Blood inflammatory phenotypes were associated with distinct clinical expressions of asthma in adults from a large population-based cohort

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Summary

Background Asthma is an inflammatory heterogeneous disease. Asthma inflammatory phenotypes based on blood eosinophil and neutrophil counts have never been identified and characterized in population-based studies.

Methods Adults with current asthma and available blood eosinophil and neutrophil counts from the French population-based CONSTANCES cohort were included. Current asthma was defined by reports of asthma attacks, symptoms or treatments in the last 12 months. Inflammatory phenotypes were based on low (L) and high (H) blood (B) eosinophil (E) (LBE/HBE: $</\geqslant 0{\cdot}25 \times 10^9$/L, respectively) and neutrophil (N) (LBN/HBN: $</\geqslant 5 \times 10^9$/L, respectively) cut-offs. Associations between inflammatory phenotypes and the clinical expressions of asthma were studied using logistic models adjusted for age, sex, smoking status, body mass index, education level, French deprivation index and treatment. Other cut-offs were applied. Stratified analyses according to age or sex were performed.

Findings Among 15,019 adults with asthma (56% women, 59%≥40 years), the LBE/LBN (reference), LBE/HBN, HBE/LBN and HBE/HBN phenotypes accounted for 57%, 6%, 33% and 4% respectively. The LBE/HBN phenotype was associated with being awaken by an attack of coughing, chronic bronchitis, and dyspnoea (adjusted(a)OR ranging from 1·21 to 1·42). The HBE/LBN and HBE/HBN phenotypes were associated with asthma attacks (aOR=1·31 [1·20-1·42], 1·25[1·02-1·53]) and asthma symptom score (p for trend<0·0001, p for trend=0·001, respectively). The HBE/LBN phenotype was also associated with being awaken with chest tightness (aOR=1·30[1·20-1·40]). Results were unchanged whatever the cut-offs used. No statistically significant heterogeneity was observed according to age or sex.

Interpretation Differences in the clinical expressions of asthma were found between the phenotypes, reproducible whatever the cut-offs used, and similar to those observed in case-control and clinical studies. Such phenotypes are of interest to improve asthma management and study its environmental risk factors.

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Introduction

Asthma is the second most common chronic respiratory disease in the world,[1] affecting around one in ten adults in France.[2–4] Although its common clinical manifestations are wheezing, shortness of breath, tightness and

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Research in context

Evidence before this study

Asthma is a heterogeneous disease that encompasses various phenotypes, among them the inflammatory phenotypes. Better understanding of these inflammatory phenotypes, particularly their distinct clinical presentation would enhance our understanding and management of the disease. We reviewed the literature reporting on blood inflammatory phenotypes of asthma in adults. We searched PubMed, using the terms "asthma" in title and "blood eosinophil* OR blood neutrophil*" and "general population OR population-based" in title or abstract with no language restrictions, and found 32 original studies. After excluding studies on severe asthma, on asthma/Chronic Obstructive Pulmonary Disease (COPD) overlap, on asthma and COVID-19, on genetics, in specific populations (only children, complement system, cytokines, longitudinal settings) and in animal models, 10 articles were retained. Among them, one studied blood eosinophils as a descriptive characteristic of asthma, three studied some characteristics of asthma in association with high eosinophil count, one studied the overlap between eosinophilic, atopic and T helper 2 (Th2) high asthma phenotypes, and five have simultaneously investigated eosinophil and neutrophil counts but without identifying and characterizing the four inflammatory phenotypes based on cut-offs.

Added value of this study

This study identified and characterized four blood inflammatory phenotypes in adults with current asthma in CONSTANCES, the largest French population-based cohort. Each blood inflammatory phenotype was associated with distinct clinical expressions of asthma. Results were unchanged whatever the cut-offs used to define the phenotypes. No statistically significant heterogeneity was observed according to age or sex.

Implication of the available evidence

Our results provide evidence in favour of the existence of a neutrophilic blood inflammatory phenotype, and confirm that the paucigranulocytic phenotype is a phenotype of interest. The identification of these phenotypes is very convenient considering that complete blood count is part of the routine examination. Such phenotypes are of interest to better understand the mechanisms by which environmental factors affect asthma.

cough, asthma is a complex and very heterogeneous disease that encompasses various phenotypes.[5] Better understanding these phenotypes, in particular their specific clinical presentation and the underlying type of inflammation, would enhance our understanding and management of the disease.[6]

Eosinophils and neutrophils are the key inflammatory cells involved in the pathogenesis of asthma, and to date, eosinophilic, severe, T helper 2 (Th2) asthma has been extensively studied. The interest in non-eosinophilic and non-inflammatory asthmas has growing more recently, and studies on inflammatory phenotypes in adults have been largely based on eosinophil and neutrophil cut-offs from induced sputum,[7] instead of blood, and more frequently from clinical studies than population-based studies. Indeed, induced sputum is difficult to obtain in large population-based studies, unlike blood eosinophils and neutrophils which are readily available in individuals of all ages and regardless of their symptoms.[8] The use of circulating granulocyte counts is biologically plausible since the infiltrating granulocytes in the airway are bone marrow-derived cells which access the airway via the circulation.[8] Our group was the first to highlight the interest to study blood inflammatory phenotypes of asthma based on both blood eosinophil and neutrophil cut-offs in epidemiological studies.[9,10] Indeed, in the epidemiological study on the Genetics and Environment of Asthma (EGEA), a case-study on asthma, we identified four inflammatory phenotypes in adults according to eosinophil and neutrophil count cut-offs of $0{\cdot}25 \times 10^9$/L and 5×10^9/L respectively. We showed that these inflammatory phenotypes were associated with distinct clinical characteristics of asthma,[9] and with different asthma evolution 10 years later.[10] Among adults from general populations, one study has investigated the overlap between eosinophilic, atopic and Th2 high asthma phenotypes,[11] and five have simultaneously investigated eosinophil and neutrophil counts but without identifying and characterizing the four inflammatory phenotypes based on cut-offs.[12–16]

The aim of the present study was to identify and to characterize the four blood inflammatory phenotypes based on cut-offs among participants with current asthma from CONSTANCES, the largest French population-based cohort. As there is no consensual definition of neutrophilic asthma, and as the eosinophil count cut-off varies across studies, we identified the four inflammatory phenotypes by using various cut-offs. We assessed the associations between blood inflammatory phenotypes and participants' characteristics as well as the clinical expressions of asthma (type of symptoms). Finally, we studied whether the clinical expressions associated with the four phenotypes varied according to sex and age.

Methods

Design

As previously described, CONSTANCES is a population-based cohort designed as a sample of the French adults.[17,18] Briefly, between February 2012 and December 2019, 199,711 participants aged 18-69 years were selected randomly from the database of the National

Pension Insurance Fund (CNAV: Caisse Nationale d'Assurance Vieillesse), and invited to participate.[18] At inclusion, the participants completed several self-administered questionnaires, including questions on respiratory health, and attended one of the 24 participating Health Prevention Centres (HPCs) located in 21 cities throughout metropolitan France for a comprehensive health examination including biological parameters.[17] At the HPCs, they also responded face-to-face to a physician-administered questionnaire on their personal and family medical history, including respiratory diseases. Additional information on sampling procedures, data collection and recruitment are available in supplementary methods, and in the full protocol.[19]

Study population

The study population included participants with current asthma who had a white blood cell (WBC) count, with (1) a sum of leukocyte components between 99% and 101%, (2) all the five leukocyte components within defined values, and (3) with absolute Z-score value of total leukocyte less than 2 (Supplementary methods).

Definition and clinical expressions of asthma

The questions on respiratory health are based on the validated and standardized British Medical Research Council/European Coal and Steel Community, American Thoracic Society, and European Community Respiratory Health Survey (ECRHS) questionnaires. Participants with ever asthma were those who answered positively to the questions "*Have you ever had asthma?* ». Among participants with ever asthma, current asthma was defined by the report in the last 12 months of asthma attacks or use of asthma medication or respiratory symptoms (wheeze, nocturnal chest tightness, attacks of breathlessness following strenuous exercise, at rest, or at night time).

Asthma treatment in the last 12 months corresponded to the positive answer to the question: "*Are you currently taking asthma medication? including inhaled products, aerosols, tablets.*".

The Asthma Symptom Score described by Sunyer et al.,[20] ranging from 0 to 5, is the sum of self-reported positive answers on the following respiratory symptoms during the past 12 months: (1) breathless while wheezing, (2) woken up with chest tightness, (3) attack of shortness of breath at rest, (4) attack of shortness of breath after exercise, and (5) woken by attack of shortness of breath.

