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RESEARCH LETTER

WILEY

A clustering analysis of eosinophilic asthmatics: Two clusters with sharp differences in atopic status and disease severity

To the Editor,

Eosinophilic airway inflammation is a major trait of asthma.[1] It is accepted that a sputum eosinophil count reaching 2%–3% is considered as a sign of significant eosinophilic inflammation.[2] A large cross-sectional study has shown that a sputum eosinophil count of at least 3% is found in almost half of asthmatics seen in a secondary care center and broadly linked to asthma severity.[3] While eosinophils are usually thought to be potent inflammatory cells and active contributors to asthma severity, some authors have suggested that lung tissue may actually harbour a population of regulatory eosinophils, the function of which might be to dampen airway inflammation.[4] A recent study has highlighted the existence of a group of patients with mild asthma and high sputum eosinophil count[5] questioning the detrimental action of eosinophils in shaping disease severity and perhaps suggesting heterogeneity in their functional roles.

While cluster analysis has been largely applied in asthma research over the last 15 years,[6] to the best of our knowledge, there has been no study that specifically focused on eosinophilic asthma as defined by sputum percentage of eosinophils.

Here, we have leveraged our large asthma clinic database to perform an unsupervised cluster analysis among asthmatics displaying a sputum eosinophil ≥3% ($N = 426$). None of our patients were receiving biologics by the time they were investigated. To this end, one of the most competitive and complex statistical analysis packages for cluster analysis was applied. In addition to handling the missing values, this analysis reduced the dimensions of variables, and after performing hierarchical clustering and k-means, the final interpretation of clustering was performed using a novel and efficient 4M method (Available at https://osf.io/kw3au/).

Demographic, functional, and inflammatory features of the whole cohort are available online (Available at https://osf.io/kw3au/?view_only=ebb4153691fa45d09a1886ade7e2e3f0). Cluster analysis revealed two subgroups identified as cluster 1 ($n = 276$) and cluster 2 ($n = 150$) (Table 1). Cluster 1 included patients whose median age was 50 years, with two thirds of atopic patients (defined as a RAST >0.35 KU/L to one of the common aeroallergens of our area), having a low treatment burden (median ICS dose 500 μg equivalent beclomethasone/day), preserved lung function (median post bronchodilation FEV1 99% predicted) and a relatively good asthma control (median ACT and ACQ 18 and 1.3 respectively) (Figure 1). Cluster 2 included older patients (median age 59 years) with a lower proportion of atopy (36%), a more frequent smoking history (57%), a higher treatment burden (median ICS dose 2000 μg/day equivalent beclomethasone), a more intense systemic and airway eosinophilic inflammation (median circulating eosinophils 379/μL, median sputum eosinophil count 16%) without higher FeNO levels, a greater systemic inflammation as reflected by higher fibrinogen and CRP levels and circulating neutrophil counts, a greater airway obstruction (median post bronchodilation FEV1 71% predicted, median FEV1/FVC 69% and median sGaw 0.6 1/kPass) and poorly controlled asthma (median ACT and ACQ 11 and 3.1 respectively) (Figure 1). There was no correlation between blood and sputum neutrophil counts and the doses of ICS in any of the two clusters. Likewise, those patients receiving OCS had not greater sputum or blood neutrophils counts (data not shown).

Besides dissimilarities in the frequency of atopic status between the two clusters, there were differences in the type of sensitization among atopic patients. Compared to cluster 2, cluster 1 displayed an increased sensitization rate to birch and grass pollens (39% vs. 18% and 58 vs. 40% respectively, $p < .01$ for both), cat (57% vs. 39% in clusters 1 and 2, respectively, $p < .01$) and dog (56% vs. 43% in cluster 1 and cluster 2 respectively, $p < .05$) whereas sensitization rate to moulds was higher in cluster 2 (41% vs. 22% in cluster 2 and cluster 1 respectively, $p < .05$). Total serum IgE did not differ between the two clusters (median (IQR) 292 (113–843) vs. 349 (154–709) in cluster 1 and cluster 2 respectively, $p > .05$).

