Gerday et al. World Allergy Organization Journal (2022) 15:100655
http://doi.org/10.1016/j.waojou.2022.100655

WORLD ALLERGY ORGANIZATION JOURNAL

Open Access

Revisiting differences between atopic and non-atopic asthmatics: When age is shaping airway inflammatory profile

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ABSTRACT

Background: Atopic asthma is one of the most common asthma phenotypes and is generally opposed to the non-atopic counterpart. There have been very few large-scale studies comparing atopic and non-atopic asthmatics in terms of systemic and airway inflammation across the age spectrum.

Methods: Here, we have undertaken a retrospective study investigating 1626 patients (924 atopic and 702 non-atopic asthmatics) recruited from our university asthma clinic who underwent extensive clinical investigations including induced sputum. Atopy was defined by any positive specific IgE to common aeroallergens (>0,35 kU/L). We performed direct comparisons between the groups and sought to appreciate the influence of age on the airway and systemic inflammatory components. The study was approved by the ethics committee of the University Hospital of Liege (Ref. 2016/276). Informed consents were obtained from healthy subjects.

Results: Atopic asthmatics were younger ($P < .001$), had a higher male/female ratio ($P < .001$), an earlier disease onset ($P < .001$) and a greater proportion of treated rhinitis ($P < .001$) while non-atopic asthmatics had greater smoke exposure ($P < .001$), lower FEV_1/FVC ratio ($P = .01$) and diffusing capacity ($P < .001$). There was no difference between the 2 groups regarding FEV_1 (% predicted), asthma control, asthma quality of life and exacerbations in the previous 12 months. Regarding inflammation, atopic patients had higher FeNO levels (median = 28 ppb, $P < .001$), were more eosinophilic both in blood (median = 2.8%, $P < .001$) and in sputum (median = 2.2%, $P < .001$) while non-atopic patients displayed greater blood (median = 57%, $P = .01$) and sputum (median = 58.8%, $P = .01$) neutrophilic inflammation. However, stratifying patients by age showed that non-atopic asthmatics above 50 years old became equally eosinophilic in the sputum ($P = .07$), but not in the blood, as compared to atopic patients. Likewise, FeNO rose in non-atopic patients after 50 years old but remained, however, lower than in atopic patients.

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http://doi.org/10.1016/j.waojou.2022.100655

Received 22 December 2021; Received in revised from 17 March 2022; Accepted 7 May 2022
Online publication date xxx

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Conclusions: We conclude that, while sharing many features, atopic group still differentiates from non-atopic asthmatics by demographics, functional and inflammatory profiles. When atopic asthmatics showed a constant eosinophilic pattern across the age spectrum, non-atopic asthmatics were found to be neutrophilic before the age of 50 but eosinophilic above 50 years old.

Keywords: Atopic asthma, Non-atopic asthma, Sputum, Neutrophils, Eosinophils

INTRODUCTION

The concept of asthma phenotype has emerged over the last decades thereby recognizing that beyond the common ground defining the disease, asthmatics may differ by several characteristics.[1] Allergic asthma is the first asthma phenotype to have been described in the first half of the previous century.[2] Allergic asthma is typically diagnosed based on symptoms triggered by allergen exposure and atopy can be confirmed by testing skin prick reactivity to common aeroallergens or by measuring specific serum immunoglobulin E (IgE).[3] This phenotype is classically opposed to the so-called "intrinsic asthma" or non-atopic asthma where triggers of airway obstruction were more often supposed to be infectious.

This definition of atopic and non-atopic asthma is widely used when designing and analyzing studies on the genetic, environmental, and immunological determinants and characteristics of asthma.[4] There is debate as to whether atopic and non-atopic asthma are immunopathologically 2 distinct entities or if both are driven by similar mechanisms.[5] Early studies in the 1990s suggested that non-atopic asthma may still, in part, be linked to IgE-mediated process.[6] Indeed, non-atopic asthma in children was found to be associated with helminth infections. There are reports of non-atopic asthma in poor children and infections by helminth and parasitosis are known to be associated with an increased total serum IgE level.[7,8] Yet, the entity of intrinsic or non-atopic asthma continues to raise questions about the possible role of IgE-mediated mechanisms in asthma pathogenesis. The clarification of this issue becomes more relevant with the current availability of anti-IgE therapy for the treatment of severe asthma.[5]

Surprisingly, there have not been many large-scale studies comparing atopic versus non-atopic asthmatics. Nieves et al[9] conducted a prospective study in patients referred to chest physicians and found that atopic asthma was more frequent (71%), more prevalent in male gender, appeared at a younger age, was more often associated with rhinitis and a family history of asthma. Conversely, non-atopic asthma had later disease onset, was more often associated with a smoking history, had more dyspnea and cough with more altered spirometric values.

To the best of our knowledge, there have been no large-scale studies investigating both systemic and airway inflammation in atopic versus non-atopic asthma. Here, we took advantage of our asthma clinic database that comprises a large cohort of well characterized patients recruited in a university secondary care center who underwent extensive clinical investigations including induced sputum. The aim of the present study was to compare atopic versus non-atopic asthmatics with respect to not only demographics and lung function but also systemic and airway inflammation and asthma control. Furthermore, as it is well known that age may impact systemic and airway inflammation as a consequence of immuno-senescence, we have performed a subgroup analysis after age stratification.[10] To this end, we have performed direct group comparisons on the whole patient groups as well as on groups obtained after age stratification.

METHODS

Subject characteristics and study design

We conducted a retrospective study on our asthma clinic database and identified 1626 asthmatics recruited from the University Asthma Clinic of Liege. Patients were eligible for the study if they

had a visit with a defined radioallergosorbent test (RAST) between March 2002 and September 2019. This study was approved by the Ethics committee of the University Hospital of Liege. For asthmatic patients, all procedures were performed in the context of clinical practice and the retrospective data collection was conducted with the approval from the above-mentioned ethics committee.

Asthma was diagnosed based on typical symptoms (wheezing, breathlessness, chest tightness, cough) and at least 1 of the following criteria; an improvement of 12% and 200 mL in forced expiratory volume in 1 s (FEV_1) following inhalation of 400 µg salbutamol or a provocative concentration of methacholine causing 20% fall in FEV_1 ($PC_{20}M$) < 16 mg/mL. FeNO was measured at flow rate of 50 mL/s (NIOX, Aerocrine, Solna, Sweden). Blood samples of patients were analyzed by the routine laboratory of the University Hospital of Liege. Sputum was induced and processed as previously described.[11] Quality life was assessed using the self-administered Asthma Quality of Life Questionnaire (AQLQ)[12] and asthma control by the Juniper Asthma Control Questionnaire (ACQ)[13] and by Asthma Control Test (ACT).[14] The number of exacerbations defined by a course of OCS of at least 3 days in the past year was registered at each visit.

