Original Article

Association Between Blood Eosinophils and Neutrophils With Clinical Features in Adult-Onset Asthma

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What is already known about this topic? It has been suggested that asthma patients with different endotypes have different risks for exacerbations. There is a lack of information differentiating various endotypes other than eosinophilic asthma.

What does this article add to our knowledge? The neutrophilic group had the highest body mass index and inhaled corticosteroid use and made unplanned respiratory visits. The neutrophilic, eosinophilic, and mixed granulocytic groups had more severe asthma. The neutrophilic and eosinophilic groups used antibiotics. The eosinophilic group had nasal polyps, suspected sinusitis, and greater FEV_1 decline.

How does this study impact current management guidelines? Information about patients' eosinophil and neutrophil counts may be useful when assessing the clinical status of patients with adult-onset asthma and planning treatment.

BACKGROUND: Asthma is a disease that can be separated into different phenotypes and endotypes based on the clinical characteristics and the molecular mechanisms of the condition, respectively.

OBJECTIVE: To assess the association between blood eosinophil and neutrophil counts with clinical and molecular features in patients with adult-onset asthma.

METHODS: Blood eosinophil and neutrophil counts were measured from 203 patients who took part in the Seinäjoki Adult Asthma Study and attended the 12-year follow-up visit. The patients were then divided into four groups (pauci-granulocytic [n = 108], neutrophilic [n = 60], eosinophilic [n = 21], and mixed granulocytic [n = 14]), according to eosinophil and neutrophil levels. The cutoff values used to define

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Abbreviations used
BMI- Body mass index
COPD- Chronic obstructive pulmonary disease
EGEA- Epidemiological Study on the Genetics and Environment of Asthma
ICS- Inhaled corticosteroid
MMP-9- Matrix metalloproteinase-9
Pre-BD- Pre-bronchodilator
T2- Type 2
URTI- Upper respiratory tract infection

the groups were $0.30 \times 10^9 \cdot L^{-1}$ for blood eosinophils and $4.4 \times 10^9 \cdot L^{-1}$ for blood neutrophils.
RESULTS: The neutrophilic group had highest body mass index. It was dispensed the highest doses of inhaled corticosteroids during the 12-year follow-up and made the most unplanned respiratory visits. The neutrophilic, eosinophilic, and mixed granulocytic groups had more severe asthma compared with the paucigranulocytic group. The neutrophilic and eosinophilic groups were associated with higher dispensed antibiotics. The eosinophilic group had more nasal polyps, more suspected sinusitis, a greater decline in lung function, and increased levels of periostin, FeNO, and IgE. The neutrophilic group had increased high-sensitivity C-reactive protein, matrix metalloproteinase-9, IL-6, leptin, and soluble urokinase plasminogen activator receptor levels. The mixed granulocytic group showed increased resistin levels together with the neutrophilic group.
CONCLUSIONS: In addition to blood eosinophils, the blood neutrophil count reflects underlying inflammatory patterns and indicates important differences in asthma clinical features and outcomes. **(J Allergy Clin Immunol Pract 2022;■:■-■)**

Key words: *Adult-onset; Antibiotic; Asthma; Eosinophil; Endotype; Exacerbation; Inflammatory marker; Neutrophil; Phenotype; Upper respiratory tract infection*

INTRODUCTION

Asthma is a chronic condition caused by inflammation of the lower respiratory tract. Asthma can be separated into different phenotypes and endotypes based on the clinical characteristics and molecular mechanisms underlying the condition. Asthma can develop at any age. The age of asthma onset is a crucial factor in separating different asthma phenotypes.[1] Childhood-onset asthma is often associated with allergies, a family history of asthma, and atopic conditions such as dermatitis and rhinitis.[1,2] Respiratory infections are a common provoker of wheezing for patients with childhood-onset asthma.[1] Adult-onset asthma is often associated with a history of smoking,[3] obesity,[4] and rhinitis.[5] Adult patients are more often women[2,6] and nonatopic.[6] In addition, adult-onset asthma is frequently severe.[6]

Asthma endotypes are complex, heterogeneous, and driven by different lineages of T helper cells (T_H1, T_H2, and T_H17). A way to distinguish them is based on the status of type 2 (T2) inflammation. Type 2-high asthma often develops in childhood and has a favorable response to corticosteroids.[7] Type 2-high asthma can develop during adulthood as well, such as in adult-onset eosinophilic asthma.[2] Blood eosinophils, serum periostin, and FeNO are markers of T2 inflammation used to identify patients with a T2-high endotype.[7,8] Originally, it was proposed that expression levels of IL-13–induced genes in airway epithelial cells could be used to divide asthmatic patients into T2-high and T2-low.[9] Skin prick tests and IgE measurements can be used to separate atopic and nonatopic T2-high asthmatic patients.[8] Type 2-low asthma is often characterized by low to normal levels of T2 markers, poor response to corticosteroids, and normal to high levels of neutrophils in sputum.[8] Blood neutrophils have less often been considered suitable markers of T2-low asthma.

It has been suggested that asthmatic patients with different endotypes have different risks for asthma exacerbations.[10,11] The Copenhagen General Population Study[10] assessed the association of high blood eosinophil and neutrophil counts with moderate and severe asthma exacerbations. The temporary use of prednisolone was regarded as a moderate asthma exacerbation and asthma-related hospitalizations as severe exacerbations of the disease. The study concluded that high blood eosinophil counts were associated with an increased risk for both moderate and severe exacerbations, whereas high blood neutrophil counts were associated only with moderate exacerbations. The Epidemiological Study on the Genetics and Environment of Asthma (EGEA)[11,12] suggested that some characteristics of asthma are differentially associated with blood eosinophil and neutrophil patterns. High blood neutrophil counts were associated with poor asthma control whereas high eosinophil levels were associated with higher bronchial hyperresponsiveness, poor lung function, and higher IgE levels.

To the best of our knowledge, no prior studies assessed the association between blood eosinophil and neutrophil levels with clinical features and inflammatory mediators in patients with adult-onset asthma, which is the aim of this exploratory analysis.

METHODS

Study design and patients

This research was the result of the Seinäjoki Adult Asthma Study, a follow-up study including 257 patients with the diagnosis of new-onset adult asthma during 1999 to 2002. The diagnosis was made by a respiratory physician based on characteristic symptoms of asthma and objective lung function measurements. Former and current smokers as well as patients with comorbidities were not excluded from the study (see Table E1 in this article's Online Repository at www.jaci-inpractice.org). A minimum age of 15 years was required and patients with a previous diagnosis of asthma were excluded. Medication was started at the time of the diagnosis according to the Finnish Asthma Programme.[13] Twelve-year follow-up visits were organized during 2012 to 2013. A total of 203 patients participated (79%). Lung function measurements, blood samples, and body mass index (BMI) measurements were taken. Moreover, patients filled out a structured questionnaire on background information, asthma control, and medication. At the follow-up visit, patients showed no signs of infection. The study was approved by the Ethics Committee of Tampere University Hospital, Tampere, Finland; all participants gave written informed consent. Specific details of the design of the Seinäjoki Adult Asthma Study can be found in a separate

publication.[14] A Consolidated Standards of Reporting Trials plot of the study can be found in Figure E1 (in this article's Online Repository at www.jaci-inpractice.org).

