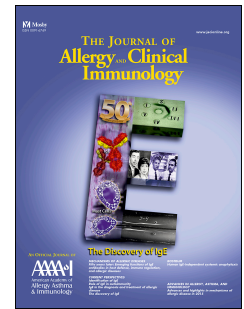


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Monitoring the inflammatory heterogeneity with multiple biomarkers for multidimensional endotyping of asthma

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Short summary

The complexity of type 2 and non-type 2 asthma requires monitoring with multiple biomarkers. We evaluated the potential of multidimensional endotyping to characterise physio-pathological clusters based on asthma severity and intensity of the blood eosinophilia.

Key words

Asthma, biomarkers, blood eosinophils, endotypes, topological data analysis

To the Editor:

Asthma phenotypes describe clinical and morphologic characteristics as well as unique responses to different treatments. Although potentially clinically relevant, phenotypes do not necessarily relate to or give insights into the underlying physiopathological processes. Asthma endotypes uncover molecular mechanisms underlying phenotypes. Identification of corresponding molecular biomarkers for each pathogenic mechanism operating within an phenotype is required to link the phenotype to a disease endotype^{1,2}. It is now timely to recognize that major pathogenic pathways such as eosinophilic and non-eosinophilic responses in asthma are highly complex and include several dynamic determinants and heterogeneous molecular mechanisms. The present study therefore embraces the concept of "*complex type 2 and non-type 2 endotypes*" and offers a multidimensional approach with multiple biomarkers. Putting them into clusters represents attempts to group asthma patients within a continuum of disease rather than clear-cut entities^{2,3}. An unbiased, multidimensional analytical approach is best suited to identify the mechanistic pathways generating asthma endotypes

In this study, we used topological data analysis (TDA) to characterize adult asthmatic patients based on exhaustive clinical characterization and blood biomarkers. TDA is a data-analytical approach that couples unsupervised pattern detection with network visualization and facilitates the identification of novel relationships in complex and heterogeneous data sets, which are allowing for a data-driven hypothesis generation that may uncover disease mechanisms⁴. TDA is sensitive to both large and small-scale patterns that often fail to be detected by other methods, such as principal component analysis, multidimensional scaling, and cluster analysis. Topological maps can more finely stratify patients and can identify patient sub-groups amenable to targeted therapy.

One hundred asthmatic patients underwent detailed clinical assessment (Table E1), evaluation of asthma severity (Global Initiative for Asthma - GINA) and control (asthma control test – ACT) and measurement of lung function, exhaled NO (FeNO), blood eosinophils and neutrophils, and serum biomarkers: total IgE, IL-4, IL-5, IL-13, periostin, eotaxin-1, eosinophil-derived neurotoxin (EDN), dipeptidyl-peptidase 4 Inhibitor (DPP4), IL-8, monocyte chemo-attractant protein-1 (MCP-1), tumor necrosis factor (TNF)- α , interferon (IFN)- γ , interferon gamma-induced protein (IP)-10, IL-17 α and IL-33. All evaluations were done outside an asthma exacerbation or a respiratory tract infection (minimum two weeks wash-out period was required, including for

oral corticosteroids). In total, TDA was performed with 21 clinical, pathophysiologic, and inflammatory parameters.

Six clusters were identified and visualized according to two dimensions, a) asthma severity (GINA) and b) blood eosinophilia (Figure 1). Within all analysed serum biomarkers, blood neutrophils, total IgE, IL-4, IFN- γ , TNF- α , IP-10, IL-17 α and IL-33 did not show a significant role in the characterization of clusters. In order to provide further dimensions and content to the clusters. TDA parameters were tested for their association to particular clusters (Table E2).

Overall, asthma control, exacerbation rate, nocturnal asthma, asthma background treatment, corticosteroid responsiveness and lung function are distributed in agreement with asthma severity (Figure 2 and Table E3).

For mild asthma (cluster A) there are no specific biomarkers (Table E2). For moderate asthma (clusters B, C and D) and severe asthma (clusters E and F), we observed a clear separation between type 2 and non-type 2 asthma (Figure 2 and Table E2).

Moderate eosinophilic asthma clusters (C and D) differ by the intensity of systemic type 2 biomarkers (IL-13, IL-5, EDN, periostin, blood eosinophilia) (Table E2, Figure E1) and this translates into lower risk of lung function decline and corticosteroid resistance in cluster C, which might represent a type 2 inflammation localized in the lung, compared to cluster D that shows consistently a high systemic type 2 inflammation. This observation further reinforces the concept of localized versus systemic type 2 inflammation, that impacts the clinical outcome.^{1,5} A strong association between type 2 biomarkers is present only for low eosinophilic asthma (Figures E2 and E3). Hence, in particular for moderate asthma, there is a need to measure multiple biomarkers besides blood eosinophils to better predict the severity and to provide evidence for the type 2 pathway as driving mechanism. As in our previous observation⁶, EDN, IL-13, IL-5 and in this study periostin measured with a different assay (Periostin ELISA Kit, Shino-test) are the best predictors of blood eosinophilia (Figure E4).