Nocturnal symptoms were the report of waking up by an attack of shortness of breath, waking up with chest tightness and waking up by an attack of coughing. Chronic bronchitis was defined by the report of cough or phlegm every day during three months. Dyspnoea was quantified using the modified Medical Research Council scale.[21]

Participants who reported rhinitis symptoms in the last 12 months and nasal allergies in their lifetime were considered as having current allergic rhinitis (Supplementary methods).

Spirometry was performed according to ATS/ERS guidelines without the administration of a bronchodilator as French HPCs are not allowed to administer any medication even for diagnostic purposes (Supplementary methods).[22]

Other variables

Current smoking habits were expressed as follows: never-smokers, ex-smokers, and current smokers. Body mass index (BMI) was categorized into four classes defined according to World Health Organization criteria: $<18{\cdot}5$, [18·5-25[, [25-30[, and ≥ 30 kg/m^2. Education level was categorized into four classes: lower than high school, high school, college and university. The contextual French Deprivation Index (Fdep, Supplementary methods) was categorized into five quintiles from the least (Q1) to the most (Q5) deprived.

Blood inflammatory phenotypes

At inclusion, a white blood cell count (WBC) was performed for each participant in each HPC by an accredited laboratory (Supplementary methods).

In the absence of consensual cut-offs, particularly for neutrophils, the values used in the EGEA study, i.e. $0{\cdot}25 \times 10^9$/L for eosinophils and 5×10^9/L for neutrophils, were used to identify the following four blood inflammatory phenotypes: low blood eosinophils and low blood neutrophils (LBE/LBN: EOS$<0{\cdot}25 \times 10^9$/L and NEU$<5 \times 10^9$/L), low blood eosinophils and high blood neutrophils (LBE/HBN: EOS$<0{\cdot}25 \times 10^9$/L and NEU$\geq 5 \times 10^9$/L), high blood eosinophils and low blood neutrophils (HBE/LBN: EOS$\geq 0{\cdot}25 \times 10^9$/L and NEU$<5 \times 10^9$/L) and high blood eosinophils and high blood neutrophils (HBE/HBN: EOS$\geq 0{\cdot}25 \times 10^9$/L andNEU$\geq 5 \times 10^9$/L).[9]

Bias

There are potential information biases related to the self-reported nature of the data collection. There may be a misclassification bias of participants in the inflammatory phenotypes based on cut-offs, as white blood cell count was performed by methods varying across centres.

Study size

An exhaustive data collection was carried out from the CONSTANCES cohort database to get our study sample. No sample size calculation was done.

Ethics statement

The CONSTANCES Cohort project has obtained the authorization of the National Data Protection Authority on March 3, 2011 (Commission Nationale de l'Informatique et des Libertés—CNIL, authorisation no. 910486). CONSTANCES was approved by the National Council for Statistical Information (Conseil National de l'Information Statistique—CNIS), the National Medical Council (Conseil National de l'Ordre des Médecins—CNOM), and the Institutional Review Board of the National Institute for Medical Research-INSERM (authorisation no. 01-011). All participants signed a written informed consent.

The present study was approved by the Inserm ethics committee (IORG0003254, FWA00005831) and the Institutional Review Board (IRB00003888) of the French Institute of medical research and Health. The study protocol was provided in supplementary material.

The data used in this study stem from the CONSTANCES database. Due to third party restrictions, CONSTANCES data are not publicly available.

Statistical methods

Associations between neutrophil and eosinophil count with age, sex, smoking, BMI, education level, FDep and any asthma treatments were studied using the Student's t-test and analysis of variance in univariate analysis, and then including all these variables in multivariate analysis.

Associations between each clinical expression of asthma and blood inflammatory phenotypes (as defined above) were estimated using non-ordinal polytomous logistic regression adjusted for age, sex, smoking habits, BMI, education level, FDep and any asthma treatment in the last year (yes/no) (main model). The asthma symptom score was entered in the models either as a categorical variable or as a continuous variable for the trend test. No statistical analyses were performed with lung function or age of onset of asthma due to the large amount of missing data (38 and 22·5%, respectively).

Several sensitivity analyses were performed. To account for a potential residual confounding, the main model was further adjusted for HPCs. To evaluate the impact of missing values on the results, the analysis was repeated after imputing missing data for explanatory variables using the multiple imputation by fully conditional specification (FCS) method.[23] The FCS method is iterative and well-adapted for large data sets including both categorical and continuous variables. Based on variables related to clinical expressions of asthma, age, sex, smoking habits, BMI, education level, FDep, and any asthma treatment, we generated ten data sets. As there is no consensus on eosinophil and neutrophil cut-offs, associations between the clinical expressions of asthma and the inflammatory phenotypes defined according to the other cut-offs were studied: (a) $0{\cdot}23 \times 10^9$/L for eosinophil and 4×10^9/L for neutrophil corresponding to the 75th percentiles in the 160,272 adults from the CONSTANCES cohort; (b) optimal cut-offs estimated by Receiver-Operating Characteristic (ROC) curves using Youden's index (Supplementary methods); and (c) EOS at $0{\cdot}3 \times 10^9$/L corresponding to the eosinophil cut-off often used in clinical studies and 5×10^9/L for neutrophil. The main model was also repeated after having restricted to participants with a sum of leukocyte components equal to 100% or in participants defined as having asthma from both the self-administered questionnaire and the physician-administered questionnaires, and using the cut-offs of eosinophil and neutrophil, $0{\cdot}25 \times 10^9$/L and 5×10^9/L, respectively.

As the prevalence of asthma differs between men and women,[24] and asthma and chronic obstructive pulmonary disease (COPD) have several symptoms in common, stratified analyses by sex or by age (<40 years and ≥ 40 years) were performed. For these analyses, tests of homogeneity were performed by fitting the interaction-term in the logistic model; to take into account multiple comparisons, the Bonferroni corrected significance p-value threshold was calculated (14 comparisons, threshold p value=0·05/14=0·004).

For all analyses, the LBE/LBN phenotype was the reference group. The multiplicity of tests was not considered except for the stratified analyses. Statistical analyses were performed using SAS version 9.4 (SAS Institute, Inc, Cary, NC). All tests were two-sided.

Role of the funding source

The funder of the study had no rule in study design, data collection, data analysis, data interpretation, or writing of the report.

Results

Participant characteristics

Among the 199,711 participants who responded to the self-questionnaires, asthma status was available for 192,648 of them: the crude prevalences of ever and current asthma were 13·5% (IC 95%: 13·4-13·7) and 9·5% (IC 95%: 9·4-9·6), respectively.

A total of 15,019 participants with current asthma were included in the analyses (Figure 1). Compared to the 3,253 participants with current asthma not included, they were younger, less often smokers, reported more often having taken any asthma treatment and reported less often university educational level and chronic bronchitis, some of these differences being small although statistically significant due to sample sizes. No other statistically significant differences were found (Supplementary Table S1). Participants with current asthma were more often women (56·5%), the mean age was

