

DR LOUIS-PHILIPPE BOULET (Orcid ID : 0000-0003-3485-9393)

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Authors:

- Isabelle Xu, MD-MSc candidate
- Marie-Eve Boulay, MSc
- Mylène Bertrand, RT
- Andréanne Côté, MD FRCPC
- Louis-Philippe Boulet, MD FRCPC

From: Institut universitaire de cardiologie et de pneumologie de Québec-Université Laval

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Corresponding author:

Dr Louis-Philippe Boulet

Institut universitaire de cardiologie et de pneumologie de Québec -
Université Laval

2725, Chemin Sainte-Foy

Québec (Québec), Canada, G1V 4G5

Tel.: 418-656-4747 Fax: 418-656-4762

Email: lpboulet@med.ulaval.ca

Authors' contribution

MEB, and LPB designed and conceive the experiments. IX and MB performed data extraction. IX, MEB, AC and LPB analyzed the data. IX, MEB, MB, AC, and LPB wrote the paper and approved its final version.

Conflicts of Interest

IX, MEB, and MB have no conflict of interest to declare.

AC considers having no conflicts of interest related to this study, but wishes to declare what can be perceived as potential conflicts of interest. *Research grants for participation to multicentre studies:* AstraZeneca, Sanofi-Regeneron. *Support for research projects initiated by the investigator:* Glaxo SmithKline. *Fee for consulting and advisory boards:* AstraZeneca, Glaxo SmithKline, Sanofi-Regeneron.

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Data availability statement: The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

Key Messages

1. Eosinophilic airway inflammation can be observed in patients from all asthma severities.
2. Even patients with very mild asthma often show high induced sputum eosinophil counts.
3. Patients with eosinophilia show distinctive features, regardless of asthma severity, suggesting a more severe/active disease.

To the Editor,

Studies looking specifically at the prevalence of eosinophilic phenotypes in mild asthma are required. In patients with corticosteroid-naïve asthma, we reported that high eosinophil counts do not necessarily translate into clinical symptoms or lower lung function¹. However, in asthma of all severities, eosinophils are linked to worst outcomes². As such, early identification of mild/very mild asthma patients with significant eosinophilia could be a marker of increased risk of such events to modulate therapeutic intervention.

This study aimed to compare the features of eosinophilic and non-eosinophilic asthma in patients with asthma of various severities and compare the characteristics of patients according to the intensity of eosinophilia. We hypothesized that patients with an eosinophilic phenotype would demonstrate a more active form of the disease, regardless of its severity.

We conducted a retrospective, cross-sectional study based on data from subjects included in the Quebec Heart and Lung Institute-Laval University asthma databank between March 1999 and June 2017. All subjects enrolled for participation in research studies on asthma pathophysiology or treatment, or attending the asthma outpatient clinic of the Institute for assessment or follow-up of their disease are asked to participate to the databank. All those agreeing to contribute sign an informed consent form.

Data from subjects fulfilling the following criteria were included: 1) aged ≥ 18 years, 2) diagnosis of asthma confirmed by a pulmonologist, defined by current asthma symptoms and either: a) forced expiratory volume in one second (FEV_1) increased by at least 12% (and ≥ 200 mL) after administration of a bronchodilator, or b) a methacholine provocative concentration inducing a 20% fall in FEV_1 (PC_{20}) < 16 mg/mL³. Subjects also had to 3) have a valid sputum differential cell count result, 4) be considered to receive optimal treatment for their asthma³, and 5) have stable asthma and no change in asthma medication for at least 4 weeks before the visit. Patients having any respiratory disease apart from asthma were excluded. Earliest and most complete data registered were used for

subjects having multiple date entries. The study was approved by the research ethics committee of the Quebec Heart and Lung Institute-Laval University (CÉR-21488) and registered on Clinical trials (NCT03696914).

Asthma severity was determined according to the treatment needed to achieve asthma control, as per the Canadian Thoracic Society guidelines³ (See supplement). The presence or absence of eosinophilic inflammation was defined as 1) eosinophilic asthma (EA, also including those with mixed eosinophilic and neutrophilic asthma) for patients having $\geq 3\%$ sputum eosinophils and 2) non-eosinophilic asthma (NEA) for patients with $< 3\%$ sputum eosinophils⁴.

Details of data collection and analyses are given in the online supplement. Demographics and asthma-related data were extracted. Clinical asthma control was obtained using the Asthma Control Scoring System (ACSS) symptom score. Atopic status and proven past or present chronic rhinosinusitis with nasal polyposis (CRSwNP) were recorded and results from induced sputum analysis, fractional exhaled nitric oxide (FeNO), expiratory flows and methacholine responsiveness were extracted. Complete blood count was used to assess systemic eosinophil and neutrophil concentrations. Descriptive statistics were performed in the entire population, then repeated according to asthma severity within and between the EA and NEA groups.

We included 911 subjects : 372 in the EA group and 539 in the NEA group (Figure S1). Detailed comparisons of the subjects according to their inflammatory phenotype is presented in Table S1 of the online supplement. Baseline comparison of EA and NEA is presented in Table S2. Compared to NEA subjects, those with EA were older, had an older age at asthma diagnosis, included a higher proportion of subjects with severe asthma and CRSwNP, had worse clinical control score and lung function in addition to higher blood eosinophils and FeNO values. Remarkably, distribution of very mild, mild-to-moderate and severe asthma was significantly different between EA and NEA.

Comparisons of features between EA and NEA according to asthma severity are presented in Table S3. Subjects with very mild NEA represented the larger group. For all severities, EA showed worse ACSS clinical sub-score in comparison to their NEA counterpart. EA subjects also had distinct features from NEA specific to each asthma severity. Those with very mild EA had a higher prevalence of atopy, lower PC₂₀, in addition to lower lung function. In subjects with mild-to-moderate asthma, those with EA were older, had a lower BMI, were older at asthma diagnosis, and had a higher prevalence of CRSwNP. They also had a lower PC₂₀ methacholine and lower lung function. Subjects with severe EA had a lower BMI and sputum neutrophil percentages; they also represented a greater proportion of subjects with CRSwNP, but PC₂₀ methacholine and lung function were comparable between severe EA and NEA.