For severe asthma, we observed a non-eosinophilic cluster with a mixed endotype (cluster F) with increased serum eotaxin and IL-8, confirming our previous observations⁶. In addition a low eosinophilic cluster characterized by low DPP4, increased exacerbation rate and late-onset asthma (cluster E) is observed. Cluster E is interesting, since it is characterized by high exacerbation rate and is mild eosinophilic, criteria used to select patients for type 2 targeted interventions in asthma (mepolizumab, lebrikizumab, dupilumab). However, IL-5 and IL-13 are not elevated in these patients and their associated biomarkers are not increased (periostin) or are significantly low (DPP4), thus they might represent non-responders. Of note, none of the severe asthma clusters is characterized by high eosinophilia, challenging the inclusion criteria used for targeted anti type 2 asthma interventions.

As an IL-13 pathway biomarker tested in the tralokinumab studies high DPP4 was linked to better improvement of lung function and quality of life.⁷ Low DPP4 can prove to be a useful biomarker as well. According to our observation, low serum DPP4 could be new biomarker for low eosinophilic non-type 2 severe asthma (cluster E), in agreement with the animal models showing that systemic inhibition of DPP4 via the oral route aggravates airway inflammation⁸.

A detailed characterization including sputum, BAL and lung tissue biomarkers would provide a wider and more insightful perspective on asthma endotypes. Our study used the same methodology as Hinks et al.⁹ to describe clinicopathobiologic clusters. Different from Hinks et al who based their analysis on blood and induced sputum measures, our aim was to provide a proof-of-concept that by using non-invasive easy accessible biomarkers from blood we might individualise groups of asthma patients based on the mechanistic pathways involved in therapeutic and prognostic implications. However these clusters have to be both longitudinally validated. In addition Hinks observed more than one eosinophilic or neutrophilic cluster, hinting to the possibility of type 2 and non-type 2 subendotypes.

The ultimate aim for future research and a clinical algorithm development should be to characterize each individual patient using both clinical characteristics and biomarkers. Based on our findings we propose the following strategy for endotyping asthma patients into clinically meaningful clusters: 1) stratify by severity (GINA) and blood eosinophils; 2) add visible properties (age at onset, exacerbation rate, nocturnal asthma, atopy, asthma control test score, lung function); 3) characterize the endotype and subendotype by measuring multiple additional biomarkers besides blood eosinophilia, both type 2 (IL-13, IL-5, DPP4, eotaxin, EDN, periostin, TSLP, IL-25) and non-type 2 (IL-8, MCP-1).

In conclusion, our study indicates that a multidimensional endotyping is needed both for type 2 and non-type 2 asthma. Therefore, the use of multiple biomarkers is recommended to provide evidence that a certain pathway is the key driver for a given patient. In particular additional biomarkers such as IL-5, IL-13, DPP4, eotaxin and periostin might refine responder selection for type 2 targeted therapy.

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Figure and tables legends

Figure 1: Topological data analysis (TDA) was used to analyze and visualize clustering of individuals based on their similarity across 21 clinical, physiologic, and inflammatory parameters simultaneously. TDA constructs a network of nodes with edges between them. The nodes represent sets of data points, and two nodes are connected with an edge if their corresponding collections of data points have a point in common. A patient can fall into several nodes, which is how two nodes are connected. More precisely, we connected two nodes with an edge if they share at least one patient. The filter function was set to principal and secondary singular value decomposition. The number of intervals was 10 and 10 and the overlapping percentage was fixed to 50.

The original TDA network is simplified by focusing on nodes with more than 4 patients. The clusters are visualized by two dimensions, asthma severity (GINA) and blood eosinophilia. Nodes are colored based on severity, and their size is proportional to the number of patients, also indicated numerically inside the node. Connecting nodes were assigned to a particular cluster either based on patient over-representation in the neighboring nodes or based on the number of connections within a cluster. Six clusters were identified. Eight patients did not cluster and appear to represent less common individual endotype.

Figure 2: The twelve most relevant clinical characteristics and serum biomarkers are visualized as box plots for the 6 TDA clusters. The dots represent outliers, the box represents the second quartile, the median and the third quartile (=50 % of the data-points). The upper whisker goes to the highest value that is within $1.5 \times \text{IQR}$, the lower whisker goes to the lowest value that is within $1.5 \times \text{IQR}$ too (opposite direction). Rests of data points are called outliers and represented as dots.

Figure E1: Heterogeneity of type 2 inflammation in moderate asthma as revealed by the classification tree. Eosinophilic, moderate asthma (clusters C and D) differs by the intensity of systemic type 2 biomarkers (IL-13, IL-5, EDN, periostin, blood eosinophilia). Of note, moderate asthma cluster D contains the patients with a consistent type 2 pattern, that show much higher type 2 activity than observed for severe patients (cluster E).

The algorithm selects variables from all variables to build nodes that best predict the clusters B, C and D. Blood eosinophils represent the most important variable (node) and 245 eosinophils/ μl value was identified as the best cut-off for separation. The combination of variables allows to build the classification rules to separate the patients. The Y-axis represents the proportion of patients in a terminal node for each cluster.

Figure E2: Type 2 biomarkers in low eosinophilic asthma (clusters A and E). There is good correlation between IL-5 and IL-13, and between EDN and blood eosinophils. As in our previous study⁶, periostin does not correlate with blood eosinophils or with the other type-2 biomarkers.

Figure E3: Type 2 biomarkers in medium and high eosinophilic asthma (clusters C and D). There is no correlation between type 2 biomarkers IL-13, IL-5, EDN, periostin and blood eosinophils.