Clustering on ICC naïve patients ($n = 114$) and those receiving high doses ICS ($n = 239$) also yielded two clusters that mainly differentiated by age, atopic status, intensity of granulocytic airway inflammation and magnitude of airflow limitation (Table 1). Among patients treated with high dose of ICS, patients from cluster 2 had greater median circulating basophil counts (50/μL vs. 32/μL, $p < .001$) and, surprisingly, greater median levels of cortisol as compared to those from cluster 1 (220/μL vs. 177/μL, $p < .001$) despite more frequent maintenance OCS (24% vs. 12%) and higher doses of ICS. The number of patients with adrenal insufficiency (morning cortisol <102 nmol/L) in patients treated with high doses ICS was, however, similar between the two clusters (22% vs. 19% in clusters 1 and 2, respectively).

After extensive clinical characterization of the patients and application of a sophisticated clustering procedure, we found two

clusters among eosinophilic asthmatics that clearly differentiate by demographics, disease activity, functional and inflammatory features. The dominant cluster, called cluster 1, features a group of patients with a high proportion of atopy and shows substantial airway eosinophilic inflammation together with mild disease as evidenced by a good level of asthma control and preserved lung function despite longer disease duration. By contrast, this cluster seems to be equally at risk of exacerbation in the year prior to the visit as compared to the other cluster, called cluster 2, where non-atopic patients with severely impaired lung function and poor asthma control account for the majority of patients. This suggests that, in atopic patients, eosinophilic airway inflammation may make the patients prone to exacerbate without necessarily leading to an irreversible decline in expiratory flow rates. The lack of lung function decline is even more remarkable that the cluster 1 has a longer disease duration as a result of earlier disease onset. Interestingly, the cluster 2 with impaired airflow limitation is a cluster which combines intense airway eosinophilic and neutrophilic inflammation. There are several cross sectional studies showing that mixed granulocytic asthmatics are those who are the most prone to show a decline in FEV1 over time, which suggests that neutrophils are acting as a cofactor for eosinophils allowing them to fully contribute to remodelling and fixed airway obstruction.[7] As cluster 2 displayed increased levels of circulating neutrophils, fibrinogen and CRP, we cannot exclude that patients from this cluster harbour greater amount of pathogens microbes. Our data would suggest that the possible protection of atopic status against disease severity may differ according to the type of sensitization as cluster 1 includes patients with higher sensitization rate towards birch and grass pollens as well as animal dander whereas cluster 2 displays a strikingly high sensitization rate to a mould mixture.

Cluster 2 displayed worse asthma control and altered lung function despite higher burden of treatment with higher dose of ICS, a greater proportion of patients with LTRA and maintenance OCS. The persistence of very high blood and sputum eosinophil counts in this cluster highlights the inability of corticoids to control eosinophilic inflammation and suppress IL-5 secretion as we know that targeting IL-5 with monoclonal antibodies may reduce the extent of eosinophilic inflammation and restore asthma control in some of these patients.[1] Interestingly, besides eosinophils, blood basophils were also clearly increased in cluster 2 despite heavy treatment with ICS, and sometimes, OCS. By contrast there was no difference in FeNO levels between the two clusters although the intensity of eosinophilic inflammation was clearly higher in cluster 2.

Key message

- The study is the first cluster analysis on a large cohort of eosinophilic asthmatics.
- Eosinophilic asthma distributes in two clusters with patients expressing very different disease severity.
- The cluster with the most severe disease mainly includes non-atopic patients with fixed airway obstruction and resistance to corticoids.

Surprisingly, levels of morning cortisol were higher in cluster 2 than in cluster 1 whereas the burden of ICS/OCS was greater in patients of cluster 2, which would suggest some kind of resistance to systemic effect of corticoids on the pituitary/adrenal axis in the patients from cluster 2. Whether this reduced systemic effect maybe, somehow, linked to a reduced molecular sensitivity to the anti-inflammatory effect of corticoids in cluster 2 or to a reduced bioavailability due to lower peripheral/alveolar ICS deposition as a consequence of fixed airway obstruction needs to be further investigated.