Atopy was characterized by the presence of at least 1 positive specific IgE (>0.35 kU/L; Phadia; Groot-Bijgaarden, Belgium) to common aeroallergens (cat, dog, grass pollen, tree pollen, house dust mite and a mixture of molds). A group of healthy subjects was recruited as a control group by advertising in the hospital among staff members or patient families. Healthy subjects denied any chronic respiratory disease, had normal spirometric values with FEV_1 above 80% and FEV_1/FVC above 70% and a $PC_{20}M > 16$ mg/mL. Informed consents were obtained from healthy subjects.

Statistical analysis

Missing data imputation

Missing values were replaced with plausible data values with specific methods for imputing values implemented in MICE package.[15] Predictive mean matching (PMM) was used for numeric variables, logistic regression was used for binary variables and polytomous logistic regression was used for categorical variables with K unordered categories. The package created multiple imputations (replacement values) for multivariate missing data. The MICE algorithm was used to impute 100 datasets and pooling procedures applied Rubin's rules[16] for subsequent statistical analyses.

Group comparisons

Data were expressed as count and percentage for categorical variables and as median (interquartile range) for quantitative variables. Comparisons were performed using a Pearson's Chi^2 test or Fisher's exact test for categorical variables, an ANOVA or a Student's *t*-test for parametric variables, and a Kruskal–Wallis test or a Mann-Whitney U test for non-parametric variables (Tables 1 and 2). Spearman correlation coefficient was used to measure the association between the percentages of sputum and blood eosinophils, age and FeNO values in asthmatic patients and healthy subjects. A *P*-value $< .05$ was considered statistically significant. Statistical analyses were performed using Rstudio Team (Rstudio: Integrated Development for R. Rstudio, Inc., Boston).

RESULTS

The functional, demographic, treatment, and inflammatory characteristics of the asthmatic cohort (n = 1626) and healthy subjects (n = 150), classified according to their atopic status are given in Tables 1–3.

Demographic status

Patients with atopy represented 57% of the asthmatic cohort. Late onset of the disease was more common in non-atopic patients ($P < .001$; Table 1). Atopic asthmatics were more often treated with inhaled corticosteroids ($P < .001$; Table 1). Moreover, age, female/male ratio, BMI and smoking history were higher in non-atopic than in atopic asthmatics. The prevalence of treated rhinitis was greater among atopic patients ($P < .001$; Table 1).

Asthma control and quality of life

There was no difference between the groups in asthma control and quality of life nor for exacerbation rate the year prior to the visit (Table 1).

http://doi.org/10.1016/j.waojou.2022.100655

	Atopic asthmatics *Median (IQR)/N (%)*	Non-atopic asthmatics *Median (IQR)/N (%)*	Healthy subjects *Median (IQR)/N (%)*	*P*-value	N/A (%)	Number of N/A (n)
N, %	*924 (57)*	*702 (43)*	*150*			
Age, years	42 (28–54)	55 (43–64)[b]	54 (45–62)	$P < .001$	0.3	5
Age at diagnosis, years	19 (5–38)	49 (31–60)	ND	$P < .001$	19.7	320
Sex (Male), N (%)	47 (431)	31 (218)[b]	47 (71)	$P < .001$	0.1	2
BMI, kg/m^2	25.2 (22.2–28.9)	26.3 (22.9–29.8)[b]	24.6 (22.4–27.7)	$P < .001$	1	18
Exacerbations, N[a]	0 (0–1)	0 (0–0)	ND	$P = .07$	40	650
Hospitalisations, N[a]	0 (0–0)	0 (0–0)	ND	$P = .53$	36.9	600
Treated rhinitis, N (%)	272 (29)	83 (12)	ND	$P < .001$	1.2	19
ACT score	16 (12–20)	15 (11–20)	ND	$P = .11$	30.2	491
ACQ score	1.7 (0.9–2.7)	1.9 (1–2.7)	ND	$P = .14$	34.3	558
AQLQ score	4.7 (3.7–5.7)	4.5 (3.5–5.5)	ND	$P = .08$	5	82
PAQ-Y	0 (0–6)	0.8 (0–20)[b]	1.1 (0–16.5)	$P < .001$	10.5	187
Smoking status, N (%)				$P < .001$	1.3	23
Non-smokers	62 (570)	49 (342)[b]	44 (66)			
Ex-smokers	21 (198)	29 (203)[b]	40 (60)			
Smokers	17 (156)	22 (157)[b]	16 (24)			
ICS, N (%)	571 (62)	372 (53)[b]	0 (0)	$P < .001$	4.3	77
ICS, mcg	500 (0–1200)	400 (0–1000)[b]	0 (0–0)	$P < .001$	4.3	77
OCS, N (%)	79 (9)	71 (10)	0 (0)	$P < .001$	1.6	29
LABA, N (%)	529 (57)	350 (50)[b]	0 (0)	$P < .001$	1.6	29
SABA, N (%)	641 (69)	446 (64)[b]	0 (0)	$P < .001$	1.7	30
LAMA, N (%)	40 (4)	36 (5)	0 (0)	$P = .02$	1.7	30
LTRA, N (%)	239 (26)	141 (20)[b]	0 (0)	$P < .001$	1.1	19

Table 1. Demographic and treatment characteristics of asthmatic patients classified by their atopic status and healthy subjects. *BMI, body mass index; ACT, asthma control test; ACQ, asthma control questionnaire; AQLQ, asthma quality of life questionnaire; PAQ-Y, quantification of cigarette smoking; ICS, inhaled corticosteroid; OCS, oral corticosteroid; LABA, long acting beta agonist; SABA, short acting beta agonist; LTRA, leukotriene receptor antagonist; LAMA, long acting muscarinic agent.* [a]*During the year prior to the visit.* [b]*$P < .05$, comparison with atopic asthmatics*

Lung function status

Detailed lung function status is given in Table 2. Atopic and non-atopic asthmatics shared similar prebronchodilation spirometric and lung volumes values. Likewise, there was no difference in bronchial hyperresponsiveness to methacholine and reversibility to salbutamol between the 2 groups.