Comparisons according to different sputum eosinophilic cut offs (NEA (<3%), low eosinophils (≥3%-≤10%), intermediate eosinophils (>10%-≤25%), and high eosinophils (>25%)) are presented in Table S4. Eosinophil levels were low in 47% of subjects, intermediate in 29%, and high in 24%. When looking at the percentage of subjects according to asthma severity, a significant proportion of patients in each EA subcategory had corticosteroid-naïve asthma (Figure 1), although most patients with the highest eosinophil counts had severe asthma. Overall, patients with >25% eosinophils were significantly older, reported older age of asthma diagnosis, had a lower FEV₁/FVC ratio and higher FeNO values. Patients with low eosinophils had a higher prevalence of atopy and a higher ACSS clinical score (better control of asthma). FEV₁ and ACSS clinical score decreased with increasing sputum eosinophil cut offs, whereas blood eosinophils, FeNO and prevalence of CRSwNP increased with increasing sputum eosinophil cut offs (Figure 2).

Our results emphasize that patients with EA have a worse phenotype than those with NEA regardless of asthma severity. They also show that marked increases in sputum eosinophils is present across all asthma severities including very mild patients. Differences in characteristics of EA across all severities also suggest the possibility that all EA do not have the same level of eosinophil

activation, or other pathophysiological features, which could explain the difference in asthma severity associated with this type of inflammation.

We observed that subjects with EA had increased respiratory symptoms, lower FEV₁, more eosinophilic systemic inflammation, and increased FeNO levels, compared to subjects with NEA of similar asthma severity. These differences were observed in all types of asthma severity, reinforcing the concept that airway eosinophilia is a major prognostic factor and a risk for chronicity of the disease. Interestingly, subjects with very mild EA showed the highest prevalence of atopy whereas subjects with severe EA had the highest prevalence of CRSwNP suggesting that different pathophysiological mechanisms may be involved according to asthma severity.

A key finding of the present analysis is that more than one-third of all subjects with EA had very mild asthma and nearly half of very mild asthma patients had EA. In addition, when stratifying subjects based on degrees of airway eosinophilia and looking at those with very high sputum eosinophils count, we observed that a considerable percentage of EA patients with corticosteroid-naïve asthma had sputum eosinophils >10%. Hence, although marked eosinophilia has been associated with the presence of severe asthma and although a sub-phenotype of severely eosinophilic asthma has been described⁵, our study shows that a sub-phenotype of less severe asthma with increased sputum eosinophils exists as well. In keeping with the literature, as much as 30% of our patients with eosinophil counts >10% were corticosteroid-naïve⁶. The outcome of this very specific population has yet to be investigated but concerns must be raised.

This study is unique by its sample size and its inclusion of asthmatic patients of all severities, particularly mild/very mild asthma. However, it also had some limitations. First, little information on patient adherence to treatment was available. Second, the inflammatory phenotype was determined by only one induced sputum measurement, as phenotype stability was not considered. Previous studies showed that the eosinophilic phenotype seems to be quite stable over a short-term period across all asthma severities, but that the non-eosinophilic one may be less stable^{7,8}. Third, a

high proportion of subjects had missing data about asthma exacerbations. A pre-specified subgroup analysis of the Novel START study showed that 65% of severe exacerbations occurring in the salbutamol-only group were in patients with blood eosinophils $\geq 300 \times 10^6/\text{mL}^9$. Long-term studies should be done in order to assess phenotype stability and determine the impact of persistent airway eosinophilia on asthma exacerbations and lung function decline. Finally, data analyzed came from previous years and covered an extended period of time. Hence, optimal asthma management can have varied slightly. However, we believe that the impact of this was mainly on the most severe asthmatic patients, therefore minimally affecting the main conclusions of our study.

This study provides data on the heterogeneity of eosinophilic asthma, particularly in relation to asthma severity. Identification of phenotype in mild/very mild asthma could be useful although more studies are needed. Recognizing that this study shows existence of both EA and NEA in this population, optimised and personalized management of patients with asthma according to their phenotypes regardless of asthma severity requires urgent considerations.

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Figure legends

Figure 1. Percentage of subjects according to asthma severity among the different eosinophil cut-off categories.

Figure 2. Comparative variables according to sputum eosinophil cut-offs.

Figure 1. Percentage of subjects according to asthma severity among the different eosinophil cut-off categories