Inflammatory parameters

White blood cell levels were measured from venous blood. Cutoff values used to define the low- and high-level groups were $0.30 \times 10^9 \cdot L^{-1}$ for blood eosinophils and $4.4 \times 10^9 \cdot L^{-1}$ for blood neutrophils. This cutoff value for eosinophil levels was previously used to define the high eosinophil phenotype,[15,16] but no equivalent value is used in asthma studies to define the high neutrophil phenotype. We evaluated several possible cutoff points for neutrophils and found this particular value to be best to differentiate among outcomes. Measurement methods of the inflammatory parameters are described in the Supplemental Methods (in this article's Online Repository at www.jaci-inpractice.org). All inflammatory parameters were measured at the 12-year follow-up visit.

Lung function, asthma control, and comorbidities

We carried lung function measurements out according to international standards[17] (see the Supplemental Methods). The decline in lung function (ΔFEV_1) was calculated by comparing the maximal pre-bronchodilator (pre-BD) FEV_1 value measured 0 to 2.5 years after the diagnosis ($Max_{0\text{-}2.5}$) with the pre-BD FEV_1 value measured at the follow-up visit. We used the Airways Questionnaire 20[18] and Asthma Control Test[19] to determine asthma symptoms. Asthma control was rated based on the Global Initiative for Asthma 2010 report.[20] Severe asthma was defined according to the European Respiratory Society and American Thoracic Society 2014 guidelines.[21] Comorbidities were self-reported or based on self-reported medication. Comorbidities included in the study and the prevalence of these comorbidities can be found elsewhere.[22]

Use of medication, adherence, and use of health care services

Information on prescribed inhaled corticosteroids (ICS) is based on data collected from asthma-related health care visits. The ICS doses were converted into budesonide equivalents. Information on dispensed ICS, oral corticosteroids, short-acting β_2-agonists, and antibiotics were collected from the Finnish Social Insurance Institution. Adherence to ICS was defined as the percentage of dispensed ICS per prescribed daily ICS in micrograms over the 12-year follow-up period. Detailed information on how adherence to ICS was determined can be found elsewhere[23] and in the Supplemental Methods. The antibiotics considered in this study are listed in the Supplemental Methods. Information on asthma- and respiratory-related health care visits and hospitalizations were gathered from medical records. Unplanned respiratory visits include health care visits for upper respiratory tract infections (URTIs) and asthma exacerbations.

Data analyses

Categorical data are expressed as population sizes and percentages. Continuous data are expressed as medians and interquartile ranges or means and SDs. Comparisons between groups according to blood eosinophil and neutrophil levels were carried out using one-way ANOVA when analyzing normally distributed data, Kruskal-Wallis test adjusted by Benjamini-Hochberg method when analyzing nonnormally distributed data, or χ^2 test with a comparison of column proportions by z-test and adjusting P values by Benjamini-Hochberg method when comparing categorical variables. Sensitivity analysis was performed by excluding patients with chronic obstructive pulmonary disease (COPD) and by excluding patients with metabolic syndrome.

We performed negative binomial regression analyses using the number of unplanned respiratory visits and dispensed antibiotics during the 12-year follow-up as dependent variables. This regression model was chosen because both dependent variables had a skewed distribution that contained 0 values. Low- and high-level groups for blood eosinophils and neutrophils, age, sex, pre-BD FEV_1, BMI (BMI < 25, $25 \leq$ BMI > 30, and BMI ≥ 30), and pack-years (≤ 10 and >10) were used as covariates. The length of follow-up was used as an offset variable. Sensitivity analysis was performed by further adjusting for dispensed ICS during 2 years before the follow-up visit. Data are presented as the incidence rate ratio and 95% CI. We performed multiple linear regression analyses to determine the association of blood eosinophils and neutrophils with ΔFEV_1. Low- and high-level groups for blood eosinophils and neutrophils, age, sex, height, change in BMI ($Max_{0\text{-}2.5}$ to follow-up), and pack-years were used as covariates. Sensitivity analysis was performed by adjusting for dispensed ICS during the entire follow-up and by adjusting for the FEV_1 value at the time of diagnosis. Data are presented as the unstandardized β coefficient and 95% CI. Data analyses were performed using SPSS software (version 26, SPSS Statistics, IBM, Armonk, NY). Results with P less than .05 were regarded statistically significant.

RESULTS

Patient characteristics

Table I lists characteristics of patients at the 12-year follow-up visit. Mean age was 58 years and most patients were overweight. Women were more represented than were men. Over half of patients were previous or current smokers, with a median of 16 pack-years. Most patients had allergic or constant rhinitis. Moreover, 76% of patients reported daily use of ICS.

Groups according to blood eosinophil and neutrophil levels

Patients were divided into four groups according to blood granulocyte levels. Patients in the eosinophilic group had high eosinophil ($\geq 0.30 \times 10^9 \cdot L^{-1}$) but low neutrophil ($<4.4 \times 10^9 \cdot L^{-1}$) counts, whereas patients in the neutrophilic group had low eosinophil ($<0.30 \times 10^9 \cdot L^{-1}$) but high neutrophil levels ($\geq 4.4 \times 10^9 \cdot L^{-1}$). The mixed granulocytic group consisted of patients with both high eosinophil and neutrophil levels. The paucigranulocytic group consisted of patients whose eosinophil and neutrophil levels remained under the cutoff values. Table I lists the group sizes.

The neutrophilic group had the highest BMI compared with the paucigranulocytic and eosinophilic groups (Table I). The neutrophilic group was dispensed a higher cumulative dose of ICS during the 12-year follow-up compared with the paucigranulocytic group (Figure 1, *A*). The neutrophilic group had higher numbers of unplanned respiratory visits (Figure 1, *B*) and purchased higher amounts of antibiotics compared with the paucigranulocytic group (Figure 1, *C*). The eosinophilic group had nasal polyps more frequently compared with the paucigranulocytic and neutrophilic groups and suspected sinusitis compared with the paucigranulocytic group (Table II). The four groups did not differ significantly regarding asthma symptoms and asthma control (Figure 1, *D* and Table II), but the neutrophilic, eosinophilic, and mixed granulocytic groups were more likely to fulfil the definition of severe asthma compared

TABLE I. Background and medication of patients at 12-y follow-up visit, classified into groups according to blood eosinophil and neutrophil levels