	Atopic asthmatics *Median (IQR)/N (%)*	Non-atopic asthmatics *Median (IQR)/N (%)*	Healthy subjects *Median (IQR)/N (%)*	*P*-value	N/A (%)	Number of N/A (n)
FeNO, ppb	28 (16–51)	18 (11–34)[a]	21 (15–29)	$P < .001$	10.7	190
FEV_1 (pre), %	89 (75–100)	88 (74–99)	104 (96–116)	$P < .001$	1.2	22
FEV_1 (post), %	94 (81–105)	92 (80–105)	110 (100–123)	$P < .001$	15	267
FVC (pre), %	97 (86–107)	96 (84–108)	ND	$P = .77$	14.8	240
FVC (post), %	99 (88–109)	99 (86–109)	ND	$P = .83$	33.3	541
FEV_1/FVC (pre),%	77 (70–83)	76 (69–81)	ND	$P = .29$	1.2	20
FEV_1/FVC (post),%	80 (73–86)	79 (72–84)	ND	$P = .01$	32.1	522
Reversibility, %	5 (1–12)	5 (1–11)	ND	$P = .30$	16.4	266
$PC_{20}M$, mg/mL	3 (0.8–16)	3.3 (1.2–14)	ND	$P = .45$	38.6	628
TLC, %	96 (86–105)	97.0 (89.0–108.0)	ND	$P = .10$	26.1	424
FRC, %	111 (92–134)	113 (94–136)	ND	$P = .56$	29.2	474
RV, %	110 (86–138)	112 (89.2–138)	ND	$P = .58$	26.4	429
DL_{CO}, %	81 (70–92)	74 (62–84)	ND	$P < .001$	29.6	482
K_{CO}, %	97 (86–110)	91 (77–104)	ND	$P < .001$	28.7	466

Table 2. Functional characteristics of asthmatic patients classified by their atopic status and healthy subjects. *FeNO, fractional exhaled nitric oxide; FEV_1, forced expiratory volume in 1 s; FVC, forced vital capacity; $PC_{20}M$, provocative concentration of methacholine causing a 20% fall in FEV_1; TLC, total lung capacity; FRC, functional residual capacity; RV, residual volume; DL_{CO}, diffusing capacity factor of the lung for carbon monoxide; K_{CO}, carbon monoxide transfer coefficient. [a]$P < .05$, comparison with atopic asthmatics*

Postbronchodilation FEV_1/FVC ratio was slightly lower in non-atopic compared to atopic asthmatics ($P = .01$; Table 2). Exhaled nitric oxide was significantly higher in atopic compared to non-atopic asthmatics ($P < .001$; Table 2) while diffusion capacity (DL_{CO}) and transfer coefficient (K_{CO}) were lower among non-atopic asthmatics ($P < .001$; Table 2).

Inflammatory status

The sputum cell counts and viability are shown in Table 3. Non-atopic asthmatics had a greater total sputum cell count ($P < .001$; Table 3). Atopic asthmatics displayed greater sputum eosinophil counts in percentage than non-atopic patients and healthy controls ($P < .001$; Table 3). By contrast, non-atopic asthmatics exhibited a more intense airway neutrophilic inflammation, both in absolute value, as well as expressed as percentage ($P < .001$ and $P = .01$ respectively; Table 3).

The blood cell counts are shown in Table 3. Total blood leukocyte count was greater in non-atopic asthmatics compared to atopic asthmatics and healthy subjects ($P < .001$; Table 3). While atopic asthmatics had higher blood eosinophil count (in absolute value and percentage) ($P < .001$; Table 3), non-atopic asthmatics were characterized by a higher neutrophilic cell count (in percentage and absolute value) ($P = .02$ and $P < .001$ respectively; Table 3) together with raised fibrinogen ($P < .001$; Table 3).

Correlation between FeNO, age, sputum and blood eosinophil count

Non-parametric correlation between blood eosinophil count (%) and FeNO (ppb) showed

http://doi.org/10.1016/j.waojou.2022.100655

	Atopic asthmatics *Median (IQR)/ N (%)*	Non-atopic asthmatics *Median (IQR)/N (%)*	Healthy subjects *Median (IQR)/N (%)*	*P*-value	N/A (%)	Number of N/A (n)
Blood leukocytes, ($\times 10^3$/µL)	7.3 (6.1–8.7)	7.5 (6.2–9.2)[a]	6.4 (5.3–7.7)	$P < .001$	5.5	97
Blood neutrophils, %	55.0 (48.0–62.0)	57.0 (50.2–63.2)[a]	55.2 (49.3–61.6)	$P = .02$	5.5	98
Blood lymphocytes, %	32.7 (26.4–39.1)	31.6 (25.7–38.0)	33.5 (28.1–37.8)	$P = .12$	5.5	98
Blood monocytes, %	6.9 (5.4–8.6)	7.0 (5.6–8.6)	7.1 (5.6–8.7)	$P = .65$	5.6	99
Blood eosinophils, %	2.8 (1.6–4.6)	2.2 (1.2–3.6)[a]	1.8 (1.0–2.9)	$P < .001$	5.6	100
Blood basophils, %	0.5 (0.3–0.7)	0.5 (0.3–0.7)	0.5 (0.4–0.7)	$P = .11$	6.2	110
Blood neutrophils,/µL	3890 (3043–5301)	4245 (3293–5474)[a]	3613 (2681–4390)	$P < .001$	5.5	98
Blood lymphocytes,/µL	2298 (1905–2787)	2352 (1881–2877)	2056 (1709–2528)	$P < .001$	5.5	98
Blood monocytes,/µL	493 (389–646)	526 (412–678)[a]	441 (338–586)	$P < .001$	5.6	99
Blood eosinophils,/µL	198 (112–348)	161 (89–286)[a]	108 (70–176)	$P < .001$	5.6	100
Blood basophils,/µL	34 (23–51)	35 (22–53)	33 (22–47)	$P = .31$	6.2	110
Fibrinogen, g/L	3.1 (2.6–3.7)	3.4 (2.9–3.9)[a]	ND	$P < .001$	21.9	356
CRP, mg/L	1.9 (0.8–4.6)	2.1 (1.0–4.7)	ND	$P = .07$	22.8	370
IgE, kU/L	214 (86–516)	42 (16–118)[a]	ND	$P < .001$	3.8	61
Total sputum cell count, ($\times 10^6$/g)	1.4 (0.6–3.4)	1.7 (0.7–4.1)[a]	0.6 (0.3–1.4)	$P < .001$	19.7	349
Sputum squamous cells, %	15.0 (6.0–30.0)	14.0 (5.0–29.0)	19.2 (11.0–31.9)	$P = .01$	18.2	324
Sputum viability, %	67.0 (50.0–79.0)	72.0 (56.0–83.0)[a]	68.0 (55.0–79.0)	$P < .001$	18.5	328
Sputum macrophages, %	28.6 (13.5–50.0)	24.1 (11.2–43.2)[a]	36.1 (20.0–59.8)	$P < .001$	20.8	370
Sputum lymphocytes, %	1.0 (0.4–2.4)	1.2 (0.4–2.4)	1.8 (0.5–3.0)	$P = .04$	20.8	370
Sputum neutrophils, %	49.2 (26.1–72.8)	58.8 (31.5–77.6)[a]	52.0 (23.2–69.9)	$P = .01$	20.5	364
Sputum eosinophils, %	2.2 (0.4–9.6)	1.4 (0.2–6.8)[a]	0.0 (0.0–0.5)	$P < .001$	20.5	364
Sputum epithelial cells, %	3.8 (1.6–8.0)	3.0 (1.3–7.0)	4.0 (2.0–10.4)	$P = .06$	20.8	370

