy that the proportion of males was significantly higher in those patients with an eosinophilic trait compared with their noneosinophilic counterpart and, in our study, it is striking that males even becomes the predominant sex in the case of diffuse eosinophilic inflammation. This is likely to reflect the different hormonal status and its effect on eosinophil biology. Few studies have evaluated the relationship between eosinophils and sex hormones. It seems that β-oestradiol significantly enhances eosinophil adhesion to human mucosal microvascular endothelial cells and their degranulation [42, 43].

By contrast, our data do not point to a peculiar association between obesity and noneosinophilic asthma as BMI was similar in all our groups of patients.

Of interest is the fact that classic systemic inflammatory markers such as fibrinogen and C-reactive protein do not link at all with the severity of eosinophilic inflammation, a phenomenon that contrasts with what is usually seen in airway diseases exhibiting neutrophilic inflammation [44, 45].

One-third of asthmatics, approximately, exhibit dissociation between airway and systemic inflammation. The reasons why there may be discordant eosinophilic inflammation between blood and sputum remains unclear. In case of intense airway eosinophilic inflammation without blood eosinophilia, we could speculate about a massive local attraction due to the release of chemotactic agents without heavy stimulation of bone marrow. In patients with high blood eosinophil count and low sputum eosinophils, there could be a lack of transendothelial migration of eosinophils due to altered receptor expression or receptor down-regulation. An alternative explanation would be that the airway eosinophilic inflammation is masked by macrophage phagocytosis of eosinophils [46]. This could explain the intermittent eosinophilic phenotype [9] and the intermediate $F_{E}NO$ we found in this subgroup, as $F_{E}NO$ is a good surrogate marker for sputum eosinophilia [47].

Even if eosinophilic inflammation appears to be important in driving asthma severity, it has to be kept in mind that half of asthmatics did not display any sign of eosinophilic inflammation, among whom 59% remained poorly controlled. Of course, for those already receiving inhaled corticoids, it could be argued that some of them may have been eosinophilic prior to initiation of ICS treatment. The current literature suggests, however, that initiating or increasing the dose of inhaled corticoids in those noneosinophilic patients is probably useless for improving asthma control [22]. This points out the need to develop and test new drugs in this asthma phenotype. Clarithromycin was shown to slightly improve quality of life in refractory neutrophilic asthmatics and a recent study suggests that azithromycin may reduce exacerbation in patients with low blood eosinophil counts [48].

The main limitation of our study is the single time-point measurement that does not take into account the possible fluctuation of the patients from one group to another over time. Indeed, some patients were shown to be intermittently eosinophilic in their sputum, which may cause bias when dealing with a single measurement. However, we believe that looking at the relationship between ACQ score and sputum eosinophils based on one single evaluation remains valid as ACQ score reflects disease control on a short time period (1 week), a period over which sputum eosinophil count was found to be reproducible [49].

Exacerbation rate, which was clearly higher in the group with combined eosinophilic inflammation, must be considered cautiously as it was based on history taking and it is not always easy to disentangle what was justified by genuine asthma worsening or by a flare up of sinusitis with cough. Nevertheless, the fact that the prospective cohort confirmed the proportion of eosinophilic subgroups and the exacerbation rates found in the retrospective cohort is quite reassuring of the validity of our classification. Of interest also is the fact that exacerbation rate decreased on average by 42% in the year after the visit to our asthma clinic, suggesting that what the clinician decided based on a comprehensive investigation has had an impact on long-term asthma control. Naturally, this is an observational finding that does not obey the tight criteria of randomised controlled trials and our finding has to be confirmed in randomised trials comparing different management strategies.

Our proportion of eosinophilic asthma is greater than that found by McGrath *et al.* [9], as they only found eosinophilic asthma in 17–36% of patients. However, their study recruited patients highly selectively, as is usually the case in clinical drug trials, thereby excluding quite a proportion of patients seen in clinical practice (*i.e.* smokers, those poorly reversible to β_2-agonists and those with comorbidity), which was not the case in our field study. Of course, we can also speculate that compliance to ICS was better in patients described by McGrath *et al.* [9], which may have also resulted in lower proportion of eosinophilic asthma. It is worth noting that our proportion of noneosinophilic *versus* eosinophilic asthma based on sputum analysis is rather similar to what was reported in another field study [3].

Conclusion

Concomitant systemic and bronchial eosinophilic inflammation contribute to poor asthma control. As there was dissociation between systemic and airway eosinophilic inflammation in 30% of patients,

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assessment of inflammation in both airways and peripheral blood provides additional information about asthma status. We believe our classification may be valid to start intervention studies using drugs mainly targeting either the airways or the blood compartment according to the patient's profile.

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