Group						
Variables	**Paucigranulocytic**	**Neutrophilic**	**Eosinophilic**	**Mixed granulocytic**	***P***	**All patients**
Subjects, n (%)	108 (53.2)	60 (29.6)	21 (10.3)	14 (6.9)		203 (100)
Blood eosinophils ($\times 10^9 \cdot L^{-1}$)	0.14 (0.09-0.21)	0.13 (0.08-0.22)	0.44 (0.39-0.63)	0.38 (0.34-0.44)		0.16 (0.10-0.27)
Blood neutrophils ($\times 10^9 \cdot L^{-1}$)	3.2 (2.5-3.7)	5.3 (4.7-6.1)	3.2 (2.7-3.8)	4.9 (4.6-5.4)		3.7 (2.9-4.8)
Background						
Age, y	58 (13)	59 (15)	61 (14)	56 (13)	.674‖	58 (14)
Women, n (%)	66 (61.1)	35 (58.3)	10 (47.6)	7 (50.0)	.631¶	118 (58.1)
Body mass index, kg/m^2	27.7 (24.4-30.6)	29.7 (25.6-32.7)**,††	26.7 (22.8-28.1)	29.3 (23.7-36.0)	.007#	28.1 (24.4-31.3)
Patients with smoking history (including current), n (%)	53 (49.1)	38 (63.3)	11 (52.4)	5 (35.7)	.178¶	107 (52.7)
Current smokers, n (%)	14 (13.0)	14 (23.3)	2 (9.5)	0 (0.0)	.084¶	30 (14.8)
History of smoking, pack-y*	16.6 (14.3)	19.4 (12.4)	18.0 (20.4)	18.9 (19.0)	.851‖	16 (6-30)
Atopy,† n (%)	38 (39.6)	15 (26.8)	9 (45.0)	6 (54.5)	.189¶	68 (33.5)
Allergic or constant rhinitis, n (%)	69 (63.9)	44 (73.3)	16 (76.2)	13 (92.9)	.107¶	142 (70.0)
Comorbidities	1.0 (0.0-2.0)	2.0 (0.0-3.0)	1.0 (0.0-2.0)	1.0 (0.0-2.3)	.118#	1 (0-2)
Medication						
Daily ICS,‡ n (%)	78 (72.2)	49 (81.7)	15 (71.4)	13 (92.9)	.226¶	155 (76.4)
Daily long-acting β_2-agonist, n (%)	47 (43.5)	35 (58.3)	6 (28.6)	8 (57.1)	.070¶	96 (47.3)
Daily self-reported daily use of short-acting β_2-agonists, n (%)	8 (7.4)	11 (18.3)	2 (9.5)	2 (14.3)	.189¶	23 (11.3)
Daily add-on medication, n (%)	51 (47.2)	36 (60.0)	8 (38.1)	8 (57.1)	.244¶	103 (50.7)
Average prescribed daily ICS dose during 12-y follow-up (μg budesonide equivalents)§	800 (595-991)	800 (621-1,149)	808 (554-1,016)	988 (842-1,160)	.087#	805 (612-1,000)
12-y adherence to ICS (%)§	64 (41)	79 (35)	65 (31)	72 (28)	.109‖	69 (38)
2-y adherence to ICS before follow-up (%)§	59 (48)	81 (44)	76 (53)	72 (34)	.081‖	68 (47)
Puffs of self-reported daily use of short-acting β_2-agonist dispensed during 2 y before follow-up	30.0 (0.0-142.5)	45.0 (0.0-207.5)	100.0 (0.0-225.0)	60.0 (0.0-235.0)	.291#	30.0 (0.0-190.0)
Medications other than asthma/allergy-related, n	1.0 (0.0-3.8)	2.0 (1.0-4.0)	1.0 (0.0-2.5)	1.0 (0.0-4.3)	.067#	2.0 (0.0-4.0)

ICS, inhaled corticosteroid.

Data are presented as n (%), means (SDs), or medians (interquartile ranges). Variables were from the 12-year follow-up visit if not otherwise mentioned.

*Information is missing for four patients.

†Atopy was determined by skin prick test. Information is missing for 20 patients.

‡Information is missing for 26 patients.

§Information is missing for 22 patients who were not prescribed regular ICS medication for the entire follow-up period.

Statistical significance was evaluated by one-way ANOVA: ‖by Pearson χ^2 test with a comparison of column proportions by *z*-test and ¶adjusting *P* values by Benjamini-Hochberg method, or #by independent-samples Kruskal-Wallis test adjusted by Benjamini-Hochberg method. Statistical significance between groups: $^{**}P < .05$ vs paucigranulocytic group; $^{††}P < .05$ vs eosinophilic group.

with the paucigranulocytic group (Figure 1, *E*). The eosinophilic group had a greater decline in FEV_1 compared with the paucigranulocytic and neutrophilic groups (Table II). The four groups did not differ significantly in the number of any single comorbidity. After excluding patients with COPD, the higher prevalence of severe asthma and suspected sinusitis lost statistical significance in the eosinophilic group (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

The neutrophilic group had the highest high-sensitivity C-reactive protein levels compared with the paucigranulocytic group (Table III) and matrix metalloproteinase-9 (MMP-9) levels compared with the paucigranulocytic and eosinophilic groups (Figure 2, *A*). The eosinophilic group had the highest periostin levels compared with the paucigranulocytic and neutrophilic groups (Figure 2, *B*), FeNO levels compared with the neutrophilic group (Figure 2, *C*) and IgE levels compared with all other groups (Table III). The neutrophilic group had increased levels of IL-6 (Figure 2, *D*) and leptin (Figure 2, *E*) compared with the paucigranulocytic and eosinophilic groups and increased levels of soluble urokinase plasminogen activator receptor compared with the paucigranulocytic group (Table III). The mixed granulocytic group and the neutrophilic group had higher levels of resistin compared with the paucigranulocytic and eosinophilic groups (Figure 2, *F*). Figure E2 (in this article's