(continued)

	Atopic asthmatics *Median (IQR)/ N (%)*	Non-atopic asthmatics *Median (IQR)/N (%)*	Healthy subjects *Median (IQR)/N (%)*	*P*-value	N/A (%)	Number of N/A (n)
Sputum macrophages, ($\times 10^3$/g)	347 (129–807)	366 (128–948)	208 (84–469)	$P = .01$	22.6	402
Sputum lymphocytes, ($\times 10^3$/g)	14 (2.3–44)	16 (3.4–52)	9 (3.3–24)	$P = .03$	22.6	402
Sputum neutrophils, ($\times 10^3$/g)	543 (193–1754)	714 (219–2229)[a]	269 (80–675)	$P < .001$	22.6	401
Sputum eosinophils, ($\times 10^3$/g)	34 (2.4–192)	17 (0–159)	0 (0–4.2)	$P < .001$	22.6	401
Sputum epithelial cells, ($\times 10^3$/g)	44 (14–125)	49 (14–134)	28 (10–62)	$P = .01$	22.6	402

Table 3. (Continued) Inflammatory characteristics of asthmatic patients classified by their atopic status and healthy subjects. *CRP, C-reactive protein; IgE, immunoglobulin E.* [a]*P < .05, comparison with atopic asthmatics*

positive correlation with spearman's rho of 0.4 in the atopic group ($P < .001$) and 0.42 in the non-atopic group ($P < .001$). Similar association between the percentage of sputum eosinophils and FeNO (ppb) was found with a positive correlation with spearman's rho of 0.35 in the atopic group ($P < .001$) and 0.41 in the non-atopic group ($P < .001$).

Relationships between FeNO levels (ppb), sputum and blood eosinophils (%) and age (years) in atopic asthmatics and non-atopic asthmatics (patients below 18 years old were excluded for these graphs in both groups) are detailed in Fig. 1. Non-parametric correlation between FeNO (ppb) and age showed positive correlation with spearman's rho of 0.21 in the non-atopic group ($P < .001$). Moreover, non-parametric correlation between blood and sputum eosinophil count (%) and age showed positive correlations ($R = 0.15$ and $R = 0.16$, respectively) in the non-atopic group ($P < .001$). No significant correlation was found in the atopic group.

Age stratification

Comparisons of FeNO levels, blood and sputum eosinophils (%) and neutrophils (%) in asthmatic patients and healthy subjects stratified by age (with 50 years old as threshold value) are presented in Figs. 2–4. In Fig. 2, FeNO level differences between the 3 groups persisted within the age range, with significantly higher levels in atopic asthmatics (Table S1). Regarding cellular inflammatory characteristics (Figs. 3 and 4), disparities between percentages of neutrophils and eosinophils tended to disappear among older subjects. Blood and sputum eosinophilic patterns were similar across the ages in atopic asthmatics. Local and systemic eosinophilic infiltration was significantly elevated in older patients compared to patients under 50 years old in non-atopic asthmatics ($P < .001$; Table S1). In patients over 50 years old, there was no difference in sputum eosinophil counts between atopic and non-atopic asthmatics (Table S1). Nevertheless, systemic eosinophilic inflammation remained more marked in older patients in the atopic group ($P = .002$; Table S1). With regard to atopic patients under 50 years of age, local and systemic neutrophilic inflammation was significantly lower than in non-atopic asthmatic patients for the same age category ($P = .001$ and $P = .009$ respectively; Table S1). This gap disappeared with increasing age.

DISCUSSION

Our study is the largest to have compared atopic and non-atopic asthmatics with a large set of clinical variables. Although, overall, the 2 groups are fairly similar, the 2 clinical phenotypes can still be distinguished by a number of variables.