Figure E4: Regression tree model. Absolute blood eosinophils were best predicted by EDN, followed by IL-13, exhaled NO, periostin, IL-5, eotaxin and DPP4.

Variable importance shows which variable predicts best high blood eosinophils levels as a relative measure of the ability of the variable to improve the outcome at each level of the tree. Results are summed at each level of the tree and for each node to compute the variable importance, which ranks relatively predictor variables.

Table E1: A thorough characterization of visible properties characterizing the phenotypes in study group is presented including lung function data and asthma background medication

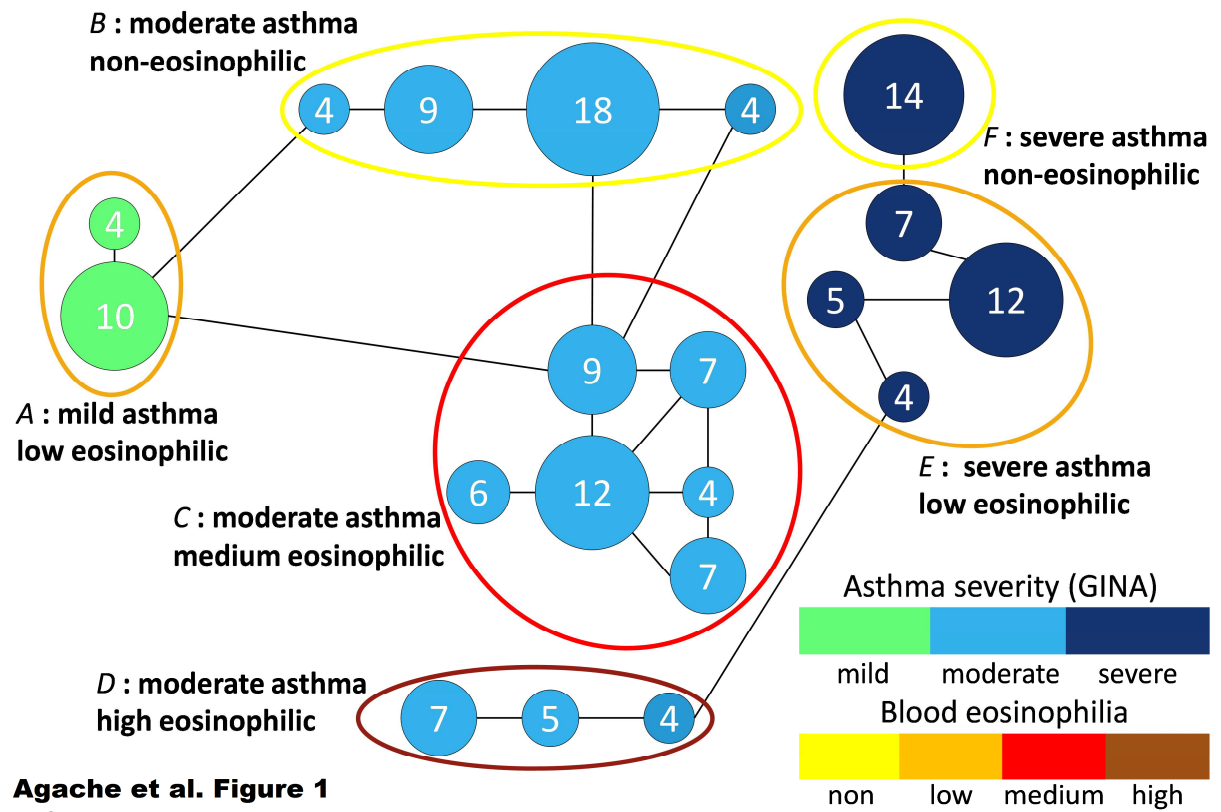
Table E2: Clinical, pathophysiological and inflammatory parameters association to particular topological clusters. Cluster A includes patients with well-controlled, mild asthma. There are no particular biomarkers. Cluster B includes patients with moderate non-eosinophilic asthma with low levels of type 2 biomarkers (IL-13, IL-5, EDN). Clusters C and D are both eosinophilic moderate asthma but are different in terms of the intensity of type 2 inflammation with significant increased serum levels of IL-13, IL-5, EDN and periostin only in cluster D. The intensity of type 2 inflammation impacts asthma severity with decreased incidence of corticosteroid resistance and fast lung decline in cluster C. Clusters E and F are both severe asthma but with a clear separation based on type 2 inflammation: low eosinophilia and significant low levels of DPP4 in cluster E and a non-eosinophilic mixed endotype in cluster F with both eotaxin and IL-8 and MCP-1 increased serum levels.