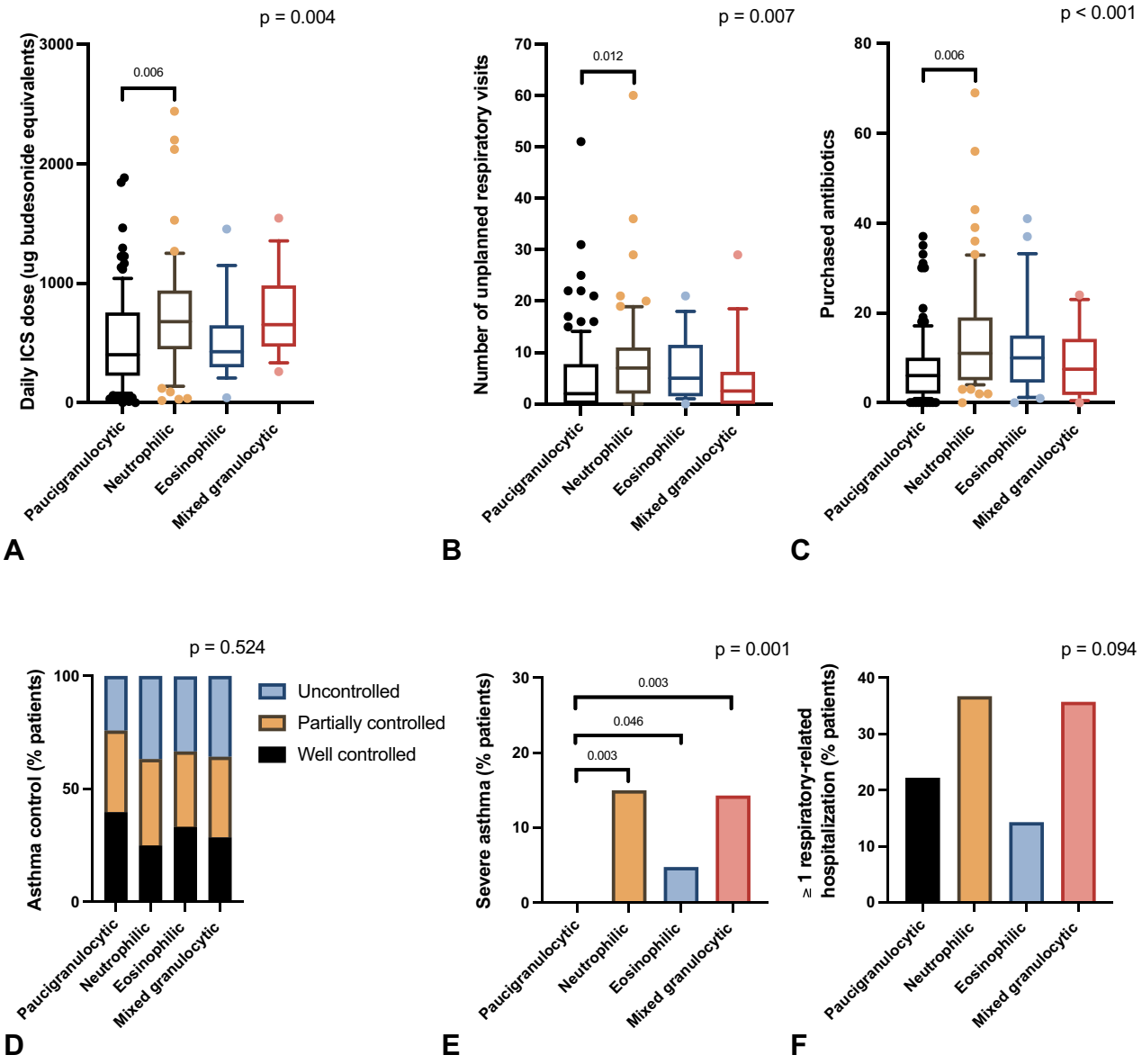

FIGURE 1. (**A**) Dispensed daily inhaled corticosteroid (ICS doses), (**B**) number of unplanned respiratory visits, (**C**) antibiotic courses, (**D**) asthma control, (**E**) proportion of patients with severe asthma according to European Respiratory Society and American Thoracic Society definition, and (**F**) respiratory-related hospitalizations of all subjects in groups according to blood eosinophil and neutrophil levels. Medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers) are shown in (A-C).

Online Repository at www.jaci-inpractice.org) provides a summary chart of these results. Figure 3 shows an equivalent chart regarding clinical manifestations.

Regression analyses

To identify factors associated with unplanned respiratory visits as well as those associated with dispensed antibiotics during the 12-year follow-up, we carried out negative binomial regression analyses. Belonging to the neutrophilic group was significantly associated with increased unplanned respiratory visits whereas belonging to the eosinophilic group had a borderline association (Table IV) relative to the paucigranulocytic group. Other factors that were significantly associated included having a history of smoking exceeding 10 pack-years relative to less than 10 pack-years, female sex, and overweight relative to a BMI of less than 25 (Table IV). After adjusting for dispensed ICS during 2 years before the follow-up visit, belonging to the eosinophilic group reached a statistically significant association whereas belonging to the neutrophilic resulted in a borderline association relative to the paucigranulocytic group (see Table E3 in this article's Online Repository at www.jaci-inpractice.org). The neutrophilic and eosinophilic groups were associated with antibiotic use during the 12-year follow-up relative to the paucigranulocytic group (see Table E4 in this article's Online Repository at www.jaci-inpractice.org). No changes in statistical significance were seen after adjusting for dispensed ICS.

TABLE II. Asthma symptoms and disease burden of patients at 12-y follow-up visit, classified into groups according to blood eosinophil and neutrophil levels

Groups					
Variables	**Paucigranulocytic**	**Neutrophilic**	**Eosinophilic**	**Mixed granulocytic**	***P***
Subjects, n (%)	108 (53.2)	60 (29.6)	21 (10.3)	14 (6.9)	
Asthma symptoms and disease burden					
Airways Questionnaire 20 score*	3 (1-7)	4 (2-7)	6 (3-9)	2 (1-7)	.269‖
Asthma Control Test score	22 (19-24)	21 (19-23)	22 (21-23)	22 (18-25)	.440‖
Asthma follow-up visits **over** 12-y period	6.0 (3.0-9.0)	7.0 (4.0-11.0)	5.0 (4.0-9.0)	6.5 (3.0-13.5)	.537‖
Purchased oral corticosteroids **over** 12-y follow-up, mg	600 (0-1,775)	1,065 (0-2,850)	1,050 (0-3,150)	1,750 (0-2,850)	.134‖
Nasal polyps mentioned at least once, n (%)	6 (5.6)	3 (5.0)	6 (28.6)#,**	3 (21.4)	.002§
Sinus ultrasounds performed,	0.0 (0.0-1.0)	0.0 (0.0-2.0)	1.0 (0.0-4.0)#	0.0 (0.0-1.0)	.037‖
Lung function					
Pre-BD FEV_1 %	89 (79-97)	86 (73-97)	85 (70-94)	86 (81-89)	.224‖
Post-BD FEV_1 %	93 (82-100)	89 (73-99)	87 (75-99)	87 (82-90)	.090‖
Pre-BD FVC %	98.9 (14.6)	95.0 (16.4)	94.3 (14.3)	92.6 (15.4)	.208‖
Post-BD FVC %	100.1 (14.3)	97.0 (16.4)	95.2 (14.4)	93.4 (15.6)	.230‖
Pre-BD FEV_1/FVC ratio	0.74 (0.68-0.79)	0.73 (0.63-0.79)	0.73 (0.68-0.77)	0.76 (0.65-0.80)	.604‖
Post-BD FEV_1/FVC ratio	0.76 (0.70-0.81)	0.72 (0.64-0.81)	0.76 (0.70-0.80)	0.78 (0.68-0.82)	.383‖
FEV_1 reversibility (**%**)	3.5 (1.5-6.8)	3.5 (0.8-6.5)	4.1 (0.4-7.3)	1.6 (-0.1-4.0)	.338‖
FEV_1 reversibility, m**L**	100 (40-150)	80 (20-167)	90 (15-220)	35 (−5 to 90)	.256‖
Longitudinal decline of pre-BD FEV_1, m**L**/y†,‡	−41 (64 to −24)	−39 (−61 to −19)	−62 (−85 to −40)#,**	−37 (−82 to −22)	.034‖

BD, bronchodilator.
Data are presented as n (%), means (SDs), or medians (interquartile ranges). Variables were from the 12-year follow-up visit if not otherwise mentioned.
*Information is missing for one patient.
†Information is missing for two patients.
‡Longitudinal decline was calculated from the point of highest measurement during the first 2.5 years after baseline to follow-up.
Statistical significance was evaluated by §Pearson χ^2 test with comparison of column proportions by *z*-test and adjusting P values by Benjamini-Hochberg method or ‖by independent-samples Kruskal-Wallis test adjusted by Benjamini-Hochberg method. Statistical significance between groups: #$P < .05$ vs paucigranulocytic group; **$P < .05$ vs neutrophilic group.

TABLE III. Inflammation markers of patients at 12-y follow-up visit, classified into groups according to blood eosinophil and neutrophil levels

Group					
Variables	**Paucigranulocytic**	**Neutrophilic**	**Eosinophilic**	**Mixed granulocytic**	***P***
Subjects**,** n (%)	108 (53.2)	60 (29.6)	21 (10.3)	14 (6.9)	
Markers of inflammation					
Total IgE ($kU \cdot L^{-1}$)*	49.5 (25.3-146.5)	57.5 (23.0-134.3)	150.0 (69.0-430.5)‖,#,**	50 (17.5-140.5)	0.025§
High-sensitivity C-reactive protein ($mg \cdot L^{-1}$)†	1.0 (0.5-1.6)	1.8 (0.8-4.9)‖	1.0 (0.4-2.5)	1.1 (0.4-3.9)	0.001§
Soluble urokinase plasminogen activator receptor ($ng \cdot mL^{-1}$)‡	2.7 (2.2-3.3)	3.0 (2.6-3.8)*	2.7 (2.1-3.8)	3.0 (2.2-3.9)	0.023§
High−molecular weight adiponectin ($\mu g \cdot mL^{-1}$)	5.0 (2.6-7.4)	4.1 (2.6-6.5)	3.8 (2.4-6.6)	2.5 (2.2-4.2)‖,#	0.042§

Data are presented as n (%), means (SDs), or medians (interquartile ranges). Variables were from the 12-y follow-up visit if not otherwise mentioned.
*Information is missing for 10 patients.
†Information is missing for one patient.
‡Information missing for two patients.
Statistical significance was evaluated by §independent-samples Kruskal-Wallis test adjusted by Benjamini-Hochberg method. Statistical significance between groups: ‖$P < .05$ vs paucigranulocytic group; #$P < .05$ vs neutrophilic group; **$P < .05$ vs mixed granulocytic group.