http://doi.org/10.1016/j.waojou.2022.100655

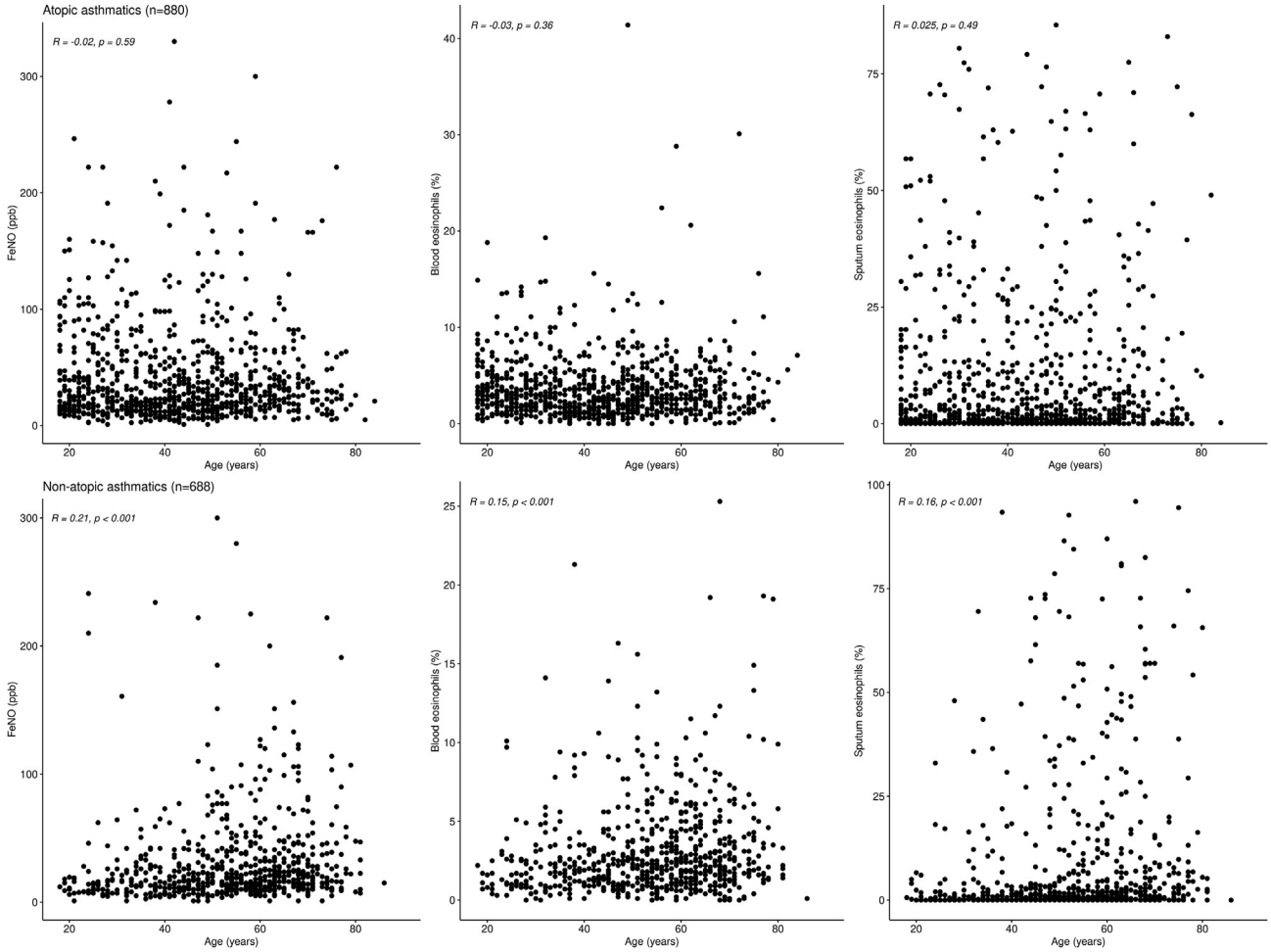

Fig. 1 Relationships between FeNO levels (ppb), sputum and blood eosinophils (%) and age (years) in atopic asthmatics (upper panel) and non-atopic asthmatics (lower panel). Patients below 18 years old were excluded for these graphs in both groups.

Younger age, early onset, male sex, lack of smoking history, treated rhinitis are factors discriminating atopic from non-atopic asthma. While showing globally well-preserved spirometric values, impairment of lung function was slightly more marked in non-atopic patients as highlighted by a lower postbronchodilation FEV_1/FVC ratio. With respect to inflammatory characteristics, atopic asthmatics displayed greater eosinophilic inflammation both at systemic and airway levels while non-atopic asthmatics had greater total and neutrophil cell counts both at systemic and airway levels together with a slightly increased plasma fibrinogen. Moreover, stratifying asthmatics by age revealed persistent eosinophilic inflammation irrespective of age in atopic patients whereas, among non-atopic asthmatics, this inflammatory profile was essentially observed in those above the age of 50 years.

A series of studies analyzed characteristics discriminating atopic from non-atopic asthma. Nieves et al[9] compared the 2 types of asthma in 751 adult asthmatics. They demonstrated, in line with our data, that atopic and non-atopic asthma definitely reflected 2 distinct phenotypes with age of the patients, age of asthma onset, and gender being different. Rhinitis was also more frequent in atopic asthmatics. Overall, those studies showed that increased age, female gender, smoking history and more severe impairment of lung function were associated with an greater chance of the patient being non-atopic.[9,17,18] Those conclusions are consistent with our observations in the present study. Furthermore, Haldar and colleagues[19] described by cluster analysis 2 different phenotypes in asthma population; an early-onset atopic group and an obese, non-eosinophilic group. In our study, BMI was slightly higher in

non-atopic asthmatics, which corroborates the results of the previously mentioned report.

As far as lung function is concerned, FEV_1 and FVC values (%) were remarkably similar between atopic and non-atopic asthmatics even if post-bronchodilation FEV_1/FVC ratio was slightly decreased in non-atopic while remaining in the normal range. This difference may be linked to the different smoking history, which was more frequent in non-atopic asthmatics. The same interpretation may also apply for the difference in the diffusion capacity and transfer coefficient which were lower in the non-atopic group, likely to reflect an early stage of emphysema. It should be noted that no difference between the groups was seen regarding sign of hyper-distension as shown by similar residual volume and total lung capacity. As spirometric values were similar between atopic and non-atopic asthmatics and as the disease duration was lower in non-atopic asthmatics because of late disease onset, this would indicate an accelerated lung function decline in the latter.

In contrast to spirometric values, there was a marked difference in exhaled nitric oxide between the 2 groups with atopic asthmatics displaying a clear increase in FeNO. Presence of atopy and eosinophilic airway infiltration can cause elevation of FeNO levels.[20] Scott et al[21] showed that FeNO behaved as a biomarker of the allergic asthma phenotype. Significant correlations have been observed in literature between Fe^{NO} and blood and sputum eosinophil counts[22-24] but it was not clear whether it also holds true in non-atopic asthmatics. Our study definitely confirmed this association both in atopic and non-atopic patients. One of the factors that may be at play in the reduced FeNO