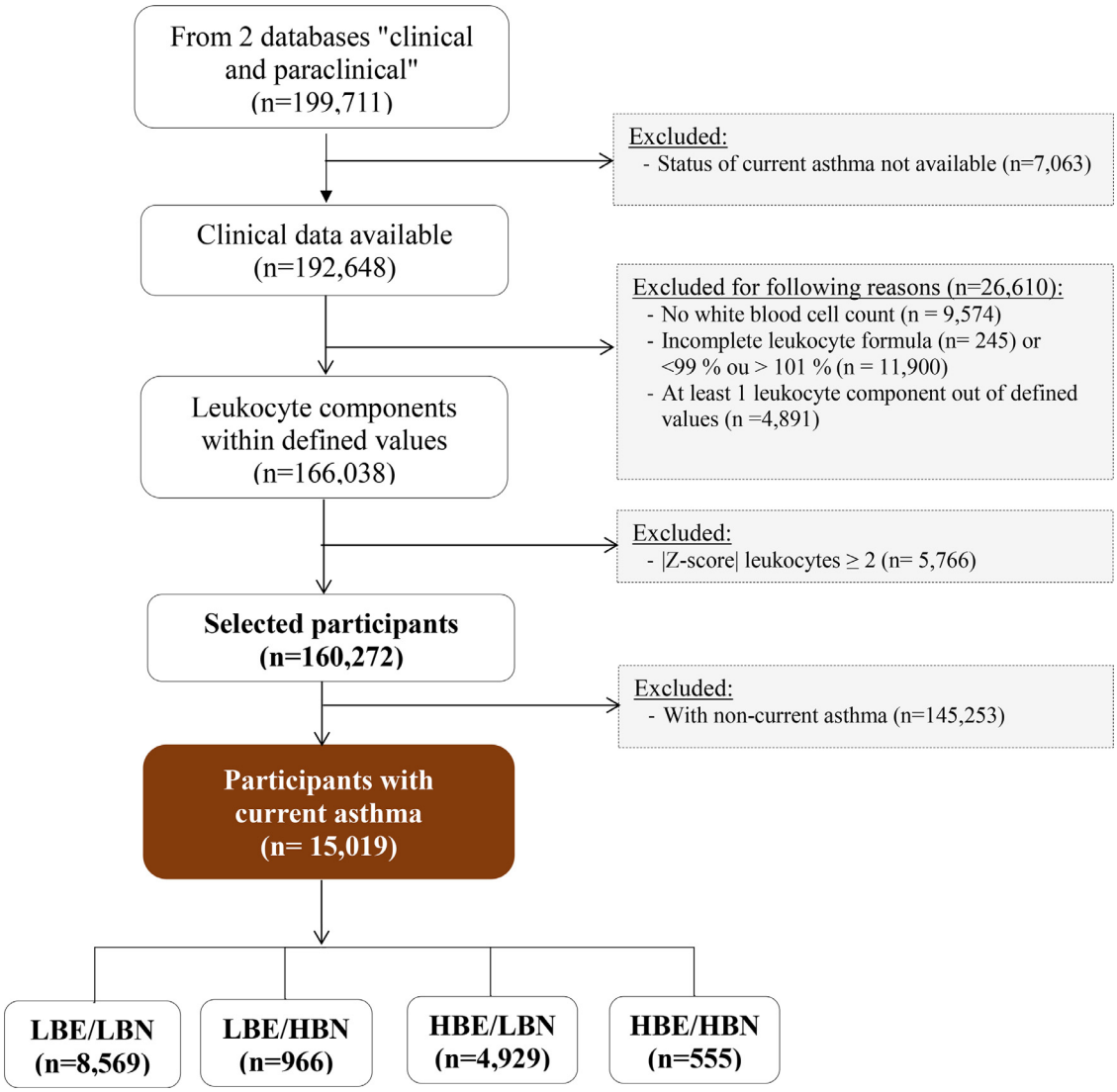

Figure 1. Flowchart. LBE: low blood eosinophils, HBE: high blood eosinophils, LBN: low blood neutrophils, HBN: high blood neutrophils.

44·3 years, 48% were overweight and 38% reported having asthma attacks in the last 12 months (Table 1).

Participants with current asthma had median (IQR) eosinophil and neutrophil counts of 0·20 (0·13-0·31) and 3·34 (2·69-4·15) × 10^9/L respectively. The neutrophil count was statistically significantly higher in women and in current smokers, increased with age, BMI and FDep, and decreased with education level (Wald test, all $p < 0·01$). In contrast, eosinophil count was statistically significantly lower in women, and decreased with age and BMI (Wald test, all $p < 0·01$). Both eosinophil and neutrophil counts were statistically significantly higher in participants who reported asthma treatment in the last 12 months (Wald test, all $p < 0·01$, Supplementary Table S2).

Blood inflammatory phenotypes, participants' characteristics and clinical expressions of asthma. The LBE/LBN, LBE/HBN, HBE/LBN and HBE/HBN phenotypes accounted for 57·1%, 6·4%, 32·8% and 3·7% respectively (Supplementary Figure S1). The proportion of women was the highest in the LBE/HBN phenotype, those of obese and current smokers were the highest in the LBE/HBN and HBE/HBN phenotypes, and the proportion of participants with a university educational level was the highest in the HBE/LBN phenotype (Table 1).

Participants with current asthma having the LBE/HBN phenotype reported more nocturnal symptoms, chronic bronchitis and dyspnoea than those with the LBE/LBN phenotype (Table 2). Participants with current asthma with the HBE/LBN phenotype reported

	Participants with current asthma, n=15,019	LBE/LBN, n=8,569 (57%)	LBE/HBN, n=966 (6%)	HBE/LBN, n=4,929 (33%)	HBE/HBN, n=555 (4%)
Age, year, mean ± SD	44·3 ± 13·7	44·7 ± 13·7	45·1 ± 13·5	43·5 ± 13·7	42·9 ± 13·8
Sex, women, n (%)	8,483 (56·5)	4,973 (58·0)	654 (67·7)	2,510 (50·9)	346 (62·3)
Smoking habits, n (%)	*n=14,512*	*n=8,276*	*n=935*	*n=4,736*	*n=538*
Never smokers	6,541 (45·1)	3,854 (46·6)	343 (36·7)	2,148 (45·1)	196 (36·4)
Ex-smokers	4,779 (32·9)	2,800 (33·8)	293 (31·3)	1,543 (32·4)	143 (26·6)
Current smokers	3,192 (22·0)	1,622 (19·6)	299 (32·0)	1,072 (22·5)	199 (37·0)
Body Mass Index (BMI), kg/m^2, n (%)	*n=14,573*	*n=8,302*	*n=937*	*n=4,793*	*n=541*
<18·5	378 (2·6)	216 (2·6)	19 (2·0)	130 (2·7)	13 (2·4)
[18·5-25[	7,196 (49·4)	4,133 (49·8)	383 (40·9)	2,450 (51·1)	230 (42·5)
[25-30[	4,602 (31·6)	2,601 (31·3)	291 (31·1)	1,529 (31·9)	181 (33·5)
≥30	2,396 (16·4)	1,351 (16·3)	244 (26·0)	684 (14·3)	117 (21·6)
Education level, n (%)	*n=14,798*	*n=8,431*	*n=952*	*n=4,870*	*n=545*
Lower than high school	3,512 (23·7)	1,950 (23·1)	316 (33·2)	1,091 (22·4)	155 (28·4)
High school	2,523 (17·1)	1,434 (17·0)	164 (17·2)	818 (16·8)	107 (19·6)
College	4,105 (27·7)	2,382 (28·3)	236 (24·8)	1,342 (27·6)	145 (26·6)
University	4,658 (31·5)	2,665 (31·6)	236 (24·8)	1,619 (33·2)	138 (25·3)
	n=15,018	*n=8,568*	*n=966*	*n=4,929*	*n=555*
FDep quintile 1, n (%)	2,996 (20·0)	1,783 (20·8)	194 (20·1)	929 (18·8)	90 (16·2)
FDep quintile 2, n (%)	3,007 (20·0)	1,695 (19·8)	174 (18·0)	1,027 (20·8)	111 (20·0)
FDep quintile 3, n (%)	2,920 (19·4)	1,681 (19·6)	175 (18·1)	959 (19·5)	105 (18·9)
FDep quintile 4, n (%)	3,101 (20·7)	1,722 (20·1)	207 (21·4)	1,058 (21·5)	114 (20·6)
FDep quintile 5, n (%)	2,994 (19·9)	1,687 (19·7)	216 (22·4)	956 (19·4)	135 (24·3)
White blood cell counts					
Eosinophils × 10^9/L, median (IQR)	0·20 (0·13-0·31)	0·15 (0·10-0·19)	0·15 (0·10-0·19)	0·36 (0·29-0·46)	0·35 (0·29-0·43)
Neutrophils × 10^9/L, median (IQR)	3·34 (2·69-4·15)	3·16 (2·57-3·81)	5·57 (5·24-6·09)	3·30 (2·71-3·95)	5·51 (5·22-5·95)
	n=9,294	*n=5,289*	*n=565*	*n=3092*	*n=348*
FEV1 % pred, mean ± SD	89·7 ± 14·4	90· 5 ± 14·2	87·9 ± 15·9	89·0 ± 14·2	87·7 ± 14·5
FVC % pred, mean ± SD	93·1 ± 12·8	93·2 ± 12·8	91·4 ± 13·5	93·3 ± 12·5	92·3 ± 13·5
FEV_1 <80% pred, n (%)	2,136 (23·0)	1,131 (21·4)	149 (26·4)	759 (24·6)	97 (27·9)
FEV_1/ FVC<0·7, n (%)	1,258 (13·5)	603 (11·4)	88 (15·6)	501 (16·2)	66 (19·0)
FEV_1/ FVC<LLN, n (%)	1,123 (12·1)	518 (9·8)	87 (15·4)	464 (15·0)	54 (15·5)
Age at onset of asthma, n (%)	*n=11,642*	*n=6,540*	*n=729*	*n=3,934*	*n=439*
≥16 years	4,827 (41·5)	2,752 (42·1)	337 (46·2)	1,557 (39·6)	181(41·2)
Asthma symptom score, n (%)	*n=14,093*	*n=8,028*	*n=892*	*n=4,661*	*n=512*
0	2,057 (14·6)	1,197 (14·9)	118 (13·2)	690 (14·8)	52 (10·2)
1	4,786 (34·0)	2,967 (37·0)	291 (32·6)	1,369 (29·4)	159 (31·0)
2	3,230 (22·9)	1,876 (23·4)	211 (23·7)	1,031 (22·1)	112 (21·9)
3	1,892 (13·4)	978 (12·2)	116 (13·0)	713 (15·3)	85 (16·6)
4	1,218 (8·6)	604 (7·5)	87 (9·8)	464 (9·9)	63 (12·3)
5	910 (6·5)	406 (5·0)	69 (7·7)	394 (8·5)	41 (8·0)
		n=7,931	*n=892*	*n=4,625*	*n=524*
Asthma attacks (last 12 months), n (%)	5,318 (38·1)	2,713 (34·2)	321 (36·0)	2,050 (44·3)	234 (44·7)
Nocturnal symptoms (last 12 months), n (%)					
		n=8,434	*n=946*	*n=4,884*	*n=550*
Woken with chest tightness	6,471 (43·7)	3,423 (40·6)	396 (41·9)	2,385 (48·8)	267 (48·6)
		n=8,418	*n=947*	n=4,845	*n=542*
Woken by an attack of shortness of breath	2,241 (15·2)	1,094 (13·0)	160 (16·9)	889 (18·4)	98 (18·1)
		n=8,456	*n=949*	*n=4,859*	*n=548*
Woken by an attack of coughing	6,052 (40·9)	3,384 (40·0)	473 (49·8)	1,925 (39·6)	270 (49·3)
		n=7,982	*n=879*	*n=4,602*	*n=519*
Chronic bronchitis, n (%)*	1,103 (7·9)	588 (7·4)	102 (11·6)	350 (7·6)	63 (12·1)
		n=8,094	*n=892*	*n=4,640*	*n=526*