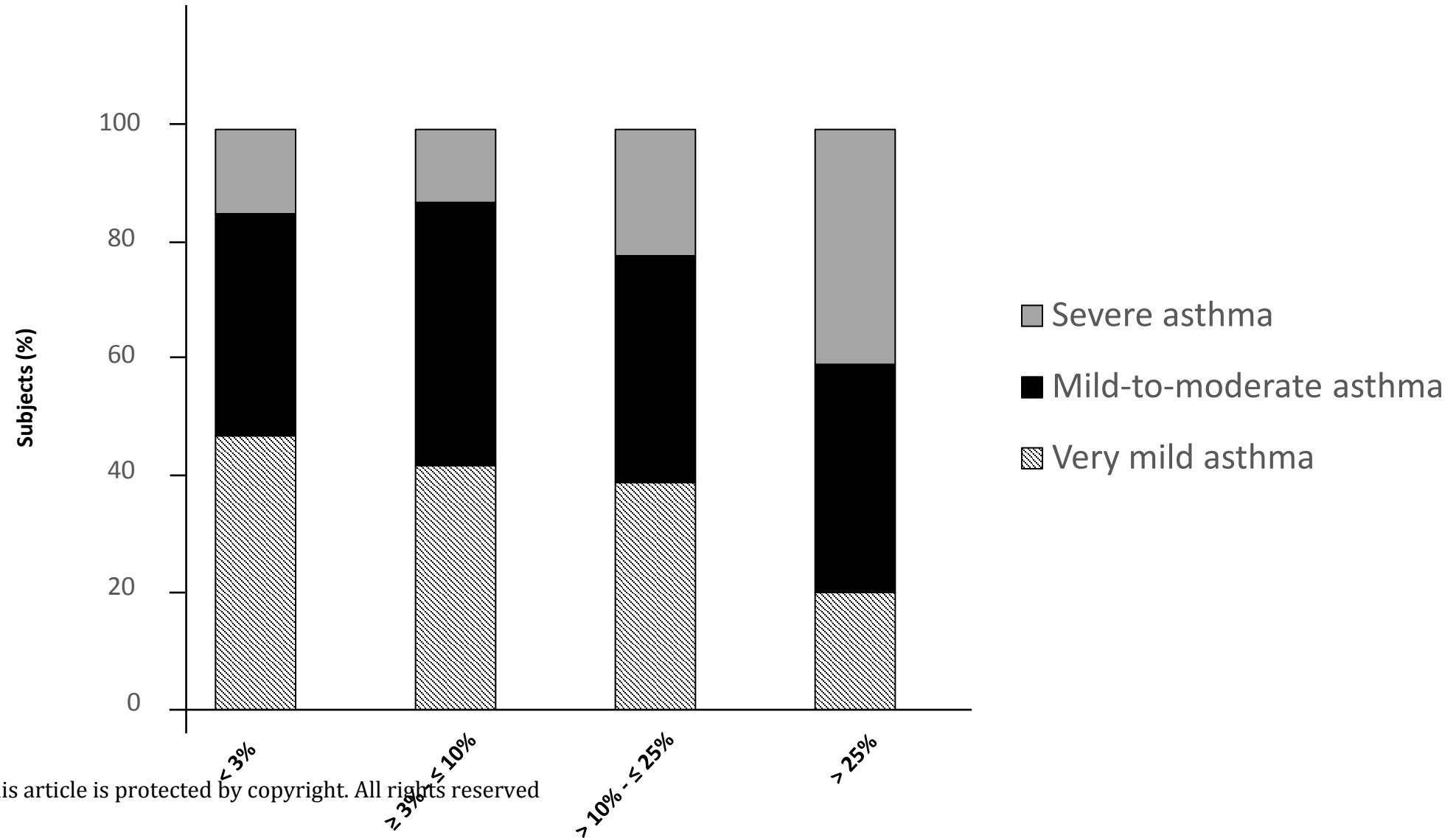
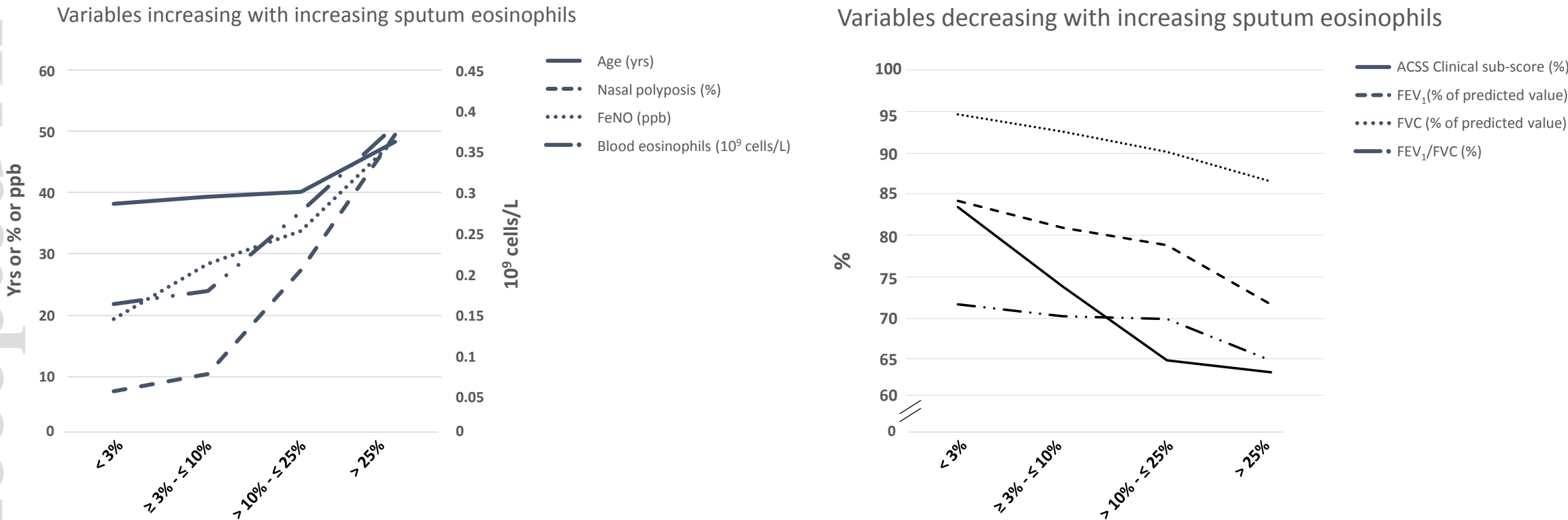


Figure 2. Comparative variables according to sputum eosinophil cut-offs



ONLINE SUPPLEMENT

Comparative features of eosinophilic and non-eosinophilic asthma.

Xu I, Boulay ME, Bertrand M, Côté A, Boulet LP.

METHODS

Definition

Asthma severity was determined according to the treatment needed to achieve asthma control, as per the Canadian Thoracic Society guidelines²³: asthma was considered as very mild (steroid-naïve) if the patient was considered by the treating physician not to require ICS on a regular basis and used only a bronchodilator as needed; mild if the patient used low-dose ICS maintenance (≤ 250 mcg/day beclomethasone dipropionate (BDP) or equivalent); moderate if the patient used medium-dose ICS maintenance (> 250 and ≤ 500 mcg/day BDP or equivalent) alone or low-to-moderate dose of ICS in combination with additional therapy (long-acting beta-agonist (LABA) or leukotriene receptor antagonist (LTRA)) and severe if the patient used high-dose ICS maintenance (> 500 mcg/day BDP or equivalent) and additional pharmacotherapy (LABA, LTRA, and/or oral corticosteroids).

Data source

Data entered in the Quebec Heart and Lung Institute-Laval University asthma databank are collected from the subjects' research file and/or medical record and are updated every time they come for a research study screening visit or a medical follow-up.