The current study presents some limitations. First, the retrospective nature of the study does not allow to be confident on the adherence of the patients nor does it allow to be sure about the accurate number of courses of OCS in the year prior to the visit that defines exacerbation rate. Second, the selection of our eosinophilic phenotype was based on a single sputum analysis whereas it is known that some asthmatics may show intermittent eosinophilic airway inflammation,[8] masked eosinophilic inflammation by isolated granules without defined cell border.[9] Thus, the considered group of eosinophilic asthmatics in our study may not be entirely representative of a whole eosinophilic asthmatic population. Third, this study is monocentric and should be replicated in other centers using sputum in clinical practice. Fourth, applying proteomic and transcriptomic in our clusters would be of great interest to unravel different molecular patterns among eosinophilic patients.

We conclude that, among eosinophilic asthmatics, there are two clusters which mainly differentiate by their age, atopic status,

TABLE 1 Median (IQR)/percentage (frequency) in each cluster and comparison between clusters.

	Whole cohort			Steroid naïve cohort			High dose ICS treated cohort		
Variable	**Cluster 1 ($n=276$)**	**Cluster 2 ($n=150$)**	***p*-Value**	**Cluster 1 ($n=66$)**	**Cluster 2 ($n=48$)**	***p*-Value**	**Cluster 1 ($n=120$)**	**Cluster 2 ($n=119$)**	***p*-Value**
Demographic									
Age (year)	50 (34–63)	59 (48–65)	**<.0001**	41 (28–51)	61 (53–70)	**<.0001**	45 (30–58)	59 (50–66)	**<.0001**
Sex (male)	47% (129)	41% (61)	.27	58% (38)	40% (19)	.09	40% (48)	41% (49)	.96
BMI (kg/m^2)	26 (23–29)	27 (23–30)	.17	25 (22–27)	27 (23–30)	**.009**	26 (23–30)	27 (24–30)	.85
Smoking									
Ex-smoker	24% (65)	41% (61)	**<.0001**	9% (6)	42% (20)	**.0002**	21% (25)	45% (54)	**<.0001**
Smoker	13% (35)	16% (27)		21% (14)	17% (8)		8% (10)	18% (21)	
Duration of smoking (year)	15 (6–26)	20 (9–42)	**<.0001**	0 (0–1)	5 (0–24)	**.0001**	0 (0–5)	6 (0–22)	**.0001**
Age at diagnosis (year)	26 (7–50)	39 (19–54)	**.022**	18 (5–31)	59 (48–63)	**<.0001**	15 (5–35)	45 (28–54)	**<.0001**
Duration of asthma (year)	14 (3–29)	18 (4–32)	.12	17 (3–26)	0 (0–5)	**<.0001**	24 (10–36)	15 (4–25)	**.004**
Atopy (yes)	67% (184)	36% (54)	**<.0001**	89% (59)	21% (10)	**<.0001**	84% (101)	27% (32)	**<.0001**
Comorbidities									
Nasal polyposis (yes)	12% (33)	8% (12)	.28	6% (4)	6% (3)	.99	13% (16)	11% (13)	.75
Allergic rhinitis (yes)	35% (97)	18% (27)	**<.0001**	50% (33)	6% (3)	**<.0001**	44% (53)	14% (17)	**<.0001**
GERD (yes)	13% (36)	13% (19)	.99	12% (8)	12% (6)	.99	12% (15)	14% (17)	.81
Treatment									
ICS (μg/day)	500 (0–1000)	2000 (1000–2000)	**<.0001**						
LABA (yes)	55% (152)	83% (125)	**<.0001**	1% (1)	4% (2)	.71	91% (109)	89% (106)	.65
LTRA (yes)	20% (55)	35% (52)	**.001**	6% (4)	8% (4)	.92	43% (52)	32% (38)	.11
LAMA (yes)	0.4% (1)	17% (25)	**<.0001**	0% (0)	4% (2)	.3	0.8% (1)	18% (21)	**<.0001**
H_1 antagonists (yes)	24% (67)	13% (19)	**.006**	21% (14)	2% (1)	**.006**	38% (46)	9% (11)	**<.0001**
SABA.SAMA (yes)	62% (170)	73% (109)	**.004**	73% (48)	48% (23)	**.04**	67% (80)	71% (84)	.29
OCS (yes)	4% (11)	25% (38)	**<.0001**	1% (1)	6% (3)	.4	12% (14)	24% (28)	**.02**
Asthma control, exacerbation and quality of life									
ACT	18 (14–22)	11 (8–16)	**<.0001**	18 (15–21)	19 (14–22)	.98	15 (11–20)	12 (8–17)	**<.0001**
ACQ	1 (0.7–2)	3 (2–4)	**<.0001**	1 (0.7–2)	1.4 (0.7–2)	.34	2 (1–3)	3 (2–4)	**<.0001**