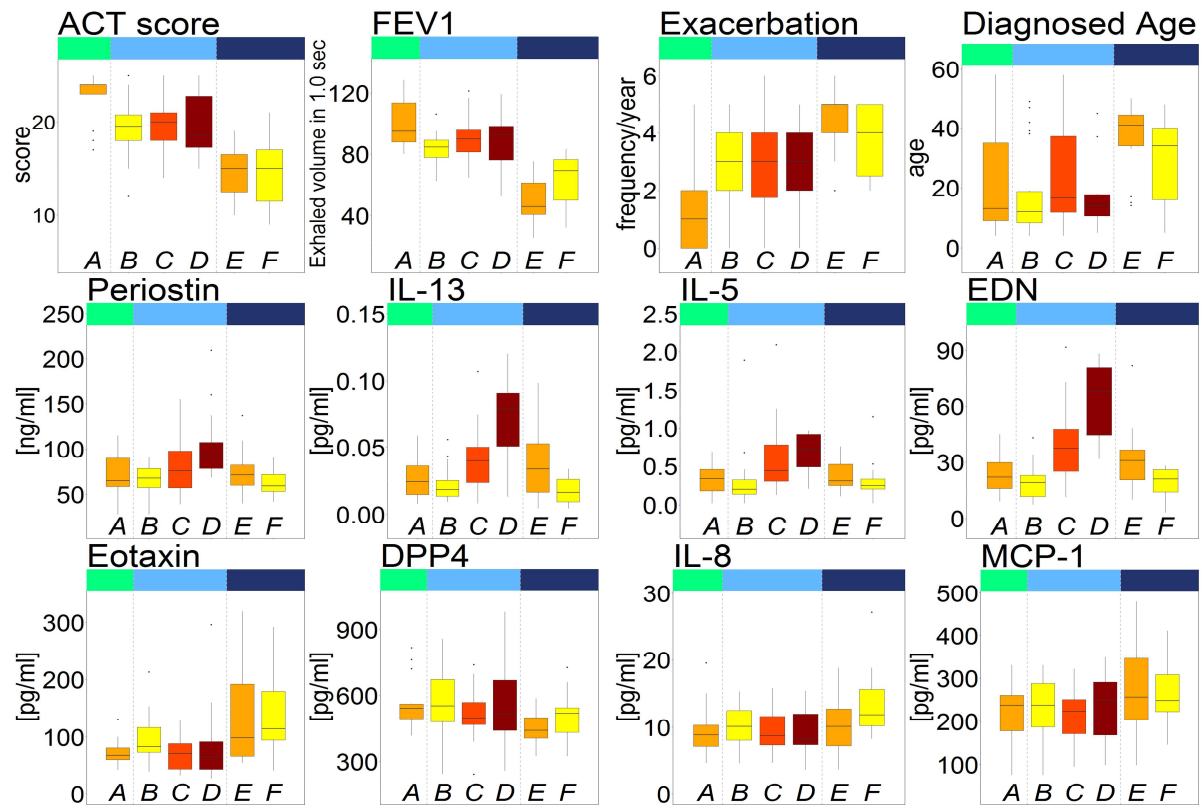
Variables were tested for association with particular clusters using Fisher exact test for nominal variables and a two-sample Kolmogorov-Smirnov test for numerical variables. For all applied tests, p-values were adjusted using the Benjamini & Hochberg procedure with the significance threshold set to 0.05. Statistically significant variables are provided in bold, with the status identifying the directionality e.g. cluster A shows significantly higher ACT score compared to other clusters.

“-” means that a given variable cannot be described as low, high because there was no significant association with the cluster.

All variables are in pg/ml except periostin.

Table E3: Asthma background treatment supports the severity stratification of the clusters with mild asthma (cluster A) receiving mainly monotherapy with ICS or LTRA, moderate asthma (clusters B,C,D) receiving the ICS/LABA or ICS/LABA/LTRA combination and severe asthma (clusters E and F) receiving the ICS/LABA/LAMA/LTRA combination





Agache et al. Figure 2

A. Methods

Serum samples were analysed as described previously (1). For serum periostin we used the ELISA-kit from Shino-test (2). The Dipeptidyl Peptidase IV (DPPIV) was quantified using a sandwich immunoassays specific for the human DPPIV (RnD Systems, DY1180). In brief, the plates were coated with capture antibody specific for human DPPIV and incubate overnight. After removal of unbound material the plate were blocked with reagent diluent containing BSA. After another wash step, serum samples were diluted in 1 to 500 in reagent diluent and incubated in the microtiter plate. After removal of unbound material a biotin-labeled secondary antibody was added to bind captured analyte. The detection of specifically bound secondary antibody was based on a colorimetric reaction of the enzyme-streptavidin label. The optical density was read at 450 nm with an Envision multi-label reader (Perkin Elmer, 2104-0020). The colorimetric signal correlating with the DPPIV samples concentration were interpreted with a calibrator curve (4000 to 62.5 pg/ml). (3)

All data analyses were performed using R 3.3.1 (4). For multidimensional exploration of the data we applied topological data analysis using the R package TDAmapper (5). This methodology allows representing the data points as a network where each node comprises patients close to each other (6,7). The most important variables were selected based on visual inspection of the principal component analysis (PCA) plots. Nodes are connected if they share at least one patient. The filter function was set to principal and secondary singular value decomposition. The number of intervals was 10 and 10 and the overlapping percentage was fixed to 50. The clusters identified were characterized using different statistical tests depending on the variable type. We applied a Fisher exact test to nominal variables and a two-sample Kolmogorov-Smirnov test to numerical variables to compare distributions between a particular cluster and all the others.

For the correlation analysis Spearman's rank correlation coefficient was used. For all applied tests, p-values were adjusted using Benjamini & Hochberg procedure and the significance threshold was set to 0.05. For the supervised analysis the R package Classification and Regression Training was used to generate a regression tree for the best predictors of blood eosinophilia and a classification tree to differentiate TDA clusters B, C and D based on biomarkers (6).