Table 1 *(Continued)*

	Participants with current asthma, n=15,019	LBE/LBN, n=8,569 (57%)	LBE/HBN, n=966 (6%)	HBE/LBN, n=4,929 (33%)	HBE/HBN, n=555 (4%)
Dyspnoea grade 3, n (%)**	3,259 (23·0)	1,920 (23·7)	309 (34·6)	882 (19·0)	148 (28·1)
		n=8,257	*n=936*	*n=4,778*	*n=540*
Any asthma treatment, n (%)	6,956 (47·9)	3,478 (42·1)	444 (47·4)	2,724 (57·0)	310 (57·4)
Allergic current rhinitis	7,932 (54·3)	4,209 (50·5)	434 (46·9)	2972 (61·8)	317 (58·4)
Other diseases, n (%)[1]					
Depression	2,953 (20·2)	1,776 (21·3)	245 (26·0)	819 (17·0)	113 (21·0)
Hypertension	1,671 (11·4)	945 (11·3)	139 (14·7)	502 (10·5)	85 (15·8)
Cancer	642 (4·4)	384 (4·6)	43 (4·6)	194 (4·1)	21 (3·9)
Respiratory disease[2]	470 (4·0)	253 (3·8)	57 (7·6)	131 (3·4)	29 (6·8)
Diabetes	362 (2·5)	193 (2·3)	37 (4·0)	108 (2·3)	24 (4·5)
Inflammatory arthritis	213 (1·5)	129 (1·6)	23 (2·5)	55 (1·2)	6 (1·1)

***Table 1*: Characteristics of the 15,019 participants with current asthma included in the analyses.**
SD, standard deviation; FEV_1= forced expiratory volume in 1 s. FVC= forced vital capacity. FDep the French deprivation index.
[1] Medical health history from the face to face physician-administered questionnaire.
[2] Chronic bronchitis or emphysema.
* defined by the report of cough or phlegm every day during 3 months.
** Dyspnoea was quantified using the modified Medical Research Council scale.LBE: low blood eosinophils, HBE: high blood eosinophils, LBN: low blood neutrophils, HBN: high blood neutrophils.

statistically significantly more chest tightness and breathlessness awakenings than those with the LBE/LBN phenotype. They also reported statistically significantly less dyspnoea grade 3 than those with the LBE/LBN phenotype. Both HBE/LBN and HBE/HBN phenotypes were associated with more frequent asthma attacks in the last 12 months, more current allergic rhinitis, and asthma symptom scores with an increasing monotonous trend (all p for the trend ≤0·001). Additional adjustment for HPCs gave similar results (Supplementary Table S3).

The main clinical expressions of each phenotype were found whatever the sensitivity analyses performed: *i.e.* after imputation of missing data, with the 75th percentile cut-offs (EOS 0·23 and NEU 4 × 10^9/L), the cut-offs obtained from the ROC curves (EOS 0·25 × 10^9/L and NEU 3·42 × 10^9/L), or the eosinophil cut-off often used in clinical studies (EOS 3 × 10^9/L, Figure 2, Supplementary Tables S4−S7). The LBE/HBN phenotype remained positively and statistically significantly associated with being awakened by an attack of coughing, chronic bronchitis and dyspnoea. The HBE/LBN phenotype remained positively and statistically significantly associated with asthma attacks, asthma symptom score and nocturnal symptoms; and remained negatively and statistically significantly associated with dyspnoea (Figure 3, Supplementary Tables S4−S7). The HBE/HBN phenotype remained positively and statistically significantly associated with asthma attacks and asthma symptom score (Figure 4, Supplementary Tables S4-S7).

Carrying out our analyses on the 14,299 participants with current asthma defined by self-administered questionnaire and with a sum of leukocyte components equal to 100, or on the 10,560 participants defined as having asthma by self-administered and physician-administered questionnaires and with a sum of leukocyte components between 99% and 101%, did not change the results (Supplementary Tables S8−S10).

Blood inflammatory phenotypes and clinical expressions of asthma stratified by sex or age. The distribution of inflammatory phenotypes differed by sex. The LBE/LBN, LBE/HBN, HBE/LBN, and HBE/HBN phenotypes accounted for 55%, 5%, 37% and 3% respectively in men, whereas in women the distribution was 59%, 8%, 30% and 4% respectively (Supplementary Figure S1).

The distribution of inflammatory phenotypes also differed by age group. The LBE/LBN, LBE/HBN, HBE/LBN, and HBE/HBN phenotypes accounted for 55%, 6%, 35%, and 4% of participants with current asthma under 40 years of age respectively, whereas for those aged 40 and over, the distribution was 58%, 7%, 31%, and 3% respectively (Supplementary Figure S1). No statistically significant heterogeneity was observed according to sex (Table 2) or age (Figure 5).

Discussion

This study identified four blood inflammatory phenotypes in adults with current asthma in a large French population-based study. Each inflammatory phenotype was associated with distinct clinical expressions of asthma. Performing various sensibility analyses did not change the conclusions. The main clinical expressions associated to each phenotype were found regardless of sex and age groups.