TABLE 1 (Continued)

Variable	Whole cohort			Steroid naïve cohort			High dose ICS treated cohort		
	Cluster 1 ($n=276$)	Cluster 2 ($n=150$)	p-Value	Cluster 1 ($n=66$)	Cluster 2 ($n=48$)	p-Value	Cluster 1 ($n=120$)	Cluster 2 ($n=119$)	p-Value
Number of exacerbation									
0	60% (166)	49% (73)	.06	61% (40)	71% (34)	.13	45% (54)	55% (65)	.21
1	16% (43)	23% (35)		14% (9)	15% (7)		24% (29)	16% (19)	
≥2	16% (44)	17% (26)		20% (13)	6% (3)		18% (22)	18% (22)	
AQLQ global	5 (4–6)	3 (3–5)	**<.0001**	5 (4–6)	5 (4–6)	.55	4.6 (3.6–6)	3.3 (3–4.5)	**<.0001**
Pulmonary function									
FEV1 pre (% predicted)	93 (83–103)	65 (52–75)	**<.0001**	98 (87–105)	88 (78–98)	**.009**	86 (73–99)	67 (53–80)	**<.0001**
FEV1 post (% predicted)	99 (90–109)	71 (57–81)	**<.0001**	104 (93–112)	95 (88–104)	**.004**	92 (79–102)	73 (59–85)	**<.0001**
FVC pre (% predicted)	102 (93–111)	84 (73–93)	**<.0001**	105 (94–113)	102 (91–111)	.12	92 (79–102)	79 (71–89)	**<.0001**
FVC post (% predicted)	101 (91–110)	79 (69–88)	**<.0001**	102 (95–112)	98 (88–108)	**.03**	98 (88–109)	84 (73–94)	**<.0001**
FEV1/FVC pre (%)	77 (70–82)	65 (58–73)	**<.0001**	77 (71–82)	73 (69–77)	**.02**	74 (67–82)	68 (59–75)	**<.0001**
FEV1/FVC post (%)	80 (75–85)	69 (61–75)	**<.0001**	83 (78–86)	76 (70–81)	**.0003**	78 (70–83)	71 (61–77)	**<.0001**
RV (% predicted)	107 (87–133)	133 (110–165)	**<.0001**	93 (80–110)	123 (94–136)	**.006**	122 (95–154)	131 (109–161)	.17
sGaw (1/kPas)	0.9 (0.6–1.2)	0.6 (0.4–0.9)	**<.0001**	0.9 (0.8–1.2)	0.7 (0.6–1.1)	.08	0.7 (0.5–1)	0.6 (0.4–1)	.07