Age, gender, body mass index (BMI), asthma duration, smoking history, asthma severity and asthma medication were extracted. Clinical asthma control was obtained using the Asthma Control Scoring System (ACSS) symptom score^{25,26}. This sub-score of the ACSS was determined according to the symptoms in the week prior to the visit (diurnal and nocturnal symptoms, rescue β_2 -agonist use and physical activity) reported by the patient.

A higher score means better control (100% = optimal control; 20% = really poor control). Atopic status, as assessed by skin prick tests and a recent or past chronic rhinosinusitis with nasal polyposis (CRSwNP) diagnosis, confirmed by nasal endoscopy or CT scan, were recorded.

FEV₁, forced vital capacity (FVC), and FEV₁/FVC ratio were measured according to the American Thoracic Society (ATS) criteria and predicted values were obtained from the European Respiratory Society Global Lung Function Initiative (GLI-2012)^{27,28}. Airway responsiveness reported as the PC₂₀, was assessed according to the method described by Juniper et al²⁹.

Differential cell count, expressed as cell percentages, was obtained following sputum induction by inhalation of hypertonic saline and processing as described by Pin *et al.*³⁰ and modified by Pizzichini *et al.*³¹. Sputum inflammatory phenotypes were defined as follows: eosinophilic (eosinophils $\geq 3\%$, neutrophils $< 64.4\%$), neutrophilic (eosinophils $< 3\%$, neutrophils $\geq 64.4\%$), mixed (eosinophils $\geq 3\%$, neutrophils $\geq 64.4\%$), paucigranulocytic (eosinophils $< 3\%$, neutrophils $< 64.4\%$)^{32,33}. Fractional exhaled nitric oxide (FeNO), performed following ATS recommendations using a NiOX Mino handheld analyzer³⁴ was also included in analysis.

Statistical analyses

Descriptive statistics were performed in the entire population then repeated in the EA and NEA groups. All descriptive continuous and dichotomic variables with non-missing data are reported as mean $\pm$ SD or as frequencies respectively. Comparative analysis between EA and NEA in the entire population were performed and then repeated according to asthma severity. Additional comparisons were done within the asthma severity considering EA and NEA as distinct populations. The comparisons were done

using one-way ANOVA and Fisher's exact test if the normality was met or not respectively. Finally, the eosinophilic population was also stratified according to eosinophilic level (NEA (<3%), low eosinophils ($\geq 3\%$ - $\leq 10\%$), intermediate eosinophils ($>10\%$ - $\leq 25\%$), and high eosinophils ($>25\%$)) and descriptive and comparison analyses between each category using the same statistical methods were performed. To maximize our sample size, multiple imputations (MI), in which 50 imputed datasets of plausible values for missing data were created, was performed with the assumption that data were missing at random. A multivariate imputation by a fully conditional specification (FCS) regression method using the predictive mean matching technique (pmm) that assumes the existence of a joint distribution for all continuous variables and a discriminant function for categorical measures was performed. This statistical approach ensures that imputed values are plausible. For all imputed variables, the graphical representation of the values used in the imputations showed no trend. Comparisons among groups were performed using generalized linear models according to the data distribution for each imputed data. Results of the analyses of imputation were combined according to Rubin's rule. All descriptive statistics for imputed data were expressed using the 95% confidence interval. All the statistical analyses were performed using SAS version 9.4 (SAS Institute Inc., Cary, NC) and R.

RESULTS

Comparisons within EA and NEA

In the EA phenotype, similar PC₂₀ methacholine and FeNO values were observed regardless of asthma severity. Subjects with very mild asthma were younger and younger at asthma diagnosis, had shorter disease duration, and included a lower proportion of ex-smokers, and a higher proportion of atopic patients compared to subjects of other asthma

severities. In addition, in this phenotype, subjects with severe asthma showed lower ACSS clinical sub-score, higher blood neutrophils and higher sputum eosinophils than subjects with steroid naïve and mild-to-moderate asthma. Lastly, with increasing severity, there was an increase in BMI, in the proportion of subjects with CRSwNP and in blood eosinophils, and a decrease in expiratory flows.

In the NEA phenotype, the PC₂₀ methacholine was also similar in all asthma severities. However, the FeNO values were significantly lower in subjects with mild-to-moderate asthma, probably due to the effect of ICS used, compared with very mild asthma. Nevertheless, as these last were all below the clinical cut off of eosinophilic inflammation for FeNO¹⁴, this was not considered clinically significant. Similarly to EA, subjects with steroid naïve asthma were younger, had a lower age of asthma diagnosis, shorter disease duration and a greater proportion of atopic patients compared to the other severities. The smoking history was only significantly different between very mild and severe asthmatics (more past smokers in severe asthma). In the NEA phenotype, only ACSS clinical sub-score showed a lower value in patients with severe asthma, no difference being observed between patients of different asthma severities for blood neutrophils and sputum eosinophils. Finally, with increasing severity, there was also in this subgroup, an increase in BMI and a decrease in expiratory flows.

ADDITIONAL COMMENTS

Previous studies looked at a mixture of mild to very mild asthma and airway inflammation was not often quantified. We previously showed that titrating ICS according to induced sputum eosinophilia could markedly reduce exacerbations in moderate-to-severe asthma but the lack of benefit in mild asthma could be due to poor observance to ICS in this population, as stressed by the meta-analysis of Barnes et al^{14,15}.

Fifty-three percent of subjects with eosinophilic asthma had more than 10% eosinophils and 24% had more than 25% eosinophils, an arbitrary cut-off but providing a categorisation allowing assessment of characteristics of these populations. Subjects with high eosinophil levels were older, reported having developed asthma at an older age and showed a more severe phenotype, characterised by increased symptoms and airway obstruction compared to those with lower sputum eosinophil percentages.