	Inflammatory phenotypes		
	LBE/HBN	**HBE/LBN**	**HBE/HBN**
All (n=15,019)	n=966 (6%)*	n=4,929 (33%)*	n=555 (4%)*
	OR (95% CI)	OR (95% CI)	OR (95% CI)
Asthma attacks (last 12 months)	0·96 (0·82-1·13)	1·31 (1·20-1·42)	1·25 (1·02-1·53)
Asthma symptom score			
0	ref.	ref.	ref.
1	0·96 (0·75-1·22)	0·99 (0·87-1·12)	1·49 (1·05-2·10)
2	1·01 (0·79-1·30)	1·10 (0·97-1·26)	1·45 (1·01-2·07)
3	0·97 (0·73-1·29)	1·33 (1·15-1·53)	1·85 (1·27-2·70)
4	1·26 (0·92-1·71)	1·36 (1·16-1·60)	2·10 (1·40-3·14)
5	1·32 (0·94-1·86)	1·62 (1·35-1·93)	1·68 (1·06-2·66)
	(p trend=0·05)	(p trend <0·0001)	(p trend=0·001)
Nocturnal symptoms (last 12 months)			
Woken with chest tightness	1·00 (0·87-1·16)	1·30 (1·20-1·40)	1·20 (0·99-1·45)
Woken by an attack of shortness of breath	1·24 (1·02-1·51)	1·36 (1·22-1·51)	1·23 (0·97-1·58)
Woken by an attack of coughing	1·27 (1·10-1·47)	0·99 (0·92-1·07)	1·24 (1·03-1·49)
Chronic bronchitis	1·42 (1·12-1·81)	0·96 (0·82-1·11)	1·26 (0·93-1·72)
Dyspnoea grade 3†	1·21 (1·02-1·43)	0·79 (0·71-0·87)	0·94 (0·75-1·18)
Men (n=6,536)	n=312 (5%)	n=2,419 (37%)	n=209 (3%)
Asthma attacks (last 12 months)	0·72 (0·53-0·99)	1·35 (1·19-1·53)	1·21 (0·87-1·69)
Asthma symptom score			
0	ref.	ref.	ref.
1	1·06 (0·71-1,60)	1·07 (0·91-1·27)	1·74 (1·08-2·82)
2	1·28 (0·84-1,96)	1·28 (1·07-1·54)	1·34 (0·79-2·28)
3	1·14 (0·69-1,88)	1·53 (1·25-1·88)	1·22 (0·66-2·25)
4	2·06 (1·25-3,39)	1·38 (1·08-1·76)	1·99 (1·08-1·76)
5	1·99 (1·16-3·41)	1·80 (1·39-2·33)	1·19 (0·55-2·55)
	(p trend =0·001)	(p trend <0·0001)	(p trend =0·53)
Nocturnal symptoms (last 12 months)			
Woken with chest tightness	1·09 (0·84-1·41)	1·33 (1·19-1·49)	0·85 (0·62-1·16)
Woken by an attack of shortness of breath	1·68 (1·23-2·31)	1·44 (1·24-1·68)	1·07 (0·70-1·62)
Woken by an attack of coughing	1·25 (0·97-1·61)	0·98 (0·88-1·11)	1·35 (0·99-1·83)
Chronic bronchitis	1·56 (1·07-2·26)	0·91 (0·74-1·12)	1·28 (0·80-2·04)
Dyspnoea grade 3†	1·25 (0·90-1·73)	0·86 (0·72-1·03)	0·95 (0·62-1·47)
Women (n=8,483)	n=654 (8%)	n=2,510 (30%)	n=346 (4%)
Asthma attacks (last 12 months)	1·05 (0·86-1·28)	1·27 (1·13-1·42)	1·23 (0·95-1·60)
Asthma symptom score			
0	ref.	ref.	ref.
1	0,85 (0·63-1,15)	0·89 (0·7-1·07)	1·30 (0·79-2·14)
2	0·85 (0·62-1,17)	0·94 (0·78-1·13)	1·50 (0·92-2·47)
3	0·83 (0·58-1,18)	1·14 (0·93-1·18)	2·19 (1·32-3·65)
4	0·94 (0·64-1·40)	1·30 (1·04-1·62)	2·20 (1·27-3·81)
5	0·97 (0·63-1·51)	1·43 (1·11-1·83)	1·95 (1·06-3·58)
	(p trend =0·93)	(p trend <0·0001)	(p trend =0·0004)
Nocturnal symptoms (last 12 months)			
Woken with chest tightness	0·97 (0·81-1·17)	1·27 (1·14-1·41)	1·48 (1·17-1·88)
Woken by an attack of shortness of breath	1·03 (0·80-1·33)	1·29 (1·12-1·49)	1·32 (0·98-1·79)
Woken by an attack of coughing	1·26 (1·06-1·51)	0·99 (0·89-1·10)	1·17 (0·92-1·47)
Chronic bronchitis	1·36 (0·99-1·85)	0·99 (0·81-1·22)	1·25 (0·82-1·88)
Dyspnoea grade 3†	1·19 (0·98-1·45)	0·74 (0·66-0·84)	0·94 (0·72-1·23)

***Table 2*: Associations between clinical expressions of asthma and inflammatory phenotypes.**

* OR (95% CI) estimated by non-ordinal polytomous logistic models, adjusted for age (continuous), smoking (non-smoker / ex-smoker / smoker), body mass index, asthma treatment, education level and French Deprivation Index.

† Dyspnoea was quantified using the modified Medical Research Council scale. LBE: low blood eosinophils, HBE: high blood eosinophils, LBN: low blood neutrophils, HBN: high blood neutrophils. P trend were calculated using Wald-test.

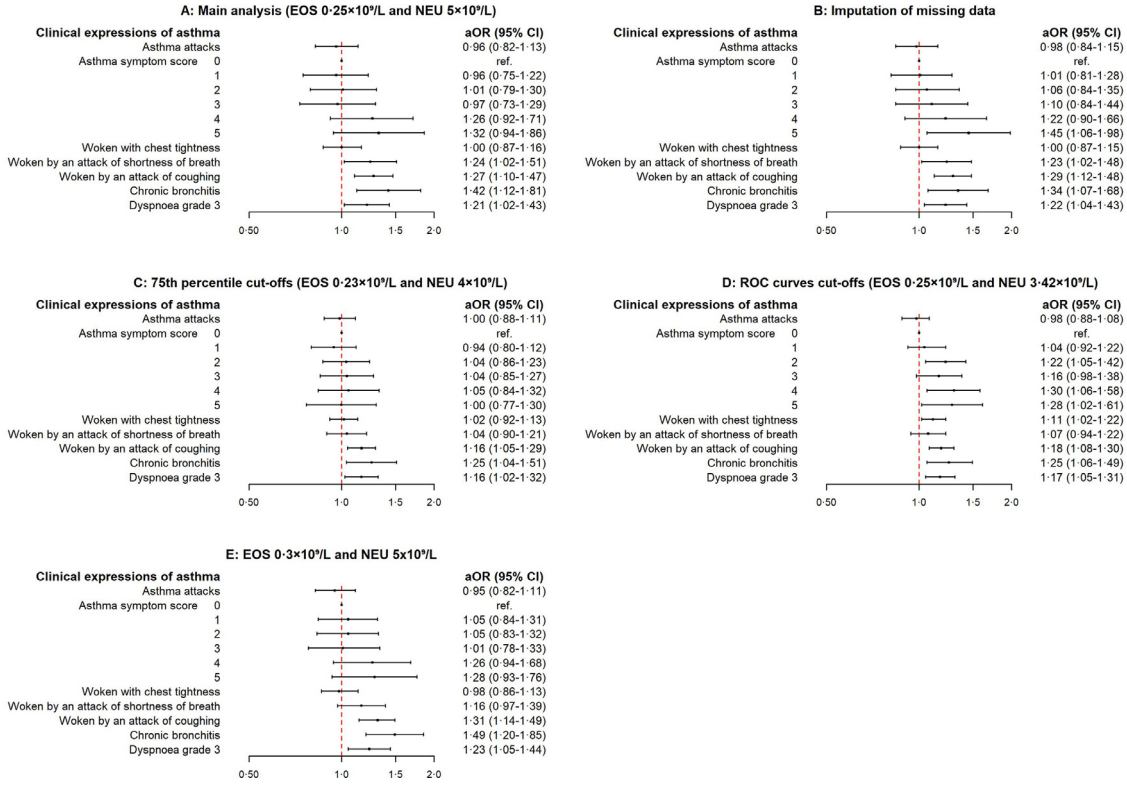

Figure 2. Associations between the low blood eosinophils and high blood neutrophils (LBE/HBN) phenotype and clinical expressions of asthma in the 15,019 participants with current asthma. Each dot represents the adjusted odds ratio (aOR) and its 95% confidence interval (95% CI) estimated by non-ordinal polytomous logistic models, adjusted for age (continuous), sex and smoking (non-smoker / ex-smoker / smoker), body mass index, asthma treatment, education level and French Deprivation index. A: Main analysis (Eosinophils 0·25 $\times$ 10^9/L and Neutrophils 5 $\times$ 10^9/L), B: Imputation of missing data, C: 75th percentile cut-offs (Eosinophils 0·23 $\times$ 10^9/L and Neutrophils 4 $\times$ 10^9/L), D: ROC curves cut-offs (Eosinophils 0·25 $\times$ 10^9/L and Neutrophils 3·42 $\times$ 10^9/L), E: Eosinophils 0·3 $\times$ 10^9/L and Neutrophils 5 $\times$ 10^9/L.