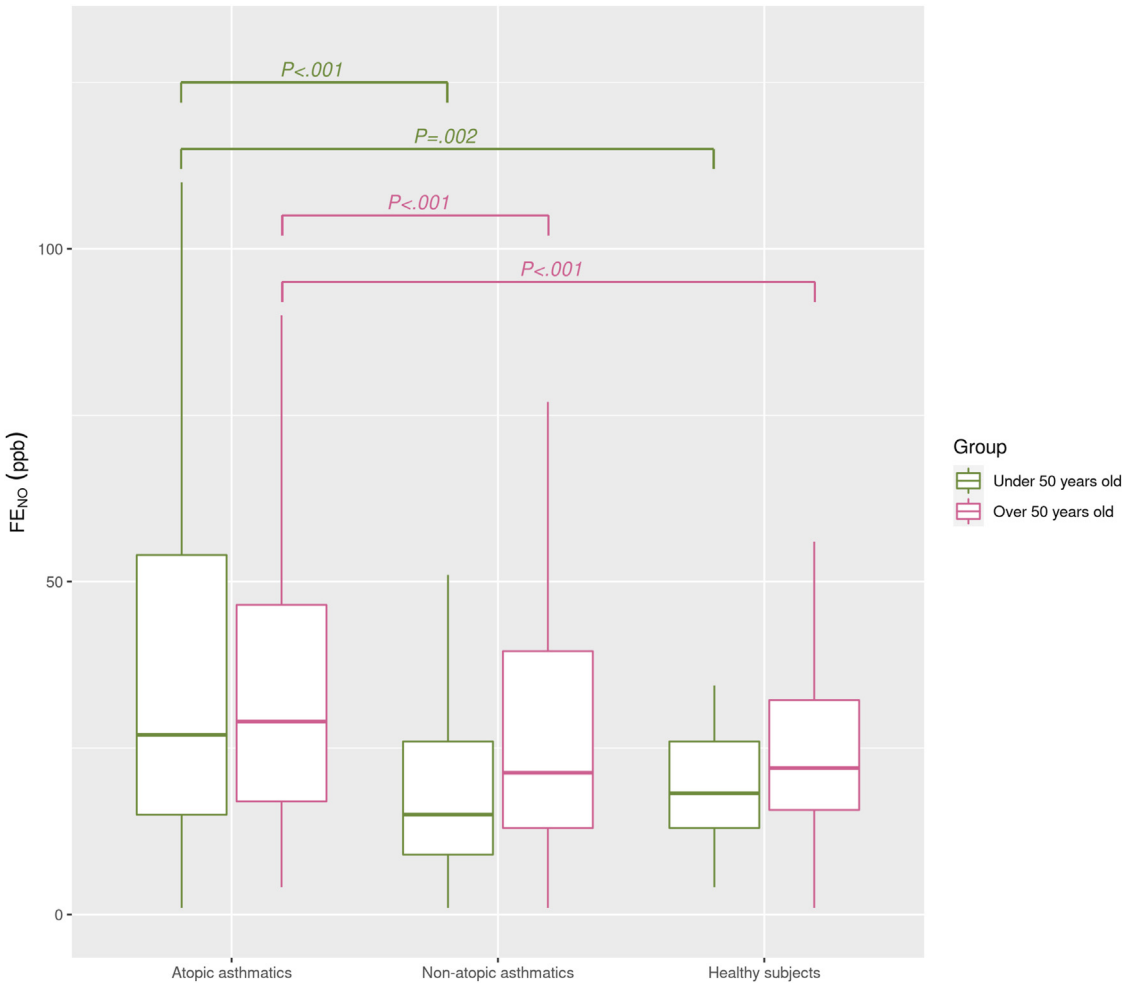

Fig. 2 Comparison of FeNO levels in asthmatic patients and healthy subjects stratified by age.

http://doi.org/10.1016/j.waojou.2022.100655

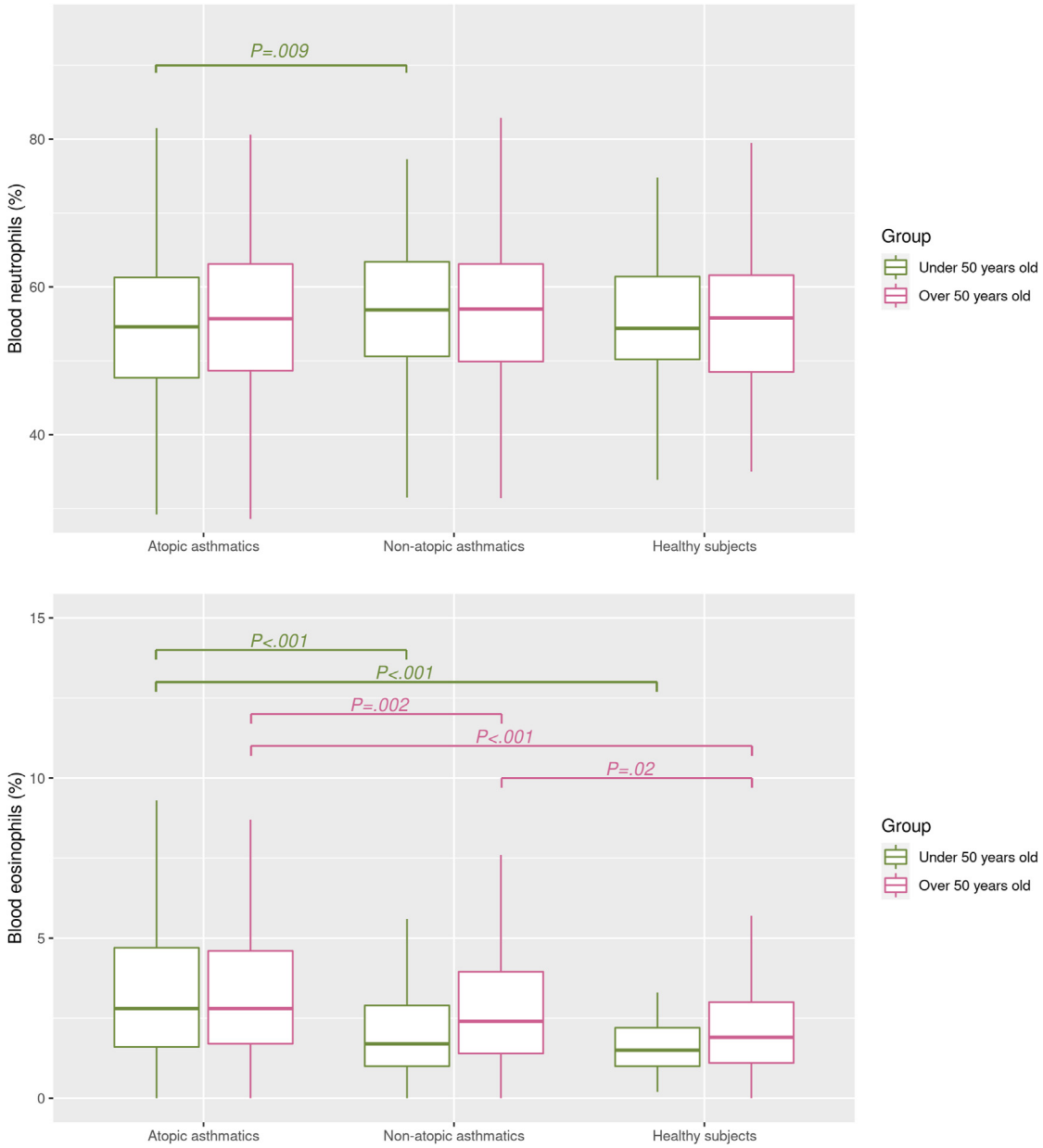

Fig. 3 Comparison of blood neutrophils and eosinophils (%) in asthmatic patients and healthy subjects stratified by age.

levels among non-atopic asthmatics is their smoking behavior. In fact, smoking has been consistently associated with reduced FeNO levels in asthmatic patients compared with their nonsmoking counterparts.[25-27] Finally, the fact that the male sex, usually displaying greater height, was more represented in the group of atopic patients may also account for part of the raised FeNO levels in this group as FeNO is known to be influenced by the height of the patient.[28]

As for inflammatory parameters, the large-scale study conducted by Nieves et al[9] did not have inflammatory data whether in the blood or airways. Here, we found that atopic asthmatics had greater eosinophilic inflammation but lower neutrophilic inflammation both in the systemic and the airway compartment. The relationship between allergic IgE-mediated reaction and the eosinophilic trait is one of the most accepted dogma in immunology.[29] Our finding showing prominent eosinophilic inflammation in atopic asthma does not come as a surprise. It should, however, be noticed that more than half of atopic asthmatics still exhibited sputum eosinophils below 3%, thereby qualifying as non eosinophilic