The results from this study reveal some important information that could contribute to improve the current clinical approach in asthma management and support the early use of ICS for improvement of asthma control^{16,17}. It is well recognized that there is a subgroup of mild asthma patients with a worst outcome^{18,19}. Indeed, a significant proportion of asthma-related mortality and/or severe asthma episodes are found in mild asthma¹⁸. Although a recent publication suggests no increased risk of exacerbation with increasing blood eosinophil counts²⁰, opposite results have been reported²¹. The present study emphasizes the need to explore the significance and long-term impact of high sputum eosinophils, particularly in very mild asthma, and especially the effects of “as needed” therapy.

Table S1. Characteristics of the participants according to the inflammatory phenotype.

	Eosinophilic		Non-Eosinophilic	
	Eosinophilic	Mix	Neutrophilic	Paucigranulocytic
<i>n</i>	326	44	115	423
Females	187 [57]	22 [50]	64 [56]	245 [58]
Age (years) <i>Mean ± SD [min-max]</i>	41.1 ± 16.3 [18-80] ^a	46.8 ± 16.3 [21-73] ^{ab}	45.5 ± 17.7 [18-78] ^b	36.2 ± 15.0 [18-80] ^c
BMI (kg/m ²)	26.7 (26.0-27.3)	26.2 (24.8-27.6)	27.6 (26.5-28.7)	27.2 (26.6-27.9)
Smoking status (Smokers/Ex smokers/Non Smokers)	25/104/197 [7.8/31.9/60.4] ^{ab}	0/9/35 [0/20.5/79.6] ^b	4/44/67 [3.5/38.3/8.3] ^a	52/100/271 [12.3/23.6/64.1] ^{ab}
Duration of asthma (yr)	10.2 (8.8-11.9) ^{ab}	10.3 (6.6-15.7) ^{ab}	12.9 (10.3-16.1) ^a	9.3 (8.1-10.6) ^b
Age at asthma onset (yr)	24.1 (22.1-26.0) ^a	28.3 (22.5-34.0) ^a	25.8 (22.0-29.6) ^a	20.7 (19.0-22.4) ^b
Asthma severity	125/129/72 [38.3/39.6/22.1] ^a	9/27/8 [20.5/61.4/18.2] ^b	34/55/26 [29.6/47.8/22.6] ^a	220/151/52 [52.0/35.7/12.3] ^c

(steroid naïve/mild-to-moderate/severe)				
ICS eq. BDP (mcg/day)	734 (618-871)	812 (494-1333)	812 (645-1020)	665 (578-762)
Atopy	83.6 (79.5-87.6)	88.0 (78.2-97.8)	80.7 (73.4-87.9)	85.2 (81.8-88.6)
Number of positive allergen on prick test	4.0 (3.4-4.5) ^{ab}	4.1 (2.7-5.8) ^{ab}	3.2 (2.5-4.1) ^a	4.2 (3.7-4.8) ^b
Diagnosis of nasal polyposis	79 [24.2] ^a	12 [27.3] ^a	19 [16.5] ^a	22 [5.2] ^b
ACSS clinical sub score (%)	68.5 (65.8-71.2) ^a	68.4 (60.5-76.4) ^a	81.2 (78.2-84.1) ^b	83.8 (82.1-85.4) ^b
FEV ₁ (% of predicted value)	80.0 (77.9-82.1) ^a	73.5 (67.7-79.4) ^b	80.7 (77.2-84.2) ^a	86.3 (84.6-88.1) ^c
FVC (% of predicted value)	94.0 (92.1-95.9) ^a	91.4 (86.2-96.4) ^a	95.5 (92.7-98.4) ^{ab}	98.5 (97.0-100.1) ^b
FEV ₁ /FVC (%)	69.5 (68.2-70.7) ^a	64.4 (60.9-67.8) ^b	68.0 (65.9-70.1) ^{ab}	72.7 (71.7-73.6) ^c
PC ₂₀ (mg/mL)	1.3 (1.0-1.6) ^a	1.4 (0.8-2.5) ^{ab}	2.3 (1.7-3.2) ^b	2.0 (1.7-2.3) ^b
Blood neutrophils (10 ⁹ cells/L)	3.978 (3.799-4.163)	4.402 (3.843-5.026)	4.081 (3.781-4.401)	3.872 (3.707-4.043)
Blood eosinophils (10 ⁹ cells/L)	0.250 (0.217-0.287) ^a	0.214 (0.148-0.309) ^{ab}	0.193 (0.154-0.240) ^{ab}	0.156 (0.138-0.177) ^b
FeNO (ppb)	34.2 (28.2-41.4) ^a	32.4 (22.9-45.9) ^a	18.5 (14.3-24.0) ^b	19.5 (16.9-22.6) ^b
Sputum total cell count (10 ⁶ cells/g)	4.1 (3.6-4.5) ^a	9.0 (6.9-11.8) ^b	11.0 (9.2-13.2) ^b	3.0 (2.8-3.2) ^c

Sputum neutrophils (%)	28.4 (27.8-29.1) ^a	74.0 (72.4-75.7) ^c	79.4 (77.6-81.1) ^b	29.3 (27.6-30.9) ^a
Sputum eosinophils (%)	20.1 (17.9-22.3) ^a	8.7 (7.2-10.1) ^c	0.9 (0.8-1.0) ^b	0.9 (0.8-1.0) ^b

Data are presented as means (95% confidence interval) for continuous variables and as numbers [%] for categorical variables, unless stated otherwise.

ACSS: Asthma Control Scoring System, BMI: Body mass index, FeNO: Fractional exhaled nitric oxide, FEV₁: Forced expiratory volume in one second, FVC: Forced vital capacity, ICS eq. BDP: Inhaled corticosteroids beclometasone dipropionate equivalent dosage, LABA: Long-acting beta-agonist, PC₂₀: Provocative concentration of methacholine inducing a 20% fall in FEV₁.