Strengths and limitations

We defined asthma on the basis of standardised and validated questionnaires. The questionnaire approach is the preferred tool in epidemiology for identifying participants with asthma in the general population; however, its use is subject to reporting or memory bias.[25] In the present study, current asthma refers to report of symptoms in the last 12 months, and results based on participants' reports had a very good concordance with those based on the responses from the face-to-face physician-administered questionnaire (93%). Furthermore, at the time participants completed the questionnaires, they did not know their phenotypes and were unaware of the study hypotheses, thus reducing the scope for differential misclassifications. The definition of current asthma was designed to exhaustively as possible ever-asthma participants with asthma manifestations during the last 12 months, based on the report of (1) an asthma attack or (2) taking treatment for asthma or (3) respiratory symptoms during this period. However, we must acknowledge that some participants with current asthma may not have been identified as such, although we have no way to estimate their number. Another potential limitation of this study is that the white blood cell count was performed by methods varying across centres, which may possibly lead to misclassification bias when applying cut-offs, but if it exists, this bias can only be non-differential and will dilute the actual association, and does not explain the associations we observed. We acknowledge that a residual confounding bias may remain even after adjustment for confounders. To approximate other potential unmeasured confounding factors, e.g. that participants from a HPC are more similar, in terms of lifestyle or environmental exposures for example, than participants from other HPCs, the HPC was used as an adjustment factor in the analyses and the results were consistent.

Interpretation of results

We observed a crude prevalence of ever asthma in adults of 13·5%, which is very close to the weighted prevalence

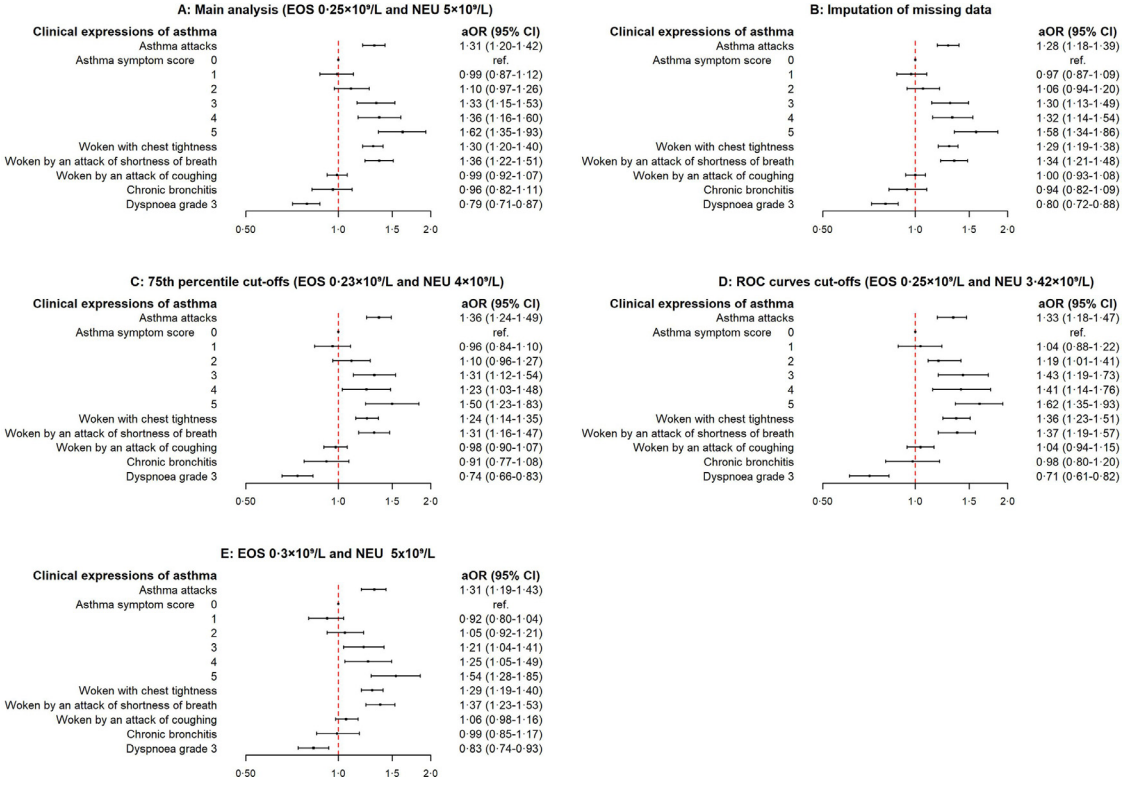

Figure 3. Associations between the high blood eosinophils and low blood neutrophils (HBE/LBN) phenotype and clinical expressions of asthma in the 15,019 participants with current asthma. Each dot represents the adjusted odds ratio (aOR) and its 95% confidence interval (95% CI) estimated by non-ordinal polytomous logistic models, adjusted for age (continuous), sex and smoking (non-smoker / ex-smoker / smoker), body mass index, asthma treatment, education level and French Deprivation index. A: Main analysis (Eosinophils 0·25 $\times$ 10^9/L and Neutrophils 5 $\times$ 10^9/L), B: Imputation of missing data, C: 75th percentile cut-offs (Eosinophils 0·23 $\times$ 10^9/L and Neutrophils 4 $\times$ 10^9/L), D: ROC curves cut-offs (Eosinophils 0·25 $\times$ 10^9/L and Neutrophils 3·42 $\times$ 10^9/L), E: Eosinophils 0·3 $\times$ 10^9/L and Neutrophils 5 $\times$ 10^9/L.

of 14% recently reported in CONSTANCES[4] and that of 12·8% reported in the ASTHMAPOP survey.[2] The prevalence of current asthma was higher in our study than in ASTHMAPOP (9·5 *vs.* 6·4% respectively), likely explained by a difference in the current asthma definition. In our study, neutrophil count was associated with age, sex and smoking habits. These results are consistent with the findings from the "Agence nationale d'accréditation et d'évaluation en santé" (Anaes, integrated in HAS) which pointed out that tobacco consumption significantly increased the different leukocyte lines, especially neutrophils.[26] The lower eosinophil count we observed in women and obese participants with asthma is consistent with previous results from epidemiological studies reporting negative correlations between BMI and blood eosinophil count in asthma.[27]

We observed that the LBE/LBN phenotype was the most common, followed by the HBE/LBN, LBE/HBN and HBE/HBN phenotypes. These results are consistent with those previously found by Nadif et al.[10] and McGrath et al.[28] in blood and in induced sputum respectively. We found that the four blood inflammatory phenotypes were associated with distinct clinical expressions of asthma: the LBE/HBN phenotype was positively associated with woken by an attack of coughing, chronic bronchitis and dyspnoea. The HBE/LBN and HBE/HBN phenotypes were positively associated with asthma attacks and asthma symptom score; the HBE/LBN phenotype was also associated with woken with chest tightness, woken by an attack of shortness of breath and dyspnoea. These results are difficult to compare with those in the literature. Indeed, apart from the EGEA study, no study has used blood eosinophils and neutrophils simultaneously to identify and characterize these inflammatory phenotypes among participants with asthma. Our results are similar to those found in the EGEA study, except for the association between the LBE/HBN phenotype and woken by an attack of coughing or dyspnoea that was not statistically significant in EGEA probably due to the small sample size (n=48).[9] Our results are also in line with the well-known characteristics of eosinophilic asthma such as asthma attacks, allergy and the severity of symptoms,[29,30] and with the association between high blood eosinophil count and