To be sure that our population is not composed by a specific phenotype we observed interactions between nominal variables by looking at the Cramer's Phi, which is, in terms of interpretation, the equivalent of correlation for nominal variables. Moreover, tests are applied after TDA to see if a given nominal variable is/can be linked to a particular cluster. Known confounders such as age, BMI, smoker, and gender were investigated using association methods, but no influence was observed

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B. Online supplemental material

Table E1 – Characteristics of study group

Gender: male/females (n)		43/57
Mean age (years)		51.3±24.6 (22-83)
Age at onset of asthma (years)		24.6±15.8 (4-58)
BMI (mean±SD, interval)		27.01±5.7 (17.6-47.3)
Atopy (%patients)		55%
Smoking status (active or ex-smokers with > 10 pack/years) (% patients)		22%
CRS (%patients)	CRS _{WNP}	34%
	CRS _{sNP}	12%
Aspirin sensitivity (% patients)		24%
ACT score (mean±SD, interval)		18.6±4.2 (9-25)
Exacerbation rate/year		3.4±2.3 (0-8)
Fast lung function decline (% patients)		46%
CS resistance (%patients)		21%
Nocturnal asthma (% patients)		56%
Exercise-induced asthma (% patients)		33%
Brittle asthma (% patients)		31%
Near-fatal asthma (% patients)		20%
GINA severity (% patients)	Mild	22%
	Moderate	41%
	Severe	37%
Asthma background treatment (% patients)	ICS	11%
	LTRA	5%
	ICS+LABA	40%
	ICS+LABA+LAMA	3%
	ICS+LABA+LTRA	18%
	ICS+LABA+LTRA+LAMA	23%
ICS dose (GINA 2017) (% patients)	Low	15%
	Medium	38%
	High	42%
FEV1 % predicted (mean±SD, interval)		82.9±22.5(25.8-128.4)
Bronchodilator response (ml) (mean±SD, interval)		167.4±118.4 (0-560)
Bronchodilator response (%) (mean±SD, interval)		7.4±5.9 (0-37.5)
Exhaled NO (ppb) (mean±SD, interval)		27.7±29.9 (5.5-106.5)
Blood eosinophils (cells/mm ³) (mean±SD, interval)		263.7±194.4 (10-1180)
Blood eosinophils (%) (mean±SD, interval)		3.6±2.6 (0.2-17.2)
Total serum IgE (IU) (mean±SD, interval)		325.9±661.1 (6.5-5264)

A thorough characterization of visible properties characterizing the phenotypes in study group is presented including lung function data and asthma background medication

ACT = asthma control test; CS= corticosteroid; CRS = chronic rhinosinusitis; CRS_{WNP} = chronic rhinosinusitis with nasal polyps; CRS_{sNP}=chronic rhinosinusitis without nasal polyps; GINA = Global Initiative for Asthma; ICS = inhaled corticosteroids; IU = international units; LABA = long acting bronchodilator; LAMA = long acting antimuscarinic agent; LTRA = leukotriene receptor antagonist; NO = exhaled nitric oxide

Table E2: Clinical, pathophysiological and inflammatory parameters association to particular clusters

Cluster	Categorical variables					Numerical variables					
	Name	p-value	Status	Percentage		Name	p-value	Status	Median	Q1	Q3
A: mild asthma low eosinophilic	CS resistant	0.018	No	Y=0	N=100	<i>IL-13</i>	<i>0.367</i>	-	0.02	0.01	0.04
	Exercise induced	0.018	No	Y=5.3	N=94.7	<i>IL-5</i>	<i>0.390</i>	-	0.34	0.18	0.46
	GINA	0	Mild	Severe=0	Mild=73.7	<i>EDN</i>	<i>0.212</i>	-	22	16	29.9
	Near fatal	0.028	No	Y=0	N=100	<i>Eos Percent</i>	<i>0.212</i>	-	2.7	1.85	3.25
	<i>Atopy</i>	<i>0.227</i>	-	Y=73.7	N=26.3	<i>Eos Cells</i>	<i>0.463</i>	-	200	140	250
	Fast decline LF	0.005	No	Y=10.5	N=89.5	<i>Periostin</i>	<i>0.425</i>	-	65	58.5	89.5
	Nocturnal asthma	0.018	No	Y=26.3	N=73.7	<i>Eotaxin</i>	<i>0.115</i>	-	67.9	60.5	81.1
						<i>DPP4</i>	<i>0.249</i>	-	542	495	561
						<i>IL-8</i>	<i>0.390</i>	-	8.85	7.15	10.32
						<i>MCP-1</i>	<i>0.577</i>	-	238	178	261
						<i>IgE</i>	<i>0.982</i>	-	129	57.8	258.5
						Exacerbation rate	0.001	Low	1	0	2
						<i>Diagnosed age</i>	<i>0.374</i>	-	13	9	35
						ACT score	0	High	24	23	24
						FEV1	0.006	High	94.8	87.9	113.5
						<i>BMI</i>	<i>0.512</i>	-	26.4	24.1	29.9
B: moderate asthma non- eosinophilic	<i>CS resistant</i>	<i>0.11</i>	-	Y=3.8	N=96.2	IL-13	0.006	Low	0.02	0.01	0.03
	<i>Exercise induced</i>	<i>0.11</i>	-	Y=11.5	N=88.5	IL-5	0.005	Low	0.2	0.14	0.32
	<i>GINA</i>	<i>0.175</i>	-	Severe=23.1	Mild=11.5	EDN	0.002	Low	19.2	11.7	23.1
	<i>Near fatal</i>	<i>0.11</i>	-	Y=3.8	N=96.2	Eos Percent	0.001	Low	2	1.4	2.7
	<i>Atopy</i>	<i>0.2</i>	-	Y=73.1	N=26.9	Eos Cells	0	Low	165	98	200
	<i>Fast decline LF</i>	<i>0.961</i>	-	Y=50	N=50	<i>Periostin</i>	<i>0.175</i>	-	67.5	57.3	78
	<i>Nocturnal asthma</i>	<i>0.839</i>	-	Y=61.5	N=38.5	<i>Eotaxin</i>	<i>0.089</i>	-	83.0	73.7	116.6
						<i>DPP4</i>	<i>0.481</i>	-	553	481	673
						<i>IL-8</i>	<i>0.860</i>	-	10.1	8.1	12.5
						<i>MCP-1</i>	<i>0.941</i>	-	238	187	288
						<i>IgE</i>	<i>0.397</i>	-	191.5	95.3	507.8
						Exacerbation rate	<i>0.786</i>	-	3	2	4
						<i>Diagnosed age</i>	<i>0.143</i>	-	12.0	8.3	18.8
						<i>ACT score</i>	<i>0.574</i>	-	19.5	18.0	20.8
						<i>FEV1</i>	<i>0.325</i>	-	84.5	78.0	89.0
						<i>BMI</i>	<i>0.786</i>	-	27.6	22.6	29.5