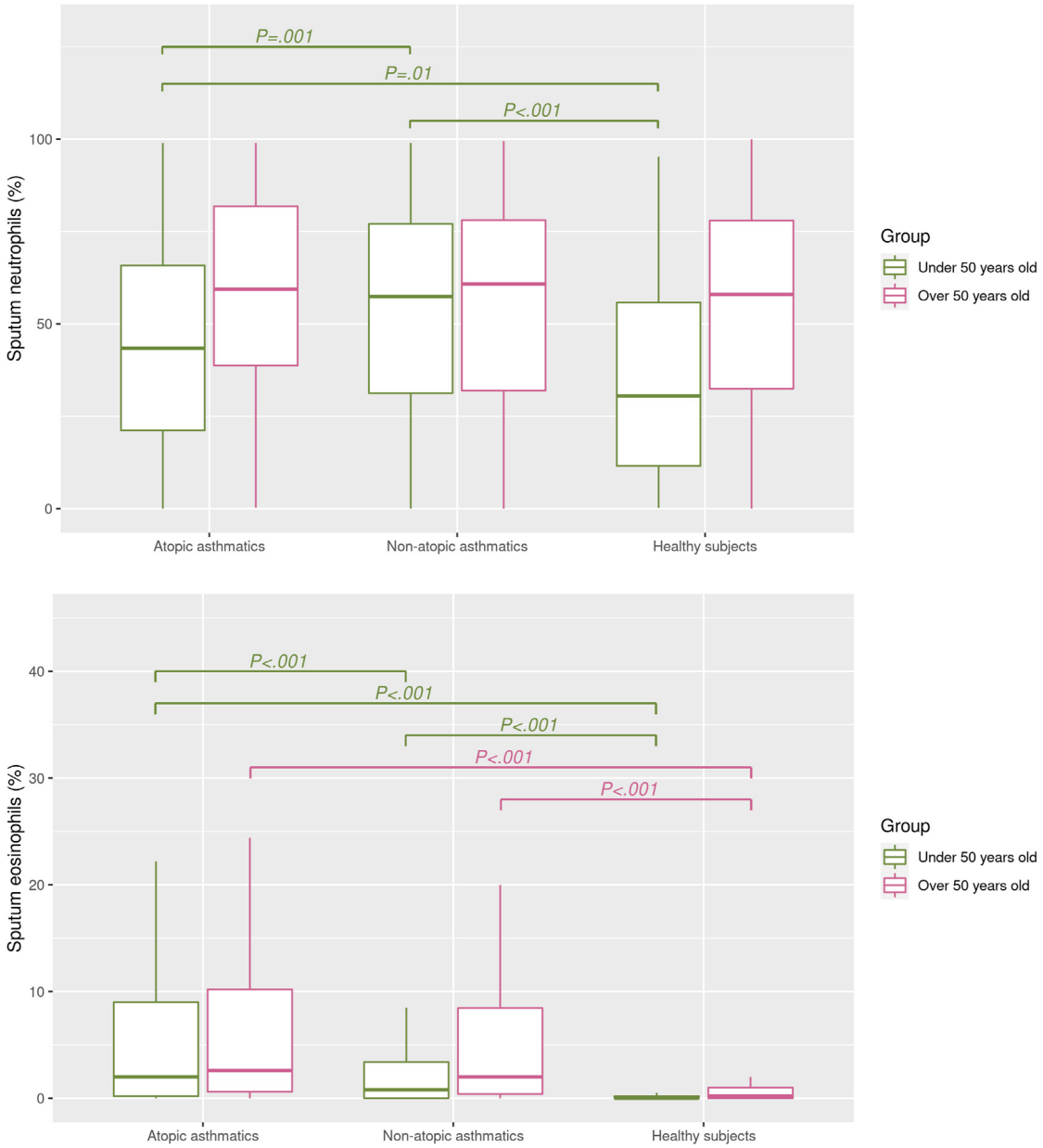

Fig. 4 Comparison of sputum neutrophils and eosinophils (%) in asthmatic patients and healthy subjects stratified by age.

asthma.[30] Of course, in some of them, low sputum eosinophilia is related to chronic treatment with ICS but we have also shown that the non eosinophilic trait is common in steroid naive asthmatics.[31] If non-atopic asthmatics had lower airway eosinophilia, it is still clearly higher than what we found in a large series of healthy subjects.[32] It has been demonstrated that atopic and non-atopic asthma may have common underlying inflammatory processes, including increased T helper type 2 (Th2) cells, mast cell activation and eosinophilic airway infiltration.[18,33] However, recent reviews have suggested a role of alarmins and ILC2 in mediating eosinophilic inflammation in non-atopic asthma.[34] In particular, recent data regarding anti-TSLP may be effective in improving asthma control in severe patients irrespective of the atopic status.[35,36]

Here, we showed that non-atopic asthmatics displayed greater number of neutrophils both in blood and in sputum. Green et al identified a group of asthmatics with high sputum neutrophilia and normal eosinophilia[37] who shared many of the

characteristics of our non-atopic group of patients, with higher severity, higher age, and late-onset of asthma. Similarly, neutrophils were found to be raised in the blood compartment in non-atopic asthmatics. This observation is in agreement with previous reports.[38,39] The reason why non-atopic asthma had greater neutrophilic inflammation may be linked to the older age and smoking history as previously reported.[30,40] Indeed, Tadao Nagasaki and Hisako Matsumoto discussed the potential effects of smoking and aging on healthy subjects and patients with asthma, particularly from the perspective of inflammatory changes. In this review, they showed that smokers/older patients with asthma may have altered baseline airway inflammation with increased neutrophilic inflammation compared with never-smokers or younger patients. Nevertheless, several studies have compared the induced sputum cell profile in children with atopic and non-atopic asthma[38,41] and found that the induced sputum cell pattern in non-atopic children was predominantly neutrophilic, while eosinophilia was the hallmark of airway inflammation in the majority of atopic patients. In addition, raised fibrinogen in non-atopic patients in the present study may also be linked to the age and would fit the concept of progressive rise in systemic inflammation with aging.[42]

As illustrated by age stratification analyses, eosinophilia in atopic asthmatic patients was not affected by the age. Atopic asthma was marked "Th2" throughout life. Remarkably, the eosinophilic trait in non-atopic patients was essentially observed in patients above the age of 50 and more pronounced in the airway compartment than the systemic compartment. In line with this finding was the clear increase in FeNO in non-atopic asthmatics after fifty years old. There was no change in circulating neutrophils according to the age in any group. This contrasts with clear increase in sputum neutrophils in atopic asthmatics and healthy subjects; similarity not found in non-atopic asthmatics. It suggests that activation of airway innate immunity occurs earlier in life in non-atopic asthmatics as opposed to atopic asthmatics and healthy subjects in whom raised sputum neutrophilia appears after the age of 50.