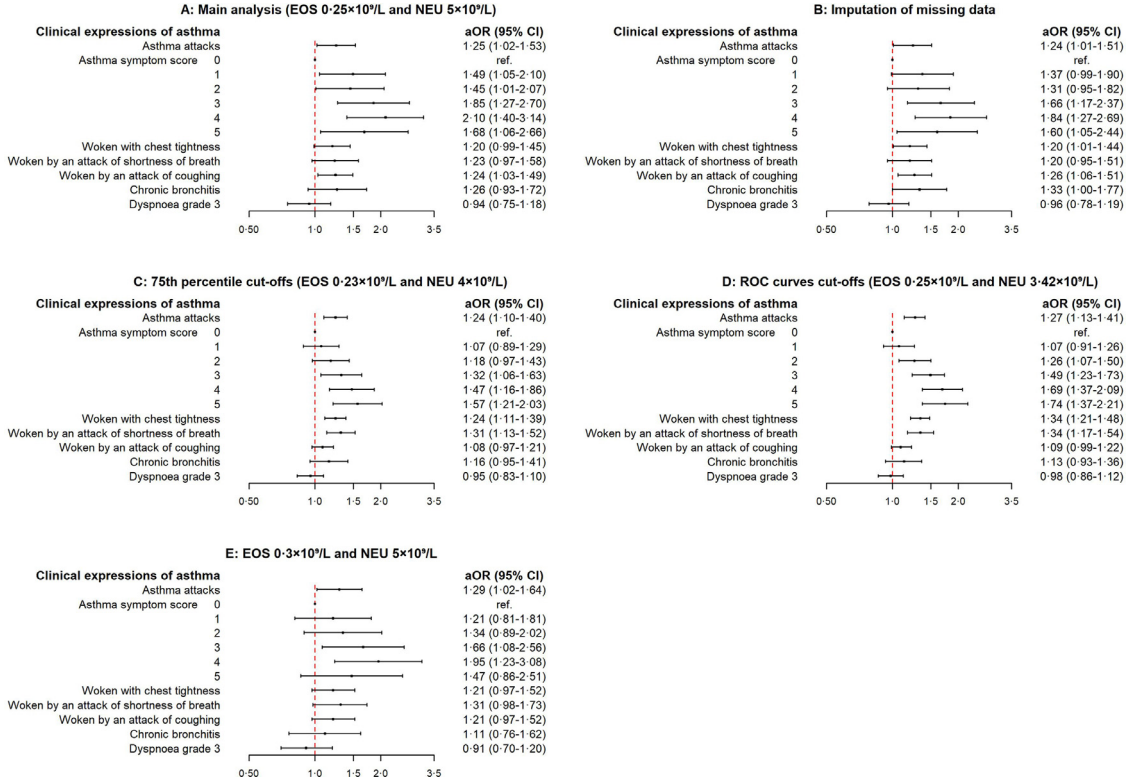

Figure 4. Associations between the high blood eosinophils and high blood neutrophils (HBE/HBN) phenotype and clinical expressions of asthma in the 15,019 participants with current asthma. Each dot represents the adjusted odds ratio (aOR) and its 95% confidence interval (95% CI) estimated by non-ordinal polytomous logistic models, adjusted for age (continuous), sex and smoking (non-smoker / ex-smoker / smoker), body mass index, asthma treatment, education level and French Deprivation index. A: Main analysis (Eosinophils 0·25 $\times$ 10^9/L and Neutrophils 5 $\times$ 10^9/L), B: Imputation of missing data, C: 75th percentile cut-offs (Eosinophils 0·23 $\times$ 10^9/L and Neutrophils 4 $\times$ 10^9/L), D: ROC curves cut-offs (Eosinophils 0·25 $\times$ 10^9/L and Neutrophils 3·42 $\times$ 10^9/L), E: Eosinophils 0·3 $\times$ 10^9/L and Neutrophils 5 $\times$ 10^9/L.

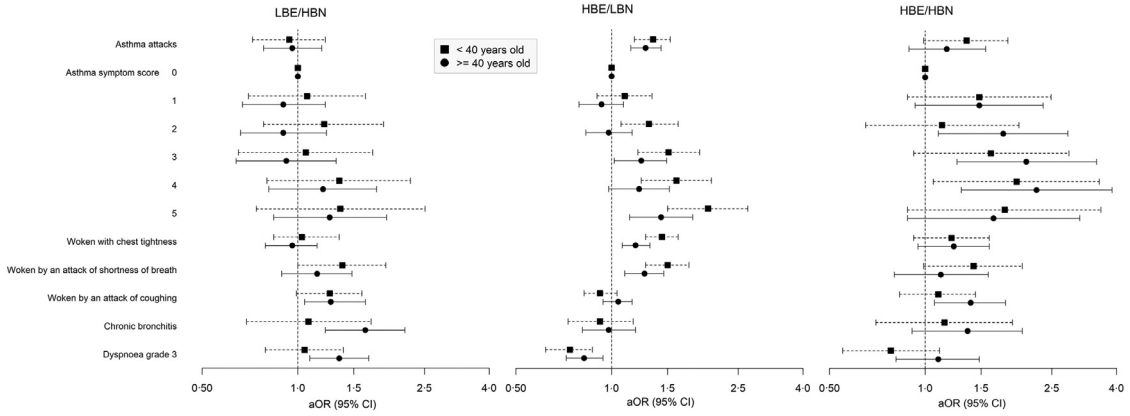

Figure 5. Associations between clinical expressions of asthma and inflammatory phenotypes stratified by age (<40/≥40 years). Each dot represents the adjusted odds ratio (aOR) and its 95% confidence interval (95% CI) estimated by non-ordinal polytomous logistic models, adjusted for age (continuous), sex and smoking (non-smoker / ex-smoker / smoker), BMI, asthma treatment, education level and Fdep. LBE: low blood eosinophils, HBE: high blood eosinophils, LBN: low blood neutrophils, HBN: high blood neutrophils.

more frequent asthma attacks reported by Tran *et al.* from US general population.[12] In addition, Vedel et al., in the Copenhagen general population study, reported that high blood eosinophil counts were associated with an increased risk of both moderate and severe asthma exacerbations.[13] In contrast to eosinophilic asthma, which is well-recognised, there is still scepticism about the existence of neutrophilic asthma. Indeed, some authors believe that neutrophilic asthma is not a relevant component of the disease and that it refers to COPD rather than asthma,[31] while others argue that it exists and is related to a more severe form of asthma, less responsive to corticosteroid therapy.[32,33] Some of the characteristics we found associated with the LBE/HBN phenotype were previously found in adult patients with high neutrophils in their sputum: be older age, late-onset asthma, less atopy (allergic rhinitis as a proxy in our study), poorer lung function, and more comorbidities.[34] A study using a data-driven method identified among women a cluster characterized by late onset of asthma, obesity, low blood eosinophils and high blood neutrophils.[35] Importantly, the associations we found remained statistically significant even after adjustment for smoking history. Overall, all these results provide evidence in favour of the existence of a neutrophilic phenotype.

Despite the lack of consensus on the cut-offs to be used especially for neutrophils, the value of $0{\cdot}25 \times 10^9$/L for eosinophils is often used in epidemiology, and a cut-off of $0{\cdot}3 \times 10^9$/L for blood eosinophils is widely used in clinical studies to identify eosinophilic asthma.[2,9] In our study, whatever the cut-offs used i.e. the CONSTANCES 75th percentile cut-offs, cut-offs obtained from the ROC curves or the cut-off of $0{\cdot}3 \times 10^9$/L for eosinophils, the main clinical expressions of each phenotype were found. After stratification on sex or on age, the main clinical expressions of each phenotype were found again, highlighting the robustness of our results.

In our study, the LBE/LBN phenotype was the most common, and this result was also observed whatever the sex and age groups. The distribution of all phenotypes differed by sex or age groups. Men had the highest proportion of HBE/LBN phenotype, which may be linked to the earlier onset of asthma in men than in women.[36] By contrast, women had the highest proportion of LBE/HBN phenotype, a result that could be related to a difference in body composition between men and women, women having mostly subcutaneous adipose tissue that secretes more leptin leading to increased neutrophilic inflammation.[35] After stratification by age groups, we observed that the proportion of the HBE/LBN phenotype was highest among participants less than 40 years old, and the proportion of the LBE/HBN phenotype highest in those of 40 years old and over. Asthma is a complex disease which expression and characteristics may considerably evolve across the life course due to, among others, the effects of sex hormones and of occupational and environmental factors.[37] In the present study, the main clinical expressions of the LBE/HBN phenotype: nocturnal symptoms, chronic bronchitis and dyspnoea, that are COPD-like symptoms, were more evidenced in participants 40 years old and over. One hypothesis to explain these results may be that the clinical expressions associated with the LBE/HBN phenotype is the result of a specific evolution of asthma.[34] Indeed, eosinophils and neutrophils in the blood are associated with different immune responses (Thelper2 -Th2 or T2- and non-T2, respectively), and these immune responses are in turn associated to distinct pathophysiological mechanisms that could partly explain the phenotypic heterogeneity of asthma.[5,38]

In conclusion, we found clinical differences according to blood inflammatory phenotypes in a large population-based cohort, similar to those observed in case-control and clinical studies. We confirm that the paucigranulocytic phenotype is a phenotype of interest, and that the neutrophilic phenotype is more than a misconception or misnomer. The identification and characterization of phenotypes based on eosinophils and neutrophils, two key inflammatory cells of asthma, could enable better patient management. Such phenotypes are of interest to better understand the mechanisms by which environmental factors affect asthma.