PC20M (mg/mL)	1.8 (0.6–8)	1.3 (0.3–5)	.31	1.5 (0.5–6)	2 (0.7–6)	.73	0.9 (0.6–6)	2.2 (1–22)	**.03**
FeNO (ppb)	37 (21–61)	35 (18–62)	.33	46 (26–78)	30 (17–59)	**.03**	47 (26–78)	34 (18–52)	.32
Sputum cell count									
Total cell counts (10^6/g)	1.46 (0.81–3.54)	2.47 (0.96–5.92)	**.009**	1 (0.7–2)	3.5 (1–7)	**.0006**	1.4 (0.6–3)	2.5 (1–7)	**.0006**
Macrophages (%)	27 (15–40)	12 (6–25)	**<.0001**	33 (21–46)	18 (10–25)	**<.0001**	28 (15–41)	11 (6–24)	**<.0001**
Lymphocytes (%)	1 (0.4–2)	0.7 (0.2–2)	**.008**	2 (0.6–4)	1 (0.6–2)	.12	0.8 (0.2–2)	0.7 (0.2–2)	.75
Neutrophils (%)	48 (31–68)	50 (28–70)	.81	37 (24–53)	65 (52–77)	**<.0001**	49 (31–69)	53 (30–69)	.89
Eosinophils (%)	9.4 (5–19)	16 (6–42)	**<.0001**	13 (7–25)	7 (5–15)	**.01**	8 (4–16)	18 (7–45)	**<.0001**
Epithelial cells (%)	3 (1–6)	3 (1–7)	.627	4 (2–8)	2 (0.7–4)	**.0005**	3 (2–8)	3 (1–7)	.18
Macrophages (10^3/g)	391 (159–892)	290 (115–762)	**.015**	396 (152–864)	455 (159–1609)	.42	338 (125–864)	323 (123–754)	.58
Lymphocytes (10^3/g)	17 (4–47)	15 (1–51)	.21	17 (4–67)	385 (16–105)	.11	9 (2–32)	17 (2–53)	.11
Neutrophils (10^3/g)	654 (252–1821)	834 (293–2774)	.17	439 (184–820)	2136 (731–4453)	**<.0001**	532 (234–1490)	954 (326–3294)	**.01**
Eosinophils (10^3/g)	172 (59–453)	401 (121–1353)	**<.0001**	189 (58–396)	283 (104–1036)	**.04**	112 (49–324)	148 (492–1561)	**<.0001**
Epithelial cells (10^3/g)	54 (16–131)	67 (24–177)	.08	44 (14–133)	39 (20–142)	.84	60 (15–126)	75 (20–175)	.22