Table S2. Baseline characteristics of the participants

	Eosinophilic	Non-Eosinophilic	<i>P</i> value
<i>N</i>	372	539	
Female	211 [57]	309 [57]	0.8917
Age (years) <i>Mean ± SD [min-max]</i>	41.8 ± 16.4 [18-80]	38.2 ± 16.1 [18-80]	0.001
BMI (kg/m ²)	26.6 (26.0-27.3)	27.3 (26.8-27.9)	0.1261
Smoking status (Smokers/Ex smokers/Non Smokers)	25/114/233/ [6.7/30.6/62.6]	56/144/339 [10.4/26.7/62.3]	0.1057
Duration of asthma (yr)	10.3 (9.0-11.8)	10.0 (8.8-11.2)	0.7243
Age at asthma diagnosis (yr)	24.5 (22.7-26.4)	21.8 (20.2-23.4)	0.03
Asthma severity (very mild/mild-to-moderate/severe)	134/156/82 [36.0/41.9/22.0]	254/207/78 [47.1/38.4/14.5]	0.001
ICS eq. BDP (mcg/day)	734 (635-849)	664 (590-747)	0.2470
LABA (yes)	342 [91.9]	514 [95.4]	0.03
Leucotriene antagonists (yes)	64 [17.2]	69 [12.8]	0.07
Oral corticosteroids (prednisone) (yes)	23 [6.2]	10 [1.9]	0.0009
Number of controllers	1.2 (1.1-1.2)	0.9 (0.8-1.0)	0.004
Atopy	83.9 (80.1-87.7)	84.2 (81.2-87.3)	0.8910

Diagnosis of nasal polyposis	92 [24.7]	41 [7.6]	< 0.0001
ACSS clinical sub score (%)	68.4 (67.3-70.4)	83.2 (81.8-84.7)	< 0.0001
FEV ₁ (% of predicted value)	79.1 (77.1-81.1)	85.1 (83.6-86.7)	< 0.0001
FVC (% of predicted value)	93.5 (91.8-95.3)	97.9 (96.5-99.3)	0.0003
FEV ₁ /FVC (%)	68.8 (67.6-70.0)	71.7 (70.7-72.6)	0.0004
PC ₂₀ (mg/mL)	1.3 (1.1-1.6)	2.1 (1.8-2.4)	0.0004
Blood neutrophils (10 ⁹ cells/L)	4.027 (3.854-4.206)	3.912 (3.768-4.062)	0.3280
Blood eosinophils (10 ⁹ cells/L)	0.245 (0.215-0.280)	0.163 (0.146-0.183)	< 0.0001
FeNO (ppb)	33.9 (28.3-40.6)	19.3 (16.8-22.2)	0.0001
Sputum total cell count (10 ⁶ cells/g)	4.5 (4.0-5.0)	4.1 (3.7-4.4)	0.1941
Sputum neutrophils (%)	33.9 (31.7-36.1)	39.9 (37.8-42.1)	0.0001
Sputum eosinophils (%)	18.6 (16.6-20.6)	0.9 (0.8-1.0)	< 0.0001

Data are presented as means (95% confidence interval) for continuous variables and as numbers [%] for categorical variables, unless stated otherwise.

ACSS: Asthma Control Scoring System, BMI: Body mass index, FeNO: Fractional exhaled nitric oxide, FEV₁: Forced expiratory volume in one second, FVC: Forced vital capacity, ICS eq. BDP: Inhaled corticosteroids beclometasone dipropionate equivalent dosage, LABA: Long-acting beta-agonist, PC₂₀: Provocative concentration of methacholine inducing a 20% fall in FEV₁.

Table S3. Characteristics of participants in the eosinophilic and non-eosinophilic group according to asthma severity

	Eosinophilic			Non-Eosinophilic		
	Corticosteroid-naïve	Mild-to-moderate	Severe	Corticosteroid-naïve	Mild-to-moderate	Severe
<i>N</i>	134	156	82	254	207	78
Females	79 [59]	89 [57]	43 [52]	158 [62]	111 [54]	40 [51]
Age (years) <i>Mean ± SD [min-max]</i>	28.9 ± 9.5 [18-64] ^a	48.5 ± 15.0 [18-74] ^b	49.9 ± 15.0 [18-80] ^b	30.2 ± 12.0 [18-77] ^a	43.9 ± 16.1 [18-79] ^c	48.8 ± 14.8 [18-80] ^{bc}
BMI (kg/m ²)	25.1 (24.2-26.0) ^a	26.7 (25.8-27.5) ^b	29.2 (27.7-30.7) ^c	25.6 (25.0-26.3) ^{ab}	28.0 (27.1-28.8) ^c	31.1 (29.1-33.0) ^d
Smoking status (Smokers/Ex smokers/Non Smokers)	14/22/98 [10.5/16.4/73.1] ^a	5/58/93 [3.2/37.2/59.6] ^{bc}	6/34/42 [7.3/41.5/51.2] ^c	41/35/178 [16.1/13.8/70.1] ^{ab}	10/73/124 [4.8/35.3/59.9] ^{bc}	5/36/37 [6.4/46.2/47.4] ^c
Duration of asthma (yr)	7.8 (6.1-9.8) ^a	11.3 (9.1-14.0) ^b	13.5 (10.5-17.4) ^b	7.9 (6.5-9.5) ^a	12.0 (10.1-14.4) ^b	12.8 (9.7-16.7) ^b
Age at asthma diagnosis (yr)	16.7 (14.5-18.8) ^a	28.8 (25.8-31.9) ^b	29.2 (25.0-33.5) ^b	17.4 (15.5-19.3) ^a	24.6 (21.9-27.3) ^c	28.7 (23.9-33.5) ^{bc}