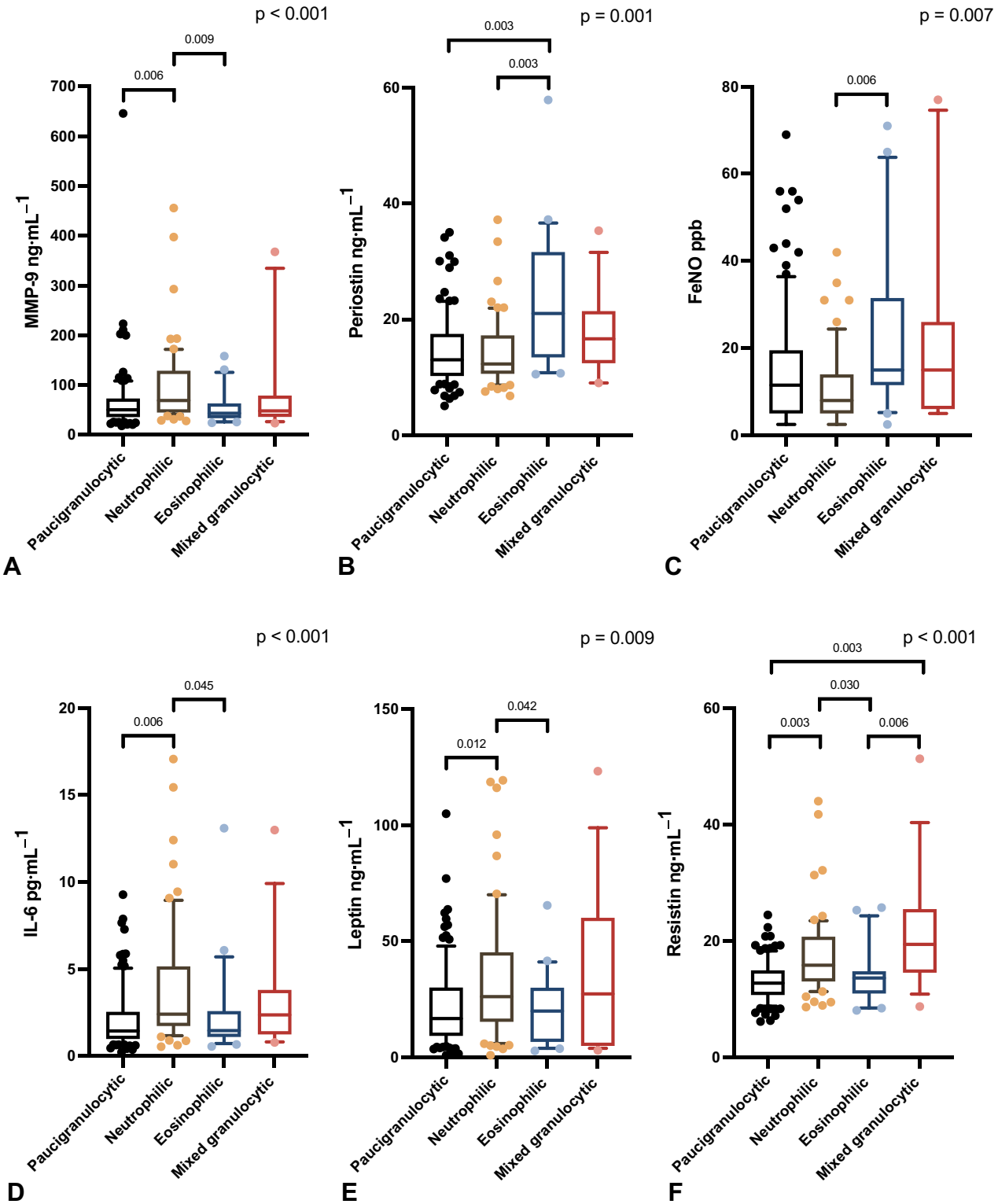

FIGURE 2. (**A**) Matrix metalloproteinase-9 (MMP-9), (**B**) periostin, (**C**) FeNO, (**D**) IL-6, (**E**) leptin, and (**F**) resistin levels of all subjects in groups according to blood eosinophil and neutrophil levels. Medians, 25th and 75th percentiles (boxes), and 10th and 90th percentiles (whiskers) are shown.

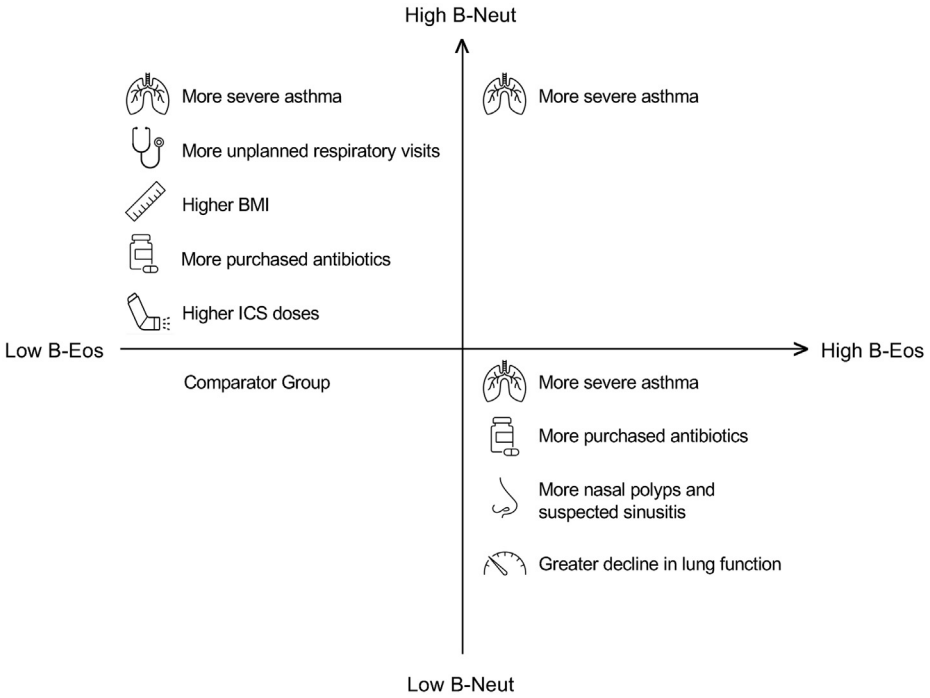

FIGURE 3. Summary chart of statistically significant results of study regarding clinical features. All statements are relative to the paucigranulotic group, referred to here as the comparator group. *BMI*, body mass index; *B-Eos*, blood eosinophils; *B-Neut*, blood neutrophils.