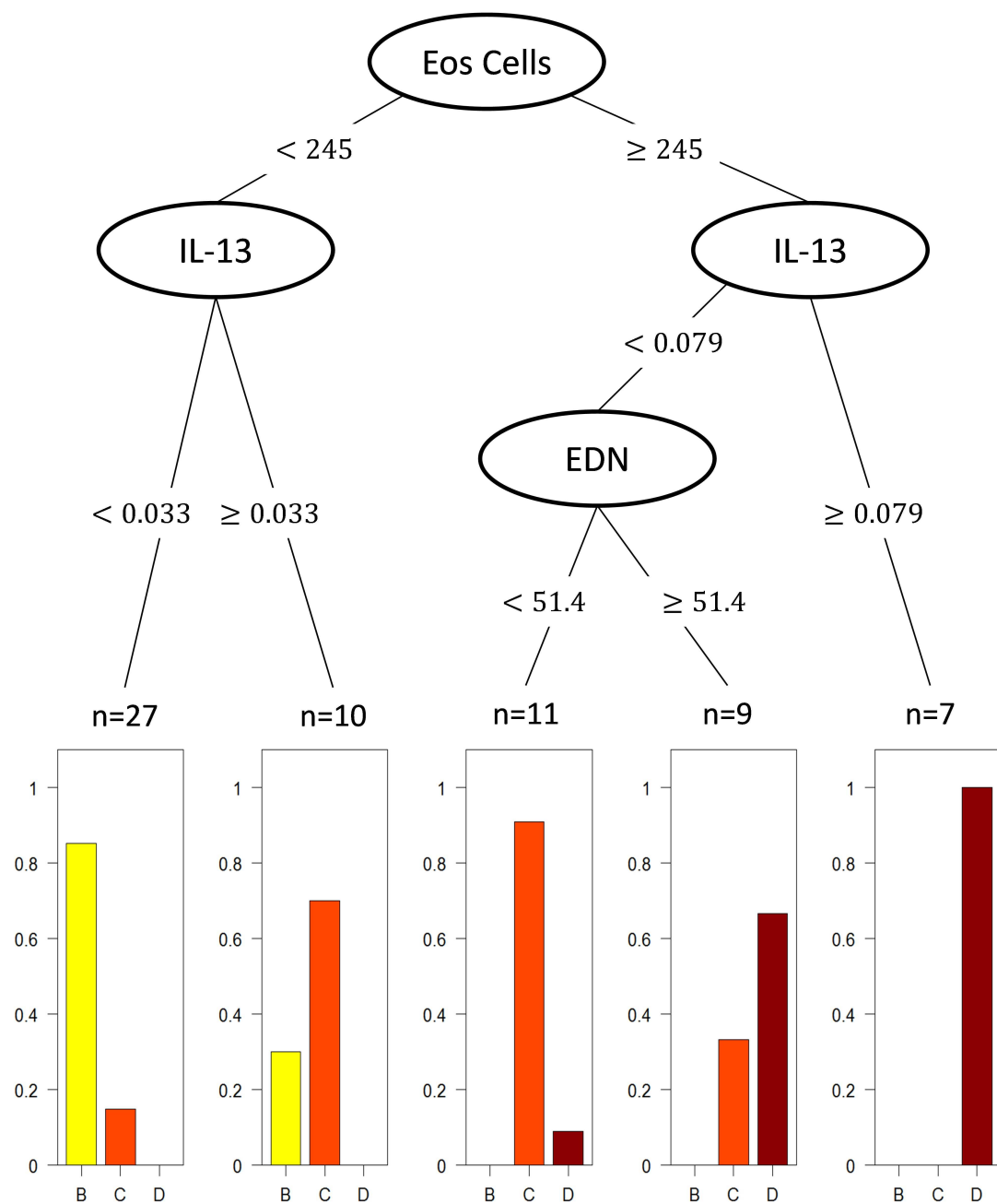
C: moderate asthma medium eosinophilic	CS resistant <i>Exercise induced</i> GINA <i>Near fatal</i> <i>Atopy</i> Fast LF decline <i>Nocturnal asthma</i>	0.012 0.883 0.431 0.115 0.883 0.012 0.883	No - - - No -	Y=0 Y=33.3 Severe=20.8 Y=4.2 Y=54.2 Y=16.7 Y=54.2	N=100 N=66.7 Mild=20.8 N=95.8 N=45.8 N=83.3 N=45.8	IL-13 IL-5 EDN Eos Percent Eos Cells Periostin Eotaxin DPP4 IL-8 MCP-1 IgE Exacerbation rate <i>Diagnosed age</i> <i>ACT score</i> <i>FEV1</i> <i>BMI</i>	0.152 0.311 0.190 0.026 0.190 0.917 0.553 0.785 0.917 0.553 0.995 0.995 0.544 0.152 0.917	- - - High - - - - - - - - - -	0.04 0.44 37.5 4.45 255 75.5 71.4 498 8.69 224 160.5 3 16.5 20 89.8 27.9	0.02 0.31 25.2 3.48 200 57.0 44.4 467 7.35 171 66.6 1.75 11.8 18 81.4 21.8	0.05 0.78 47.9 5.03 355 97.5 88.6 568 11.61 251 301.2 4 37.3 21 95.7 30.9
D: moderate asthma high eosinophilic	CS resistant <i>Exercise induced</i> GINA <i>Near fatal</i> <i>Atopy</i> Fast LF decline <i>Nocturnal asthma</i>	1 1 0.912 1 1 0.949 0.912	- - - - - -	Y=21.4 Y=35.7 Severe=28.6 Y=21.4 Y=64.3 Y=35.7 Y=42.9	N=78.6 N=64.3 Mild=7.1 N=78.6 N=35.7 N=64.3 N=57.1	IL-13 IL-5 EDN Eos Percent Eos Cells Periostin Eotaxin DPP4 IL-8 MCP-1 IgE Exacerbation rate <i>Diagnosed age</i> <i>ACT score</i> <i>FEV1</i> <i>BMI</i>	0 0.008 0 0 0 0.021 0.278 0.914 0.561 0.914 0.506 0.914 0.506 0.914 0.510 0.914	High High High High High High - - - - - - - -	0.08 0.77 68.5 6.05 450 91.5 66.0 516.5 8.26 249 86.7 3 14 18.5 92.3 27.6	0.05 0.53 44.7 4.71 370 78.0 43.7 440.5 7.40 168 48.2 2 10.5 17.3 76.2 21.5	0.09 0.93 81.1 7.67 583 107.3 91.7 669.5 11.98 291 187.8 4 17.75 22.8 98.2 30.5
E: severe asthma low eosinophilic	CS resistant <i>Exercise induced</i> GINA <i>Near fatal</i> <i>Atopy</i> Fast LF decline <i>Nocturnal asthma</i>	0 0.035 0 0 0.011 0 0.141	Yes Yes Severe Yes No Yes -	Y=73.3 Y=60 Severe=100 Y=66.7 Y=20 Y=100 Y=80	N=26.7 N=40 Mild=0 N=33.3 N=80 N=0 N=20	IL-13 IL-5 EDN Eos Percent Eos Cells Periostin Eotaxin	0.810 0.713 0.965 0.596 0.713 0.810 0.220	- - - - - -	0.03 0.31 30.8 2.6 210 71 98.4	0.02 0.25 20.6 1.5 110 60 66.9	0.05 0.52 36.8 3.9 260 82 191.9

