Blood granulocyte patterns as predictors of asthma phenotypes in adults from the EGEA study

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SUPPLEMENTARY DATA

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Methods

Study design

Data used for the analyses were collected in the framework of the Epidemiological study on the Genetics and Environment of Asthma (EGEA, https://egeanet.vjf.inserm.fr/). EGEA is a French cohort study based on an initial group of asthma cases and their first-degree relatives, and controls (388 families, 415 controls, first survey EGEA1). The protocol and descriptive characteristics have been described previously [1, 2]. A 12-year follow-up of the initial cohort was conducted between 2003 and 2007 (EGEA2) [3]. All participants responded to a questionnaire based on international standardized tools to diagnose asthma and to determine respiratory and allergic symptoms, treatments, and environmental exposures.

Respiratory phenotypes

Inclusion criteria used to define asthma in cases were based on positive self-reported response to the four questions: "*Have you ever had attacks of breathlessness at rest with wheezing?*", "*Have you ever had asthma attacks?*", "*Was this diagnosis confirmed by a physician?*", and "*Have you had an asthma attack in the last 12 months?*", or a positive response to at least two questions and a positive review of their medical record. Asthma in relatives of cases was defined as a positive answer to at least one of the first two questions. Among subjects with asthma, "current asthma" was defined by a report of respiratory symptoms in the past 12 months (wheeze, nocturnal chest tightness, attacks of breathlessness following strenuous activity, at rest or at night time, and asthma attacks) or use of inhaled and/or oral medicines because of breathing problems. Upper and lower respiratory tract infections within the last four weeks were recorded.

Allergic sensitization was defined by a positive skin prick test (SPT+) with a mean wheal diameter ≥3mm than the negative control for at least one of 12 aeroallergens (indoor: cat,

Dermatophagoides pteronyssinus, Blattela germanica, outdoor: olive, birch, Parieteria judaica, timothy grass, Cupressus and ragweed pollen, and molds: Aspergillus, Cladosporium herbarum, Alternaria tenuis). Subjects were classified as sensitized if they have one or more SPT+.

Total Immunoglobulin E (IgE) determination was assessed by UniCAP system (Pharmacia®) from blood samples in a centralized laboratory, and expressed in international units (IU) per milliliter. Skin prick tests (SPT) to 12 aeroallergens were also performed.

Blood granulocyte patterns

Participants were asked to avoid smoking for at least 1 h and to avoid use of their inhaler for at least 4 h prior to testing.

Receiver‐Operating Characteristic (ROC) curve was generated in order to determine the ability of neutrophil counts to discriminate between participants with poor asthma control (partly-controlled or uncontrolled asthma) and those with controlled asthma.

Results

The ROC curve is shown in Figure 1. An increase of 500 cells/mm^3 in neutrophil counts was associated with an increased risk of poor asthma control of OR=1.15 [1.07-1.25]. The area under the curve was 0.618 (95%CI 0.566‑0.669; P<0.01). Cut-off points given the higher percentage of correctly classified participants were selected: the correct criterion is only based on the proportion of correctly classified observations and the two cut-off points of 4780 and 5080 cells/mm^3 gave the best percentage (63.0%). The Youden index is the smallest vertical distance from the uninformative diagonal to the cut-off point. The optimal cut‑off points were between 4360 and 5080 cells/mm^3 including the cut-off point of 5000 cells/mm^3 of our study. For each cut-off point, the sensitivity, specificity, positive predictive value and negative predictive value were calculated. Associations between neutrophil inflammation and asthma control for each cut-off point were then calculated at EGEA2 in participants having high neutrophil counts at baseline (OR1), or high neutrophil counts at baseline and follow-up (OR2), and gave similar findings as those obtained with the cut-off point of 5000 cells/mm^3.

Figure legends

Figure 1. Receiver‐operating characteristic (ROC) curve for asthmatics with poor asthma control and those with controlled asthma (n=474).

Sens=sensibility, Spec=specificity, PPV=positive predictive value, NPV=negative predictive value, adjusted OR1= association between high neutrophil counts at EGEA2 and poor asthma control at EGEA2, adjusted OR2= association between high neutrophil counts at EGEA1 and EGEA2 (stable pattern) and poor asthma control at EGEA2.