TABLE IV. Factors associated with number of unplanned respiratory visits in negative binomial regression analysis

Variable	Incidence rate ratio	95% CI	*P*
Paucigranulocytic asthma	1		
Neutrophilic asthma	1.62	1.06-2.46	.025
Eosinophilic asthma	1.78	0.96-3.29	.069
Mixed granulocytic asthma	0.84	0.40-1.78	.652
Female sex	2.20	1.45-3.35	<.001
Age, y	0.99	0.98-1.01	.183
Pre-bronchodilator FEV_1 %	1.00	0.98-1.01	.550
Body mass index $\geq$ 30	1.30	0.81-2.07	.274
25 $\leq$ body mass index < 30	1.58	1.00-2.49	.050
Smoking >10 pack-years	1.55	1.01-2.36	.043

We carried out linear regression analyses to identify factors associated with a decline in lung function. The eosinophilic group was associated with a decline in lung function (Table V) relative to the paucigranulocytic group. Other associated factors included height and a change in BMI (Table V). No changes were seen in statistical significance regarding the eosinophilic group after adjusting for dispensed ICS during the entire follow-up or after adjusting for the FEV_1 value at the time of diagnosis.

DISCUSSION

In this real-life study, we showed that different granulocyte subtype counts are associated with distinct differences in the clinical features of adult-onset asthma. The paucigranulocytic group had the lowest prevalence of severe asthma, was dispensed the least ICS and antibiotics, and made the fewest unplanned respiratory visits. The neutrophilic group had the highest BMI, was dispensed the highest doses of ICS, and had the most unplanned respiratory visits. The neutrophilic, eosinophilic, and mixed granulocytic groups were more likely to fulfil the definition of severe asthma compared with the paucigranulocytic group. The neutrophilic and eosinophilic groups were dispensed the most antibiotics. The four groups did not differ by asthma control.

We found an association between high eosinophil levels and a greater decline in lung function. Previous studies support this result.[24,25] Although patients in the mixed granulocytic group had a decline in FEV_1 similar to that in the eosinophilic group, the association between the mixed granulocytic group and the decline in FEV_1 was lost after adjustments. Type 2 inflammation may have been more pronounced in patients in the eosinophilic group compared with patients in the mixed granulocytic group. This is seen when comparing blood eosinophil, IgE, and periostin levels between these groups. However, in a previous study, the association between high blood eosinophil levels and a decline in lung function was even stronger when considering only patients using ICS.[24] In our study, the association remained similar regardless of the adjustment for dispensed ICS. In the current study, we saw no association between neutrophil levels and FEV_1 decline, in line with a previous result.[24]

In our study, the neutrophilic group was associated with increased unplanned respiratory visits, including those related to URTIs and asthma exacerbations, and the eosinophilic group had a borderline association. The eosinophilic group did not reach statistical significance most likely owing to the lower group size. This result was broadly in line with the Copenhagen General Population Study[10] and EGEA2 study.[11] Among these three studies, asthma exacerbations had slightly different

TABLE V. Factors associated with decline of pre-bronchodilator FEV_1 (mL) from $Max_{0\text{-}2.5}$ to follow-up in linear regression analysis

Variable	B	95% CI	*P*
Paucigranulocytic asthma	1		
Neutrophilic asthma	3.56	−7.50 to 14.62	.526
Eosinophilic asthma	−22.16	−38.14 to −6.18	.007
Mixed granulocytic asthma	1.80	−16.90 to 20.50	.850
Female sex	3.42	−11.50 to 18.25	.651
Age, y	−0.38	−0.78 to 0.01	.057
Height, cm	−0.87	−1.66 to −0.09	.030
Change in body mass index ($Max_{0\text{-}2.5}$ to follow-up)	−1.93	−3.55 to −0.32	.019
Smoking (>10 pack-years)	−8.37	−19.55 to 2.81	.141

Data are presented as the unstandardized β coefficient. $Max_{0\text{-}2.5}$ = point of highest measurement during the first 2.5 years after baseline.

definitions. In our study, asthma exacerbations and URTIs were addressed as one variable based on health care visits, because it is difficult to distinguish asthma exacerbations and URTIs reliably from one another. In the Copenhagen General Population Study, high eosinophil levels were associated with both moderate and severe exacerbations, whereas high neutrophil levels were associated only with moderate exacerbations. Temporary use of prednisolone was regarded as a moderate exacerbation, and asthma-related hospitalizations as severe exacerbations.[10] In the EGEA2 study, high neutrophil levels were associated with severe exacerbations, whereas high eosinophil levels were associated with mild exacerbations. Asthma attacks were regarded as mild exacerbations, and temporary use of prednisone, respiratory-related hospitalizations, and respiratory-related emergency clinic visits as severe exacerbations.[11] Because of differences in the definitions of exacerbations, it is difficult to compare results. Some differences among these three studies may also result from differing cutoff values used to define the low- and high-level groups. Nevertheless, neutrophil levels were associated with a high risk for exacerbations in all three studies.

In our study, the eosinophilic and neutrophilic groups were dispensed the most antibiotics during the entire follow-up. Patients with only high eosinophil levels were dispensed a median of 0.90 antibiotic treatments per year, and patients with only high neutrophil levels were dispensed a median of 0.89 antibiotic treatments per year. This suggests that antibiotics are overprescribed for patients with asthma. Such concerns are supported by research conducted in the United States evaluating the use of antibiotics to treat patients with asthma exacerbations in emergency departments.[26] Data from the National Hospital Ambulatory Medical Care Survey[27] revealed that 22% of acute asthma-related hospital visits during a 12-year period resulted in antibiotic treatment. The study included patients with childhood- and adult-onset asthma. Other previous studies on the use of antibiotics to treat asthma exacerbations focused on pediatric patients.[28,29] Another explanation for the current finding is that asthma or its exacerbations is of a different type, and the prescription of antibiotics reflects that.

Our study suggests that blood eosinophil and neutrophil counts might provide useful information on the type of asthma exacerbations, which may also help to target treatment. The neutrophilic group was dispensed significantly more ICS during the 12-year follow-up compared with patients in the eosinophilic group. Corticosteroids inhibit the apoptosis of neutrophils and may increase their numbers.[30,31] Thus, it is not known whether neutrophilic asthma is a true endotype that leads to heavy corticosteroid use or whether it is an endotype resulting from heavy corticosteroid use. Patients with T2-low asthma may have a transformed microbiome.[32,33] One study[32] concluded that endobronchial brush samples were enriched with different fungal species depending on whether the patient had T2-high or T2-low asthma. In addition, fungal diversity was lower in patients with T2-high asthma. Another study[33] concluded that patients with T2-low asthma had a higher bacterial burden in bronchial brush samples compared with patients with T2-high asthma. It remains unanswered whether the etiology of asthma exacerbations could be different among patients with differing asthma endotypes owing to the contrasting microbiomes. Could it be that patients with neutrophilic asthma need antibiotic treatments more often? Previous research regarding COPD suggests using blood biomarker measurements to select a treatment line during COPD exacerbations,[34] an idea that could be applied to treat asthma in the future.

The same variability among different granulocyte subtype counts was seen when we focused on molecular features. The eosinophilic group had the highest periostin and FeNO levels. Periostin and FeNO levels were not statistically significantly higher in the mixed granulocytic group compared with the paucigranulocytic and neutrophilic groups, which may be explained by the small group sizes. A previous study[35] suggested that periostin levels are the best single predictor of airway eosinophilia in asthma, followed by FeNO analyses. Another study[36] supported using periostin as a biomarker for patients with eosinophilic asthma, because periostin appears to be stable over 8 weeks. Our study supports a close association among blood eosinophil, periostin, and FeNO levels.