This study has certain limitations. First, its design was cross-sectional and monocentric with blood and sputum analyzed at only 1 time point. Second, some patients (62% in the atopic group and 53% in the non-atopic group) were on ICS treatment with a wide dosing range. ICS treatment can modify asthma endotype and sputum cellularity. Indeed, inhaled corticosteroids (ICS) have demonstrated their ability to control airway inflammation by reducing eosinophilic airway infiltration.[43] Previous studies showed that an initiation of ICS reduced sputum eosinophils.[44–46] Third, there was no extensive characterization of possible other aeroallergens than the common aeroallergens prevalent in our region. This could lead to incorrect classification of non-atopic asthmatic patients.

CONCLUSION

While sharing many demographic, lung function and inflammatory features, atopic and non-atopic patients showed clear differences with respect to the age of onset, smoking history and FeNO levels. They also displayed subtle differences in the profile of granulocytic inflammation according to the age, of which the most conspicuous is the change in the granulocytic profile in non-atopic patients above the age of 50 years.

Funding
This work was supported by the "Excellence of Science" programme (FNRS, Belgium).

Availability of data and materials
The data-sets analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions
SG and RL designed the study; SG, FS, VP, FG, MH and RL collected the data; SG analyzed the data; SG and RL interpreted the data; SG and RL drafted the manuscript; FS revised the manuscript critically for important intellectual content; all authors read and approved the final manuscript.

Ethics approval and consent to participate
The study was approved by the ethics committee of the University Hospital of Liege (Ref. 2016/276). For asthmatic patients, all procedures were performed in the context of clinical practice and the retrospective data collection was conducted with the approval from the above-mentioned ethics committee. Informed consents were obtained from healthy subjects.

Submission declaration

The authors confirm that this manuscript is original, has not been published before, is not currently being considered for publication elsewhere, and has not been posted to a preprint server.

Declaration of competing interest

SG, FG, VP, MH: no competing interests. FS reports grants from GSK, Astrazeneca, Teva, Chiesi and Novartis; consulting fees from GSK, Astrazeneca, Amgen, Chiesi and Novartis; lecture payments from GSK, Astrazeneca, Teva, Chiesi and Novartis, outside the submitted work. RL reports grants from GSK, AZ, Novartis, Chiesi and Teva; royalties from patent AU2016328384, CA2997506, EP 3337393, US2020345266; consulting fees and lecture payments from GSK, AZ, Novartis, Sanofi and Chiesi, outside the submitted work.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.waojou.2022.100655.

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Supplemental Appendix

Table S1 – Comparisons of FE_{NO} levels, blood and sputum eosinophils (%) and neutrophils (%) in asthmatic patients and healthy subjects stratified by age (with 50 years old as the threshold value).

Category	Parameter	Group 1	Group 2	*P-value*	Method
Under 50	FeNO	Healthy subjects	Non-atopic asthmatics	*P=.34*	Mann-Whitney U test
		Healthy subjects	Atopic asthmatics	*P=.002*	
		Non-atopic asthmatics	Atopic asthmatics	*P<.001*	
Over 50		Healthy subjects	Non-atopic asthmatics	*P=.47*	
		Healthy subjects	Atopic asthmatics	*P<.001*	
		Non-atopic asthmatics	Atopic asthmatics	*P<.001*	
Healthy subjects	FeNO	Under 50	Over 50	*P=.20*	Mann-Whitney U test
Non-atopic asthmatics				*P<.001*	
Atopic asthmatics				*P=.52*	
Under 50	Blood neutrophils (%)	Healthy subjects	Non-atopic asthmatics	*P=.30*	T-test
		Healthy subjects	Atopic asthmatics	*P=.72*	
		Non-atopic asthmatics	Atopic asthmatics	*P=.009*	
Over 50		Healthy subjects	Non-atopic asthmatics	*P=.29*	
		Healthy subjects	Atopic asthmatics	*P=.66*	
		Non-atopic asthmatics	Atopic asthmatics	*P=.43*	
Healthy subjects	Blood neutrophils (%)	Under 50	Over 50	*P=.78*	T-test
Non-atopic asthmatics				*P=.63*	
Atopic asthmatics				*P=.18*	
Under 50	Blood eosinophils (%)	Healthy subjects	Non-atopic asthmatics	*P=.38*	Mann-Whitney U test
		Healthy subjects	Atopic asthmatics	*P<.001*	
		Non-atopic asthmatics	Atopic asthmatics	*P<.001*	
Over 50		Healthy subjects	Non-atopic asthmatics	*P=.02*	
		Healthy subjects	Atopic asthmatics	*P<.001*	
		Non-atopic asthmatics	Atopic asthmatics	*P=.002*	
Healthy subjects	Blood eosinophils (%)	Under 50	Over 50	*P=.24*	Mann-Whitney U test
Non-atopic asthmatics				*P<.001*	
Atopic asthmatics				*P=.38*	
Under 50	Sputum neutrophils (%)	Healthy subjects	Non-atopic asthmatics	*P<.001*	Mann-Whitney U test
		Healthy subjects	Atopic asthmatics	*P=.01*	
		Non-atopic asthmatics	Atopic asthmatics	*P=.001*	
Over 50		Healthy subjects	Non-atopic asthmatics	*P=.78*	
		Healthy subjects	Atopic asthmatics	*P=.40*	
		Non-atopic asthmatics	Atopic asthmatics	*P=.21*	
Healthy subjects	Sputum neutrophils (%)	Under 50	Over 50	*P<.001*	Mann-Whitney U test
Non-atopic asthmatics				*P=.23*	
Atopic asthmatics				*P<.001*	
Under 50	Sputum eosinophils (%)	Healthy subjects	Non-atopic asthmatics	*P<.001*	Mann-Whitney U test
		Healthy subjects	Atopic asthmatics	*P<.001*	
		Non-atopic asthmatics	Atopic asthmatics	*P<.001*	
Over 50		Healthy subjects	Non-atopic asthmatics	*P<.001*	
		Healthy subjects	Atopic asthmatics	*P<.001*	
		Non-atopic asthmatics	Atopic asthmatics	*P=.07*	
Healthy subjects	Sputum eosinophils (%)	Under 50	Over 50	*P=.003*	Mann-Whitney U test
Non-atopic asthmatics				*P<.001*	
Atopic asthmatics				*P=.11*	