Atopy	96.9 (93.9-99.9) ^a	78.6 (72.1-85.1) ^b	72.8 (63.1-82.5) ^b	90.1 (86.5-93.8) ^c	81.4 (76.1-86.8) ^b	72.6 (62.6-82.6) ^b
Diagnosis of CRSwNP	7 [7.2] ^{ad}	44 [28.9] ^b	40 [48.8] ^c	8 [3.2] ^a	20 [9.7] ^{ad}	13 [16.7] ^{bd}
ACSS clinical sub score (%)	70.4 (66.4-74.4) ^a	72.4 (68.7-76.1) ^{ad}	57.7 (52.6-62.9) ^b	85.3 (83.3-87.4) ^c	82.9 (80.7-85.1) ^c	77.3 (73.0-81.6) ^d
FEV ₁ (% of predicted value)	87.0 (84.0-90.0) ^a	77.5 (74.7-80.4) ^b	69.3 (64.9-73.7) ^c	91.2 (89.1-93.3) ^d	82.0 (79.6-84.4) ^e	73.8 (69.5-78.1) ^{bc}
FVC (% of predicted value)	98.6 (95.9-101.3) ^a	93.2 (90.7-95.7) ^b	85.9 (81.8-90.0) ^c	102.4 (100.5-104.3) ^d	96.0 (93.2-98.1) ^{ab}	88.4 (84.6-92.2) ^c
FEV ₁ /FVC (%)	74.6 (72.8-76.4) ^a	66.6 (64.9-68.4) ^b	63.4 (60.9-66.0) ^c	75.1 (73.9-76.3) ^a	69.4 (67.9-70.9) ^d	66.4 (64.0-68.8) ^{bc}
PC ₂₀ (mg/mL)	1.1 (1.0-1.4) ^a	1.3 (0.9-1.7) ^a	1.8 (1.1-2.8) ^{ab}	2.1 (1.8 (2.5) ^b	1.8 (1.4-2.3) ^b	2.7 (1.7-4.4) ^b
Blood neutrophils (10 ⁹ cells/L)	3.774 (3.513-4.049) ^a	3.978 (3.725-4.244) ^a	4.574 (4.156-5.026) ^b	3.762 (3.549-3.985) ^a	4.006 (3.778-4.245) ^a	4.169 (3.764-4.608) ^{ab}
Blood eosinophils (10 ⁹ cells/L)	0.208 (0.164-0.262) ^a	0.256 (0.215-0.306) ^{ab}	0.295 (0.221-0.392) ^b	0.157 (0.133-0.184) ^c	0.169 (1.42-0.200) ^{cd}	0.173 (0.127-0.234) ^{cd}
FeNO (ppb)	37.9 (35.6-40.2) ^a	29.7 (27.5-32.0) ^{ab}	36.6 (34.3-39.0) ^a	22.6 (20.4-24.8) ^{bd}	16.5 (14.4-18.6) ^{ce}	17.5 (15.2-19.8) ^{de}
Sputum total cell count (10 ⁶ cells/g)	2.7 (2.3-3.2) ^a	5.0 (4.2-5.8) ^b	8.2 (6.4-10.4) ^c	3.3 (3.0-3.7) ^a	5.0 (4.3-5.7) ^b	6.7 (5.2-8.5) ^{bc}

Sputum neutrophils (%)	28.1 (24.6-31.5) ^a	38.9 (35.5-42.4) ^b	33.8 (29.4-38.2) ^{ab}	34.2 (31.3-37.1) ^{ab}	43.2 (39.5-46.8) ^{bc}	50.2 (44.2-56.2) ^c
Sputum eosinophils (%)	14.2 (11.6-16.8) ^a	17.8 (14.9-20.7) ^a	27.5 (22.5-32.5) ^b	0.9 (0.8-1.0) ^c	0.8 (0.7-0.9) ^c	0.8 (0.6-1.0) ^c

Data are presented as means (95% confidence interval) for continuous variables and as numbers [%] for categorical variables, unless stated otherwise.

^{a,b,c,d,e}Superscript letters indicate statistical significance between groups $P < .05$.

ACSS: Asthma Control Scoring System, BMI: Body mass index, CRSwNP: chronic rhinosinusitis with nasal polypsis, FeNO: Fractional exhaled nitric oxide, FEV₁: Forced expiratory volume in one second, FVC: Forced vital capacity, LABA: Long-acting beta-agonist, PC₂₀: Provocative concentration of methacholine inducing a 20% fall in FEV₁.

Table S4. Characteristics of the participants according to the degree of eosinophilic inflammation.

	< 3% eosinophils	≥ 3% - ≤10%	> 10% - ≤25%	>25% eosinophils
<i>N</i>	538	174	110	89
Females	309 [57]	102 [59]	66 [60]	43 [48]
Age (years) <i>Mean ± SD [min-max]</i>	38.2 ± 16.1 [18-80] ^a	39.3 ± 16.0 [18-74] ^a	40.2 ± 15.6 [18-72] ^a	48.3 ± 16.4 [18-80] ^b
BMI (kg/m ²)	27.3 (26.8-27.9)	26.9 (26.0-27.8)	26.4 (25.4-27.5)	26.4 (25.2-27.6)
Smoking status (Smokers/Ex smokers/Non Smokers)	56/144/338 [10.4/26.8/62.8]	13/47/114 [7.5/27.0/65.5]	8/35/67 [7.3/31.8/60.9]	4/32/53 [4.5/36.0/59.6]
Duration of asthma (yr)	10.0 (8.8-11.2)	10.4 (8.4-12.7)	10.2 (8.0-12.9)	10.3 (7.8-13.5)
Age at asthma diagnosis (yr)	21.8 (20.2-23.4) ^a	21.9 (19.1-24.6) ^a	23.9 (20.8-26.9) ^a	30.5 (26.5-34.6) ^b
Asthma severity (steroid naïve/mild-to-moderate/severe)	254/206/78 [47.2/38.3/14.5] ^a	73/79/22 [42.0/45.4/12.6] ^a	43/43/24 [39.1/39.1/21.8] ^a	18/35/36 [20.2/39.3/40.5] ^b
ICS eq. BDP (mcg/day)	734 (618-871) ^a	544 (425-695) ^a	811 (594-1109) ^{ab}	991 (806-1219) ^b
Atopy	84.2 (81.1-87.3) ^a	90.7 (86.3-95.0) ^b	83.1 (76.0-90.2) ^{abc}	71.9 (70.9-72.9) ^c