						DPP4	0.021	Low	441	406	499
						<i>IL-8</i>	0.976	-	10.1	7.2	12.7
						<i>IgE</i>	0.319	-	56.8	25.8	251.5
						<i>MCP-1</i>	0.154	-	256	204	346
						Exacerbation rate	0.049	High	5	4	5
						Diagnosed age	0.019	High	41	34	44.5
						ACT score	0.006	Low	15	12.5	16.5
						FEV1	0	Low	46.0	41.0	61.5
						<i>BMI</i>	0.713	-	23.7	20.6	31.0
F: severe asthma non-eosinophilic	CS resistant	0.001	Yes	Y=66.7	N=33.3	IL-13	0.024	Low	0.02	0.01	0.03
	Exercise induced	0.042	Yes	Y=60	N=40	<i>IL-5</i>	0.130	-	0.25	0.2	0.34
	GINA	0.012	Severe	Severe=73.3	Mild=0	EDN	0.004	Low	21	14.2	26.0
	Near fatal	0.011	Yes	Y=53.3	N=46.7	Eos Percent	0.008	Low	1.9	0.85	2.35
	<i>Atopy</i>	0.308	-	Y=40	N=60	Eos Cells	0.049	Low	150	60	195
	Fast decline	0.001	Yes	Y=93.3	N=6.7	<i>Periostin</i>	0.191	-	59	53	71.5
	LF					Eotaxin	0.002	High	114	95	179
	<i>Nocturnal asthma</i>	0.052	-	Y=86.7	N=13.3	<i>DPP4</i>	0.692	-	518	433	545
						<i>IL-8</i>	0.076	-	11.9	10.2	15.6
						<i>MCP-1</i>	0.597	-	248	223	309
						<i>IgE</i>	0.130	-	181.0	84.6	309.5
						Exacerbation rate	0.475	-	4	2.5	5
						Diagnosed age	0.374	-	34	16	40
						ACT score	0.005	Low	15	11.5	17
						FEV1	0.001	Low	69.2	50.1	76.5
						<i>BMI</i>	0.222	-	26.8	24.2	28.6