Declaration of interests

Dr. N.Roche reports grants and personal fees from GSK, Boehringer Ingelheim, Pfizer and Novartis; personal fees from Teva, AstraZeneca, Chiesi, Sanofi and Zambon outside the submitted work. The other authors declare no potential conflicts of interest.

Contributors

T.Tsiavia, L.Orsi and R.Nadif have verified the underlying data, designed and conducted the study; J.Henny, M.Goldberg, M.Zins, L.Orsi and R.Nadif contributed to the data acquisition; T.Tsiavia, L.Orsi and R.Nadif interpreted the data; T.Tsiavia, L.Orsi and R.Nadif drafted the article; T.Tsiavia, J.Henny, M.Goldberg, M.Zins, N. Roche, L.Orsi and R.Nadif contributed to the critical review of important intellectual content; all authors edited and approved the final manuscript.

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Data sharing

Access to sensitive and personal data, such as those from CONSTANCES cohort, is restricted by French law. The CONSTANCES coordination team makes the data available, upon request, to qualified researchers who have obtained prior authorization from the French national data protection authority (Commission de l'informatique et des libertés, CNIL). Information for applicants to CONSTANCES data is available on the website: https://www.constances.fr/CFP.pdf. CONSTANCES investigators may be contacted at following address: contact@constances.fr

Supplementary materials

Supplementary material associated with this article can be found in the online version at doi:10.1016/j.ebiom.2022.103875.

References

1 GBD Chronic Respiratory Disease Collaborators. Prevalence and attributable health burden of chronic respiratory diseases, 1990–2017: a systematic analysis for the Global Burden of Disease Study 2017. *Lancet Respir Med*. 2020;8(6):585–596.
2 Raherison-Semjen C, Izadifar A, Russier M, et al. Prévalence et prise en charge de l'asthme de l'adulte en France en 2018 : enquête ASTHMAPOP. *Rev Mal Respir*. 2019;36:A7.
3 Delmas MC, Fuhrman C. L'asthme en France : synthèse des données épidémiologiques descriptives. *Rev Mal Respir*. 2010;27(2):151–159.
4 Delmas MC, Bénézet L, Ribet C, et al. Prévalence de l'asthme chez l'adulte en France, données de la cohorte Constances. *Rev Mal Respir*. 2021. juinS0761842521002461.
5 Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med*. 2012;18(5):716–725.
6 Bush A. Pathophysiological mechanisms of asthma. *Front Pediatr*. 2019;7:68.
7 Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology*. 2006;11(1):54–61.
8 Gibson PG. Tackling asthma phenotypes in community studies. *Thorax*. 2009;64(5):369–370.
9 Nadif R, Siroux V, Oryszczyn MP, et al. Heterogeneity of asthma according to blood inflammatory patterns. *Thorax*. 2009;64(5):374–380.
10 Nadif R, Siroux V, Boudier A, et al. Blood granulocyte patterns as predictors of asthma phenotypes in adults from the EGEA study. *Eur Respir J*. 2016;48(4):1040–1051.
11 Tran TN, Zeiger RS, Peters SP, et al. Overlap of atopic, eosinophilic, and TH2-high asthma phenotypes in a general population with current asthma. *Ann Allergy Asthma Immunol*. 2016;116(1):37–42.
12 Tran TN, Khatry DB, Ke X, Ward CK, Gossage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. *Ann Allergy Asthma Immunol*. 2014;113(1):19–24.
13 Vedel-Krogh S, Fallgaard Nielsen S, Lange P, Vestbo J, Nordestgaard BG. Association of blood eosinophil and blood neutrophil counts with asthma exacerbations in the copenhagen general population study. *Clin Chem*. 2017;63(4):823–832.
14 Li J, Ye L, She J, Song Y. Clinical differences between early- and late-onset asthma: a population-based cross-sectional study. *Can Respir J*. 2021;2021:8886520.
15 Musk AW, Knuiman M, Hunter M, et al. Patterns of airway disease and the clinical diagnosis of asthma in the Busselton population. *Eur Respir J*. 2011;38(5):1053–1059.
16 Backman H, Lindberg A, Hedman L, et al. FEV1 decline in relation to blood eosinophils and neutrophils in a population-based asthma cohort. *World Allergy Organ J*. 2020;13(3):100110.
17 Zins M, Goldberg M, CONSTANCES team. The French CONSTANCES population-based cohort: design, inclusion and follow-up. *Eur J Epidemiol*. 2015;30(12):1317–1328.
18 Henny J, Nadif R, Got SL, Lemonnier S, Ozguler A, Ruiz F, et al. The CONSTANCES cohort Biobank: an open tool for research in epidemiology and prevention of diseases. *Front Public Health*. 2020;8:605133.
19 CONSTANCES Cohort: Scientific protocol. Available at: https://www.constances.fr/_assets/_pdf/Scientific-protocol-01-2015.pdf Accessed 5 January 2022.
20 Sunyer J, Pekkanen J, Garcia-Esteban R, et al. Asthma score: predictive ability and risk factors. *Allergy*. 2007;62(2):142–148.
21 Yorke J, Garrow A, Tyson S, et al. Evaluation of individual activity descriptors of the MRC dyspnoea scale: do they add up? 112 Clin Probl COPD. sept 2015;PA681.
22 Miller MR, Hankinson J, Brusasco V, et al. Standardisation of spirometry. *Eur Respir J*. 2005;26(2):319–338.
23 Lee KJ, Carlin JB. Multiple imputation for missing data: fully conditional specification versus multivariate normal imputation. *Am J Epidemiol*. 2010;171(5):624–632.
24 Chowdhury NU, Guntur VP, Newcomb DC. Wechsler ME. Sex and gender in asthma. *Eur Respir Rev*. 2021;30(162):210067.
25 Althubaiti A. Information bias in health research: definition, pitfalls, and adjustment methods. *J Multidiscip Healthc*. 2016;9:211–217.
26 Haute Autorité de Santé. Lecture critique de l'hémogramme : valeurs seuils à reconnaître comme probablement pathologiques et principales variations non pathologiques. SaintDenis La Plaine; 1997.
27 Caspard H, Tran TN, Ambrose C. Population-based evaluation of blood eosinophil counts and association with patient characteristics in US adults with asthma. *J Allergy Clin Immunol*. 2018;141(2):AB103.
28 McGrath KW, Icitovic N, Boushey HA, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. *Am J Respir Crit Care Med*. 2012;185(6):612–619.
29 Carr TF, Zeki AA, Kraft M. Eosinophilic and noneosinophilic asthma. *Am J Respir Crit Care Med*. 2018;197(1):22–37.
30 Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet Lond Engl*. 2006;368(9537):804–813.
31 Nair P, Surette MG, Virchow JC. Neutrophilic asthma: misconception or misnomer? *Lancet Respir Med*. 2021;9(5):441–443.
32 Gibson PG, Foster PS. Neutrophilic asthma: welcome back!. *Eur Respir J*. 2019;54(5):1901846.

33 Zhang J, Zhu Z, Zuo X, et al. The role of NTHi colonization and infection in the pathogenesis of neutrophilic asthma. *Respir Res*. 2020;21:170.

34 Crisford H, Sapey E, Rogers GB, et al. Neutrophils in asthma: the good, the bad and the bacteria. *Thorax*. 2021;76(8):835–844.

35 Hsiao HP, Lin MC, Wu CC, Wang CC, Wang TN. Sex-specific asthma phenotypes, inflammatory patterns, and asthma control in a cluster analysis. *J Allergy Clin Immunol Pract*. 2019;7(2):556–567.

36 Postma DS. Gender differences in asthma development and progression. *Gend Med*. 2007;4(Suppl B):S1333–S1346.

37 Kuruvilla ME, Vanijcharoenkarn K, Shih JA, Lee FEH. Epidemiology and risk factors for asthma. *Respir Med*. 2019;149:16–22.

38 Kuruvilla ME, Lee FEH, Lee GB. Understanding asthma phenotypes, endotypes, and mechanisms of disease. *Clin Rev Allergy Immunol*. 2019;56(2):219–233.