To date, there is lack of information regarding biomarkers that could be used to recognize endotypes of asthma, other than those of eosinophilic asthma. In our study, the neutrophilic group had the highest high-sensitivity C-reactive protein, MMP-9, IL-6, leptin, and soluble urokinase plasminogen activator receptor levels. This is comparable to the results of a previous study in which patients with severe asthma and sputum neutrophilia had increased exhaled MMP-9 levels.[37] The mixed granulocytic group had the highest levels of resistin, together with neutrophilic group. The current study suggests that distinct findings in inflammatory markers also characterize different endotypes of asthma.

This study had multiple strengths. Previous studies[10,11] selected patients with self-reported asthma, whereas in our study all patients who were included have a diagnosis of asthma provided by a respiratory physician and confirmed by objective lung function measurements. Patients included in this study represent a realistic sample of asthma patients in the general population, because former and current smokers as well as patients with coexisting COPD were not excluded. The 12-year follow-up was exceptionally long; moreover, we had access to all data regarding the use of medication and health services throughout the entire follow-up. There were also limitations to our study. The study population could have been larger, especially regarding the mixed granulocytic group. Although we included a question regarding symptoms compatible with asthma at some point during childhood and we considered all available medical records, we cannot rule out the possibility that a few

patients might have had undiagnosed childhood-onset asthma. Blood eosinophil and neutrophil counts were measured once at the 12-year follow-up visit. Therefore, we cannot be certain of the stability of findings during the entire follow-up. It is known that neutrophil counts increase during infections; therefore, some doubt the use of blood neutrophils in asthma phenotyping. However, a previous study showed that over 50% of a study population maintained the same granulocyte patterns over long periods.[11] In addition, the elevating effect of infections on neutrophil levels can be avoided by determining the distribution of granulocytes during a stable phase of the condition.

The value of measuring blood eosinophil and neutrophil counts for predicting inflammatory phenotypes in asthma has been disputed. Previous research[38] concluded that both absolute blood eosinophil counts and blood eosinophil percentages are significant predictors of sputum eosinophilia. In that study, the positive relationship between blood neutrophil levels and sputum neutrophil levels was weaker than the relationship between blood eosinophil levels and sputum eosinophil levels, but it was still seen. The blood neutrophil percentage had 61.5% sensitivity and 68.8% specificity for predicting sputum neutrophilia. However, another study[39] reported that blood eosinophil and blood neutrophil counts do not accurately predict sputum eosinophil and neutrophil percentages in patients with mild, moderate, or severe asthma. Further studies are needed to obtain an overall perception of the association between sputum granulocyte and blood granulocyte counts. However, sputum sampling is unavailable at all health care units, and sputum samples cannot be routinely obtained from all asthma patients, unlike blood samples.

Our study indicates that assays of blood eosinophil and neutrophil counts provide useful information for assessing and treating patients with adult-onset asthma. These granulocyte counts reflect the underlying inflammatory pattern and reveal important differences in asthma clinical features and outcomes. Nevertheless, more research is needed regarding biomarkers used to identify different endotypes of asthma.

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ONLINE REPOSITORY

SUPPLEMENTAL METHODS

Use of medication and adherence to inhaled corticosteroids

Information on prescribed inhaled corticosteroids (ICS) and oral corticosteroids (OCS) is based on data collected from asthma-related health care visits. Information on dispensed ICS and OCS was collected from the Finnish Social Insurance Institution. The ICS doses were converted into budesonide equivalents and methyl prednisolone was converted into prednisolone equivalents. Only documentation of OCS with an indication for asthma was considered.[E1] The indication was presumed to be asthma on the condition that the indication was missing from medical records and no other indication was identified. Average annual use of OCS was calculated by dividing the total amount of dispensed OCS during the entire follow-up period by the patient-specific years of follow-up. Adherence to ICS was determined by comparing prescribed and dispensed ICS doses, as described in a separate study.[E1] Annual adherence was calculated by comparing yearly dispensed doses of ICS (in micrograms) with yearly prescribed doses of ICS (in micrograms). The 12-year adherence was calculated by comparing cumulative dispensed doses of ICS (in micrograms) with cumulative prescribed doses of ICS (in micrograms). Average prescribed and dispensed daily doses of ICS were calculated by combining yearly ICS doses (in micrograms) and dividing the sum by the length of the follow-up period (in days). Antibiotics considered in this study were amoxycillin, amoxycillin plus clavulanate, azithromycin, cefalexin, clarithromycin, doxycycline, erythromycin, phenoxy methyl-penicillin, roxithromycin, sulfadiazine, telithromycin, and trimethoprim.

Lung function and markers of inflammation

Lung function measurements were completed with a spirometer (Vmax Encore 22, Viasys Healthcare, Palm Springs, Calif) that was calibrated daily. Finnish lung function values were used as references.[E2] Postbronchodilator measurements were taken 15 minutes after inhalation of salbutamol (400 mg). We carried out FeNO measurements according to American Thoracic Society standards.[E3] (flow rate of 50 $mL \cdot s^{-1}$) (NIOX System, Aerocrine, Solna, Sweden). Measurements were completed using a portable rapid-response chemiluminescent analyzer. We measured IgE levels using the ImmunoCAP blood test (Thermo Scientific, Uppsala, Sweden). High-sensitivity C-reactive protein was measured using the Roche Cobas 800 automated clinical chemistry analyzer (Roche Diagnostics, Basel, Switzerland). The detection limit was 0.3 $mg \cdot L^{-1}$ for high-sensitivity C-reactive protein. Enzyme-linked immunosorbent assay kits were used following the manufacturer's instructions to record the levels of plasma soluble urokinase plasminogen activator receptor (suPARnostic, ViroGates, Birkeroed, Denmark), and IL-6, leptin, high−molecular weight adiponectin, resistin, periostin, and matrix metalloproteinase-9 (R&D Systems Europe Ltd, Abingdon, UK). The detection limit was 0.8 pg mL^{-1} for soluble urokinase plasminogen activator receptor, 0.7 pg mL^{-1} for IL-6, 15.6 pg mL^{-1} for leptin, 3.9 pg mL^{-1} for high−molecular weight adiponectin, 15.6 pg mL^{-1} for resistin, 15.6 pg mL^{-1} for periostin, and 7.8 pg mL^{-1} for matrix metalloproteinase-9.

TABLE E1. Inclusion and exclusion criteria of Seinäjoki Adult Asthma Study

Inclusion criteria	Diagnosis of new-onset asthma made by a respiratory physician Diagnosis confirmed by at least one of the following objective lung function measurements*: FEV_1 reversibility in spirometry of at least 15% and 200 mL Diurnal variability (≥20%) or repeated reversibility (≥15%/60 L/min) in PEF follow-up Significant decrease in FEV_1 (15%) or PEF (20%) in response to exercise or allergen Significant reversibility in FEV_1 (at least 15% and 200 mL) or mean PEF (20%) in response to trial with oral or inhaled glucocorticoids Symptoms of asthma Age ≥15 y
Exclusion criteria	Physical or mental inability to provide signed informed consent Of note: Patients with comorbidities of other lung disease or any other significant disease were not excluded. Patients were not excluded because of smoking, alcohol use, or any other lifestyle factor.