Diagnosis of CRSwNP	41 [7.6] ^a	18 [10.3] ^a	30 [27.3] ^b	44 [49.4] ^c
ACSS clinical sub score (%)	83.2 (81.7-84.7) ^a	73.6 (70.3-76.9) ^b	64.7 (59.9-69.6) ^c	63.2 (58.0-68.3) ^c
FEV ₁ (% of predicted value)	85.1 (83.6-86.7) ^a	81.9 (79.2-84.6) ^{ab}	79.8 (76.1-83.6) ^{bc}	72.7 (68.3-77.1) ^c
FVC (% of predicted value)	97.9 (96.5-99.3) ^a	95.7 (93.0-98.3) ^{ab}	93.3 (90.1-96.5) ^b	89.7 (86.2-93.2) ^c
FEV ₁ /FVC (%)	71.7 (70.7-72.6) ^a	70.2 (68.6-71.8) ^a	69.9 (67.7-72.1) ^a	64.8 (62.0-67.6) ^b
PC ₂₀ (mg/mL)	2.1 (1.8-2.4) ^a	1.3 (1.0-1.6) ^b	1.2 (0.9-1.7) ^b	1.5 (1.0-2.2) ^{ab}
Blood neutrophils (10 ⁹ cells/L)	3.916 (3.771-4.066)	3.903 (3.666-4.151)	4.131 (3.809-4.474)	4.123 (3.768-4.503)
Blood eosinophils (10 ⁹ cells/L)	0.164 (0.146-0.183) ^a	0.179 (0.147-0.218) ^a	0.277 (0.221-0.345) ^b	0.382 (0.298-0.490) ^c
FeNO (ppb)	19.3 (16.8-22.2) ^a	28.3 (22.6-35.4) ^b	33.7 (24.5-46.3) ^b	48.6 (35.0-67.5) ^c
Sputum total cell count (10 ⁶ cells/g)	4.1 (3.7-4.4) ^a	3.7 (3.2-4.2) ^a	4.1 (3.3-5.0) ^a	8.2 (6.4-10.5) ^b
Sputum neutrophils (%)	40.0 (39.7-40.2) ^a	38.8 (35.4-42.2) ^{ab}	33.9 (33.5-34.3) ^b	24.1 (23.8-24.4) ^c
Sputum eosinophils (%)	0.9 (0.8-1.0) ^a	5.5 (5.2-5.8) ^b	16.1 (15.3-16.9) ^c	47.3 (43.5-51.1) ^d

Data are presented as means (95% confidence interval) for continuous variables and as numbers [%] for categorical variables,

unless stated otherwise.

^{a,b,c,d,e}Superscript letters indicate statistical significance between groups $P < .05$.

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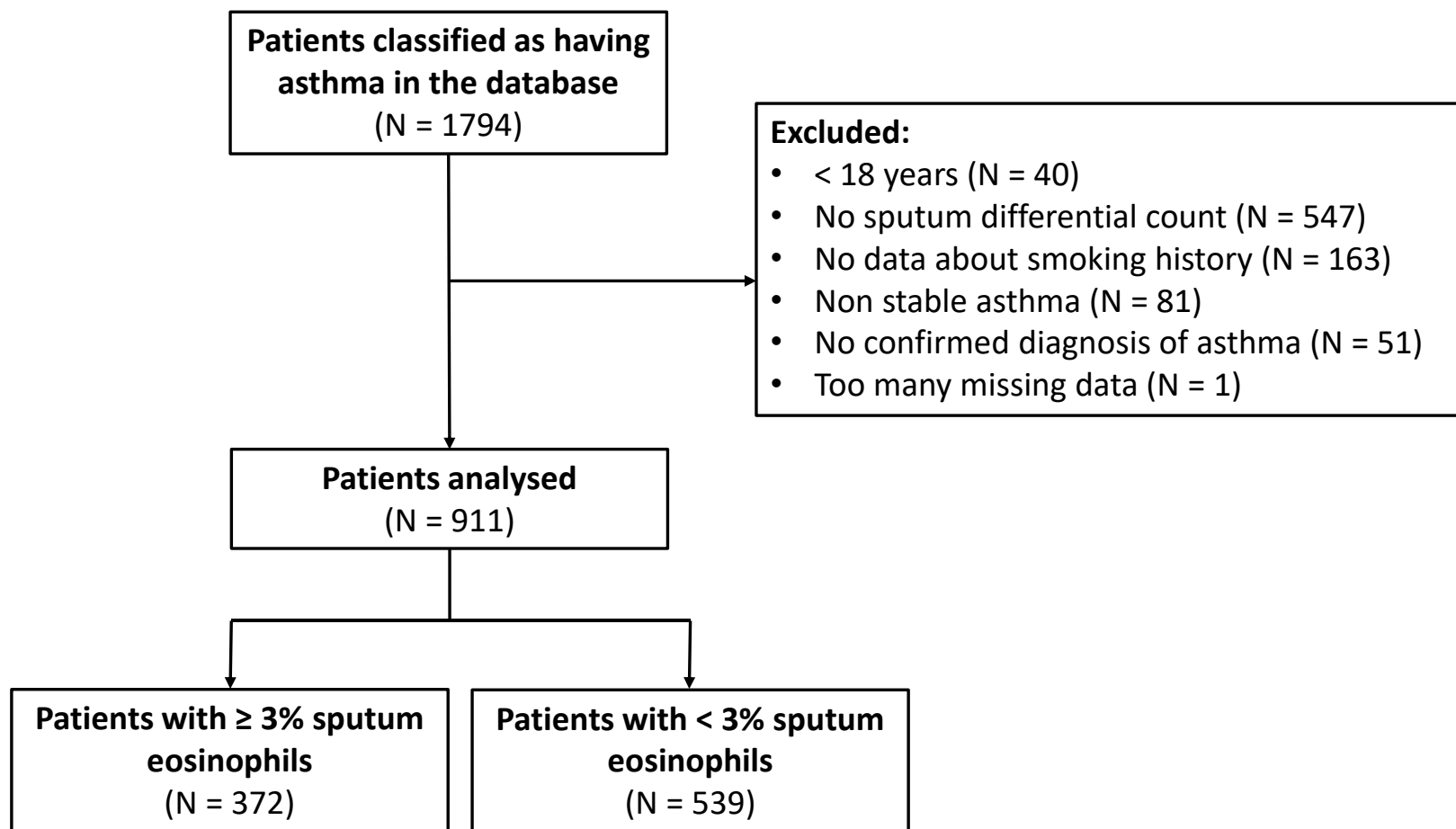


Figure S1. Flow of study participants