Table 1. Comparison of main characteristics at baseline between subjects included in the longitudinal analysis, non selected subjects and all subjects

	Subjects included in the longitudinal analysis (n=242)		**Non selected subjects (n=139)**		**All subjects (n=381)**	
	n	Percent, mean	n	Percent, mean	n	Percent, mean
Age, year, mean ± SD	242	36.4 (13.1)	139	36.5 (13.3)	381	36.5 (13.1)
Cases	242	57.8	139	43.2#	381	52.5
Sex, women	242	53.3	139	43.2	381	49.6
Age of asthma onset, ≥ 16 years	237	49.4	126	44.4	173	47.7
Total IgE, IU/ml, GM	234	161 (73.0-413)	139	174 (69.0-387)	373	166 (71.0-398)
Skin prick test positivity (any of 12 allergens)*	234	75.2	135	74.8	369	75.1
White blood cell counts						
Eosinophils/mm^3, mean (SD)	242	284 (210)	139	261 (182)	381	275 (200)
Neutrophils/mm^3, mean (SD)	242	4235 (1681)	139	4146 (1646)	381	4202 (1667)
FEV_1 % predicted, mean (SD)	241	92.7 (19.5)	138	94.7 (20.4)	379	93.5 (19.9)
FEV_1 < 80% predicted, %	241	22.4	138	19.6	379	21.4
Methacholine challenge, PD 20 ≤ 4 mg†	114	76.3	71	73.2	185	75.1
Asthma attacks in the last 12 months	234	75.2	124	66.9	358	72.3
Smoking habits						
smokers	41	17.1	41	29.7#	82	21.7
ex-smokers	69	28.9	32	23.2	101	26.8
non-smokers	129	54.0	65	47.1	194	51.5
Body Mass Index (BMI), kg/m^2, mean (SD)	241	23.0 (3.69)	139	23.7 (4.02)	380	23.3 (3.82)
Respiratory infection (last 4 weeks)	241	16.6	139	11.5	380	14.7
Inhaled corticosteroids (last 12 months)	242	53.3	139	50.4	199	52.2

GM= geometric mean is shown with interquartile range. * Skin Prick Test positivity (SPT+) was defined by a mean wheal diameter ≥3mm than the negative control for at least one of 12 aeroallergens. † Methacholine challenge test was not performed if baseline FEV_1 <80% predicted. P values comparing the distributions of the characteristics between the subjects included in the longitudinal anlysis with those who were not included were obtained from independent T Tests for continuous and Chi2 test for qualitative variables. P values >0.05 are not shown. # P≤0.05.

Table 2. Characteristics of adults with asthma according to their blood granulocyte pattern (cross-sectional analyses, n=474)

	EOS^{lo}/NEU^{lo} (n=232)	**EOS^{lo}/NEU^{hi} (n=50)**	**EOS^{hi}/NEU^{lo} (n=150)**	**EOS^{hi}/NEU^{hi} (n=42)**
Age, year, mean ± SD	38.5 ± 15.7	43.0 ± 18.4	36.4 ± 15.7	37.8 ± 16.2
Cases, %	49.6	52.0	56.0	59.5
Sex, women, %	47.4	52.0	44.0	59.5
Body Mass Index (BMI), kg/m^2, %				
<20	10.0	8.0	16.4	14.6
[20-25[	53.0	54.0	51.4	61.0
[25-30[	26.1	26.0	24.7	12.2
≥30	10.9	12.0	7.5	12.2
Smoking habits, %				
smokers	22.4	20.0	26.0	30.9
ex-smokers	28.9	28.0	18.0	16.7
non-smokers	48.7	52.0	56.0	52.4
Age of asthma onset, %				
≤ 4 years	31.2	14.6	38.3	30.0
4-16 years	37.1	41.7	33.6	35.0
> 16 years	31.7	43.7	28.1	35.0
Total IgE, IU/ml, GM	129 (48.6-316)	111 (54.7-224)	208 (98.0-545)	223 (99.2-517)
Skin prick test positivivity *	82.9	70.2	83.3	88.9
FEV_1 % predicted, mean ± SD	98.8 ± 18.5	91.6 ± 20.3	94.6 ± 17.1	92.7 ± 16.2
FEV_1 < 80% predicted, %	13.5	20.0	13.1	17.1
Methacholine challenge, PD 20 ≤ 4 mg †	66.0	64.0	79.3	90.0
Inhaled corticosteroids (last 12 months), %	35.5	54.0	52.0	54.8
Respiratory infections (last 4 weeks), %	13.0	22.0	13.0	24.4

GM= geometric mean is shown with interquartile range. *Skin Prick Test positivity (SPT+) was defined by a mean wheal diameter ≥3mm than the negative control for at least one of 12 aeroallergens. †Methacholine challenge test was not performed if baseline FEV_1 <80% predicted

Table 3. Change in blood granulocyte patterns between EGEA1 and EGEA2 in adults with asthma (longitudinal analyses, n=242)

	Neutrophil pattern % (n)		**Eosinophil pattern % (n)**	
	Low pattern at EGEA2	High pattern at EGEA2	Low pattern at EGEA2	High pattern at EGEA2
Low pattern at EGEA1	**83.7** (159)	16.3 (31)	**72.8** (99)	27.2 (37)
High pattern at EGEA1	**53.8** (28)	**46.2** (24)	**48.1** (51)	**51.9** (55)

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