PEF, peak expiratory flow.
*Objective lung function criteria reflect those of national and international guidelines valid in 1999 to 2002 and may not exactly follow those currently valid. The table was previously elsewhere.[E4]

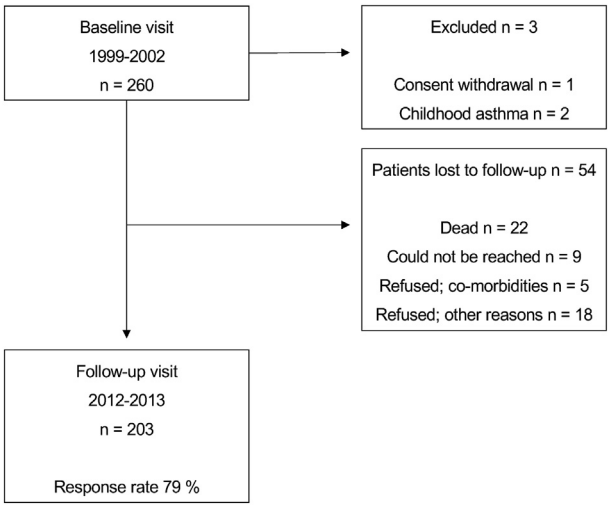

FIGURE E1. Consolidated Standards of Reporting Trials plot of the study.

TABLE E2. Statistically significant results of study regarding clinical manifestations excluding patients with chronic obstructive pulmonary disease.

Group					
Variables	**Paucigranulocytic**	**Neutrophilic**	**Eosinophilic**	**Mixed granulocytic**	***P***
Subjects, n (%)	94 (56.3)	45 (26.9)	18 (10.8)	10 (6.0)	
Blood eosinophils ($\times 10^9 \cdot L^{-1}$)	0.14 (0.09-0.21)	0.13 (0.08-0.22)	0.44 (0.39-0.63)	0.38 (0.34-0.44)	
Blood neutrophils ($\times 10^9 \cdot L^{-1}$)	3.2 (2.5-3.7)	5.3 (4.7-6.1)	3.2 (2.7-3.8)	4.9 (4.6-5.4)	
Body mass index, kg/m^2	27.1 (24.4-30.2)	31.3 (27.0-33.6)**,‡‡	26.9 (22.8-28.8)	31.0 (22.7-37.5)‡‡	.001#
Severe asthma, n (%)	0	7 (15.6)**	0 (0.9)	1 (10.0)**	.001‖
Longitudinal decline of pre- bronchodilator FEV_1, mL*,†	−38.7 (−62.4 to −22.1)	−32.6 (−55.0 to −18.1)	−59.3 (−79.2 to −41.3)**,††,§§	−35.1 (−44.1 to −12.7)	.023#
Average dispensed daily inhaled corticosteroid dose during 12-y follow-up, μg budesonide equivalents‡	388 (229-770)	767 (478-998)**	420 (255-790)	651 (469-982)	.001#
Unplanned respiratory visits§	2.0 (0.0-7.0)	8.0 (2.0-11.0)**	3.5 (1.0-11.3)	2.5 (0.0-6.5)	.013#
Purchased antibiotics	6.0 (2.0-10.0)	10.0 (4.5-20.0)**	9.5 (4.0-15.0)	7.5 (3.3-14.3)	.002#
Polyps mentioned at least once, n (%)	6 (6.4)	3 (6.7)	5 (27.8)**,††	2 (20.0)	.021‖
Sinus ultrasounds performed	0.0 (0.0-1.0)	0.0 (0.0-2.0)	1.0 (0.0-4.25)	0.50 (0.0-1.25)	.175‖

Data are presented as n (%), means (SDs), or medians (interquartile ranges). Variables were from the 12-y follow-up visit if not otherwise mentioned.
*Information is missing for two patients.
†Longitudinal decline calculated from the point of highest measurement during the first 2.5 y after baseline to follow-up.
‡Information missing for 20 patients who were not prescribed regular inhaled corticosteroid medication for the whole follow-up period.
§Unplanned respiratory visits include health care visits regarding upper respiratory tract infections and asthma exacerbations.
‖Statistical significance was evaluated by Pearson χ^2 test with comparison of column proportions by z-test and adjusting P values by Benjamini-Hochberg method or by #independent-samples Kruskal-Wallis test adjusted by Benjamini-Hochberg method. Statistical significance between groups: **$P < .05$ vs paucigranulocytic group; ††$P < .05$ vs neutrophilic group; ‡‡$P < .05$ vs eosinophilic group; §§$P < .05$ vs mixed granulocytic group.

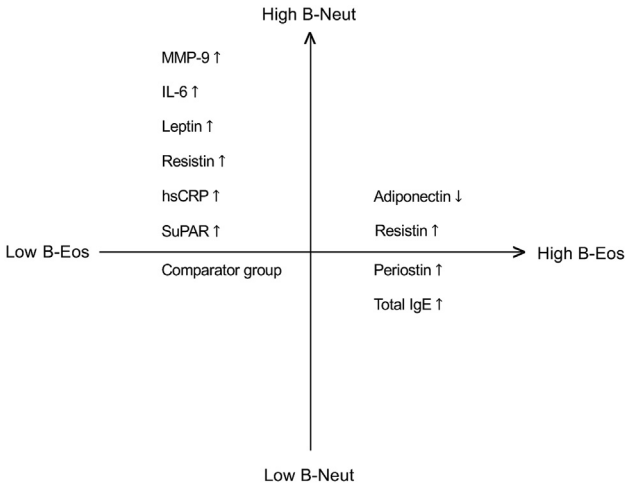

FIGURE E2. Summary chart of statistically significant results of the study regarding inflammatory markers. All statements are relative to the paucigranulotic group, which is referred to here as the comparator group. *B-Eos*, blood eosinophils; *B-Neut*, blood neutrophils; *hs-CRP*, high-sensitivity C-reactive protein; *MMP-9*, matrix metalloproteinase-9; *SuPAR*, soluble urokinase plasminogen activator receptor.

TABLE E3. Factors associated with unplanned respiratory visits in negative binominal regression analysis after adjusting for dispensed inhaled corticosteroids

Variable	Incidence rate ratio	95% CI	*P*
Paucigranulocytic asthma	1		
Neutrophilic asthma	1.43	0.95-2.16	.088
Eosinophilic asthma	1.87	1.02-3.44	.045
Mixed granulocytic asthma	0.85	0.41-1.77	.664
Female sex	2.08	1.38-3.12	<.001
Age	0.99	0.97-1.00	.042
Pre-bronchodilator FEV_1 %	1.00	0.99-1.01	.742
Body mass index $\geq$ 30	1.24	0.79-1.96	.348
25 $\leq$ body mass index $>$ 30	1.54	0.99-2.41	.057
Smoking >10 pack-years	1.54	1.02-2.33	.039
Dispensed inhaled corticosteroids during 2 y before follow-up	2.12	1.23-3.65	.007

TABLE E4. Factors associated with dispensed antibiotics during 12-y follow-up

Variable	Incidence rate ratio	95% CI	*P*
Paucigranulocytic asthma	1		
Neutrophilic asthma	1.68	1.25-2.25	.001
Eosinophilic asthma	1.64	1.09-2.47	.018
Mixed granulocytic asthma	1.08	0.66-1.78	.758
Female sex	1.70	1.29-2.25	<.001
Age	0.99	0.98-1.00	.187
Pre- bronchodilator FEV_1 %	0.99	0.99-1.00	.109
Body mass index $\geq$ 30	1.17	0.85-1.62	.327
25 $\leq$ body mass index $>$ 30	1.23	0.90-1.67	.189
Smoking >10 pack-years	1.35	1.00-1.82	.054

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