TABLE 1 (Continued)

Variable	Whole cohort			Steroid naïve cohort			High dose ICS treated cohort		
	Cluster 1 ($n=276$)	Cluster 2 ($n=150$)	p-Value	Cluster 1 ($n=66$)	Cluster 2 ($n=48$)	p-Value	Cluster 1 ($n=120$)	Cluster 2 ($n=119$)	p-Value
Blood cell count									
Leucocytes ($10^3/\mu L$)	7 (6–8)	9 (7–11)	**<.0001**	7 (6–8)	7 (6–9)	**.003**	7 (6–9)	8 (7–10)	**.003**
Neutrophils (%)	53 (47–59)	56 (49–63)	**.008**	51 (46–56)	53 (48–60)	.19	56 (47–62)	55 (49–62)	.74
Lymphocytes (%)	34 (28–40)	30 (23–36)	**<.0001**	36 (32–41)	33 (26–38)	.05	32 (25–38)	30 (24–36)	.14
Monocytes (%)	7.6 (6.5–9.1)	7.7 (6–9)	.71	7 (6–9)	8 (6–10)	.64	8 (7–9)	7 (6–9)	.50
Eosinophils (%)	4 (2–5)	4 (3–7)	**.005**	3 (2–4)	3 (2–5)	.84	4 (3–6)	4 (3–7)	.12
Basophils (%)	0.5 (0.3–0.7)	0.6 (0.4–0.8)	**.008**	0.6 (0.4–0.7)	0.6 (0.4–0.7)	.52	0.5 (0.3–0.7)	0.6 (0.4–0.8)	**.004**
Neutrophils ($1/\mu L$)	3701 (2867–4726)	4663 (3577–6403)	**<.0001**	3305 (2644–3969)	3916 (3290–4733)	**.004**	4029 (3157–5279)	4444 (3529–6106)	**.03**
Lymphocytes ($1/\mu L$)	2341 (1896–2769)	2381 (1940–3004)	.16	2367 (1988–2754)	2370 (1934–2920)	.83	2355 (1794–2809)	2368 (1983–2943)	.28
Monocytes ($1/\mu L$)	533 (442–669)	666 (533–813)	**<.0001**	512 (392–610)	589 (480–746)	**.006**	566 (471–695)	625 (493–801)	.06
Eosinophils ($1/\mu L$)	242 (173–377)	379 (209–627)	**<.0001**	220 (177–292)	259 (181–375)	.3	275 (191–421)	371 (190–613)	**.02**
Basophils ($1/\mu L$)	32 (22–50)	49 (32.25–67)	**<.0001**	32 (22–47)	39 (28–60)	.1	32 (23–50)	50 (32–68)	**<.0001**
Serum IgE									
Birch (t3) % >0.35 (KU/L)	28% (78)	7% (11)	**<.0001**	44% (29)	2% (1)	**<.0001**	37% (45)	2% (3)	**<.0001**
Mould (MIX1) % >0.35 (KU/L)	14% (40)	15% (22)	.99	14% (9)	4% (2)	.15	27% (33)	9% (11)	**<.0001**
Grass (GX3) % >0.35 (KU/L)	39% (108)	15% (22)	**<.0001**	58% (38)	0% (0)	**<.0001**	55% (66)	8% (9)	**<.0001**
Dog (e5) % >0.35 (KU/L)	38% (104)	15% (23)	**<.0001**	58% (38)	4% (2)	**<.0001**	56% (67)	3% (3)	**<.0001**
Cat (e1) % >0.35 (KU/L)	38% (104)	14% (21)	**<.0001**	56% (37)	6% (3)	**<.0001**	52% (63)	5% (6)	**<.0001**
DPT (d1) % >0.35 (KU/L)	470% (130)	21% (32)	**<.0001**	65% (43)	10% (5)	**<.0001**	62% (74)	13% (16)	**<.0001**
Total IgE (KU/L)	215 (66–528)	154 (54–364)	**.035**	285 (102–452)	104 (27–256)	**.0006**	383 (103–983)	137 (48–284)	**<.0001**
Systematic inflammation									
CRP (mg/L)	2 (1–4)	2 (1–6)	**.002**	1 (0.6–4)	2 (1–3)	.15	1.7 (0.8–4)	2.8 (1–7)	**.01**
Fibrinogen (g/L)	3.2 (2.7–3.6)	3.6 (3–4)	**<.0001**	3 (2.41–3.51)	3.3 (2.9–3.9)	**.01**	3.14 (2.6–3.6)	3.6 (3.1–4)	**<.0001**
Adrenal function									
Cortisol (nmol/L)	199.5 (156–251)	231 (179–301)	**.016**	222 (189–301)	243 (195–293)	.89	177 (141–221)	220 (176–301)	**<.0001**

Note: Bold indicates statistical significant value ($p < .05$).

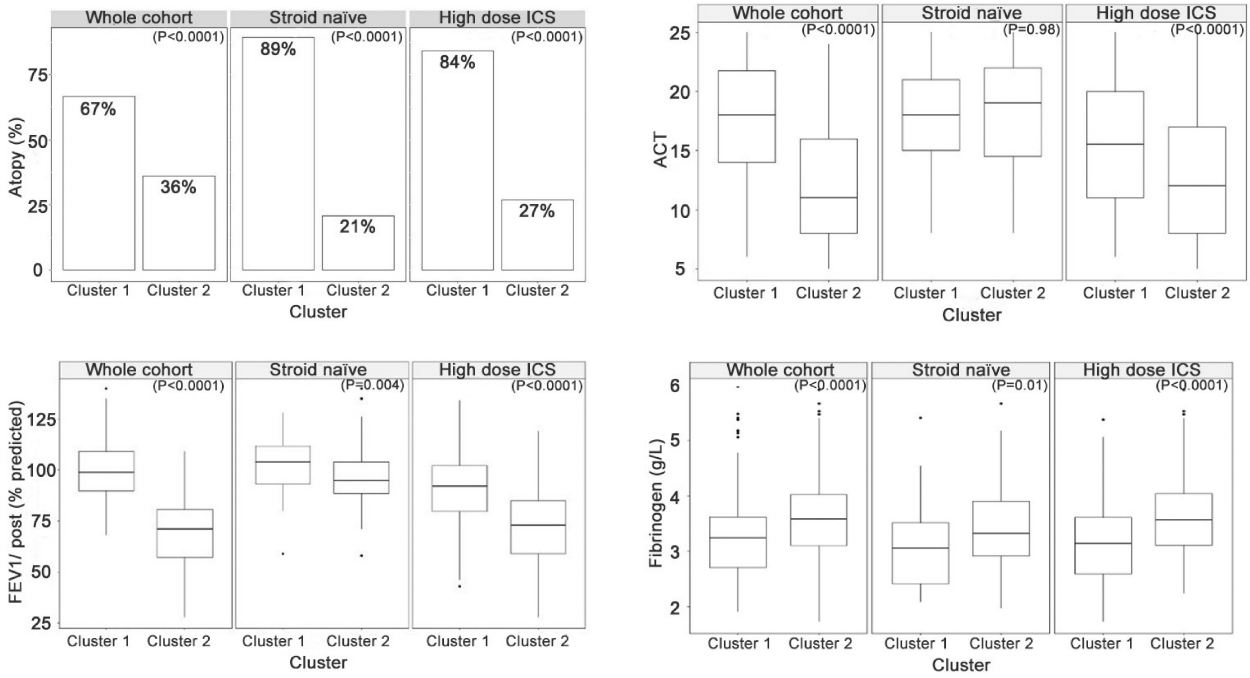

FIGURE 1 Box plot of important variables in two clusters of eosinophilic asthmatics and their subgroups.

their level of functional impairment, the magnitude of granulocytic inflammation, and asthma control. The cluster with the lower proportion of atopic patients is clearly the most severe and resistant to corticoids. Whether eosinophils are phenotypically and functionally different among the two clusters warrant further investigation.

AUTHOR CONTRIBUTIONS

Halehsadat Nekoee Zahraei: Performing statistical analysis of the datasets and computations, writing the manuscript. Florence Schleich: Patient recruitment, drafting the manuscript. Sara Gerday: Construction of the clinical data base. Françoise Guissard, Virginie Paulus: Sputum induction. Monique Henket: Sputum processing and cell count analysis. Catherine Moermans: Contribution to the revised version and answering reviewer's comments. Anne-Françoise Donneau: Supervision of the statistical analysis and computation. Renaud Louis: Patient recruitment, management and supervision of the project, drafting the manuscript.

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CONFLICT OF INTEREST STATEMENT

RL and FS have received grants and lectures/adboard fees from GSK and AZ and Chiesi outside the submitted work.

DATA AVAILABILITY STATEMENT

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

Halehsadat Nekoee Zahraei[1,2]
Florence Schleich[2]
Sara Gerday[2]
Françoise Guissard[2]
Virginie Paulus[2]
Monique Henket[2]
Catherine Moermans[2]
Anne-Françoise Donneau[1]
Renaud Louis[2]

[1]*Biostatistics Unit, Department of Public Health, University of Liège, Liege, Belgium*
[2]*Department of Pneumology, GIGA, University of Liège, Liege, Belgium*

Correspondence
Renaud Louis, Department of Pneumology, GIGA, University of Liège, Liege, Belgium.
Email: r.louis@uliege.be

Anne-Françoise Donneau and Renaud Louis contributed equally to this study.

ORCID

Catherine Moermans https://orcid.org/0000-0002-9558-5878

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