Table E3: Comparison between clusters for asthma background medication

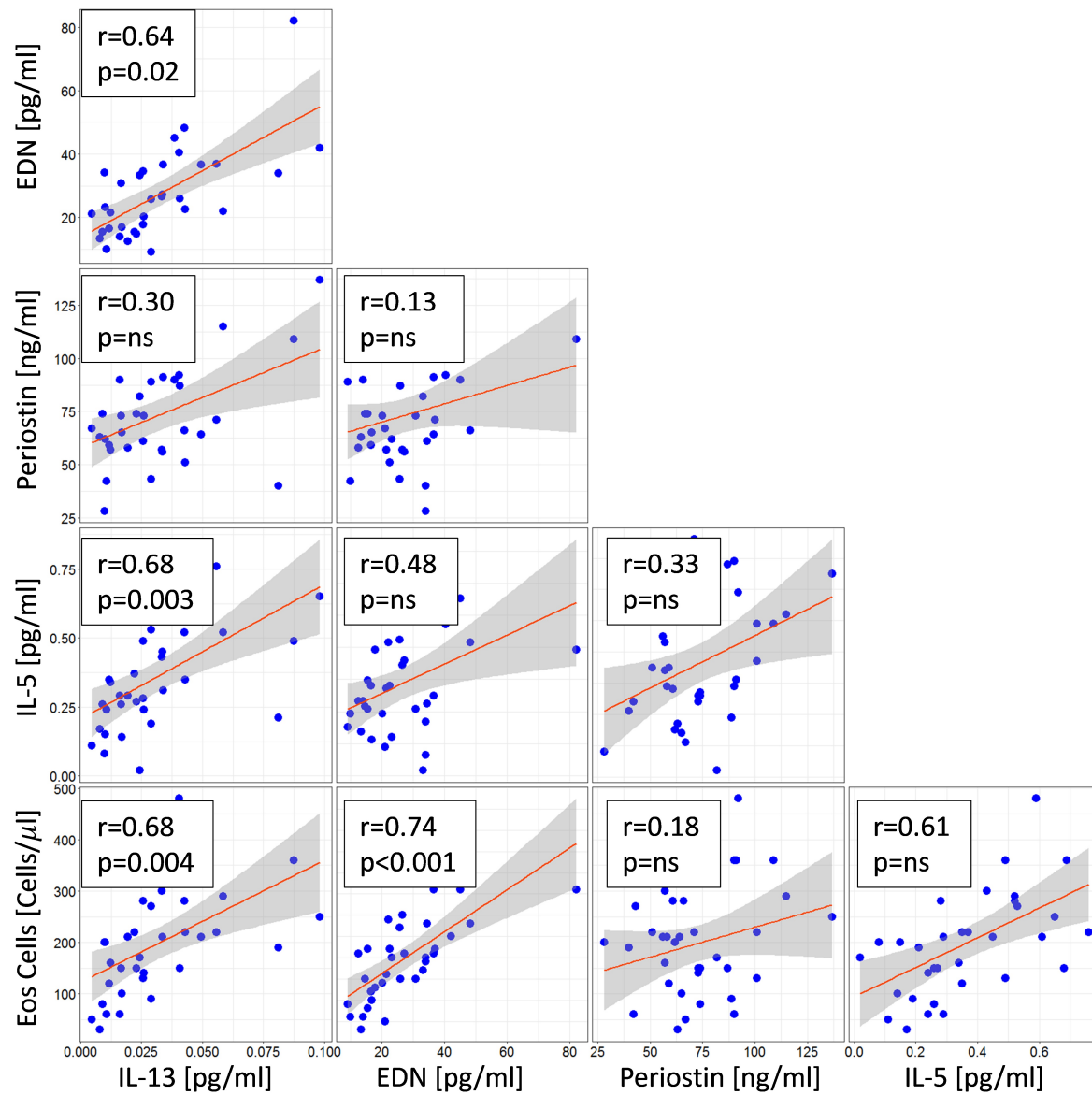
ICS dose	A	B	C	D	E	F
Low	10	3	4	0	0	0
Medium	5	14	12	9	2	5
High	1	9	7	5	13	10
Asthma controller drugs	A	B	C	D	E	F
ICS	9	2	0	1	0	0
LTRA	3	0	1	0	0	0
ICS/LABA	5	15	15	5	1	4
ICS/LTRA	0	0	1	0	0	0
ICS/LABA/LAMA	1	1	1	0	0	2
ICS/LABA/LTRA	1	4	5	5	4	0
ICS/LABA/LAMA/LTRA	0	4	1	3	10	9

Asthma background treatment supports the severity stratification of the clusters with mild asthma (cluster A) receiving mainly monotherapy with ICS or LTRA, moderate asthma (clusters B,C,D) receiving the ICS/LABA or ICS/LABA/LTRA combination and severe asthma (clusters E and F) receiving the ICS/LABA/LAMA/LTRA combination
ICS = inhaled corticosteroids; LABA = long acting bronchodilator; LAMA = long acting antimuscarinic agent; LTRA = leukotriene receptor antagonist



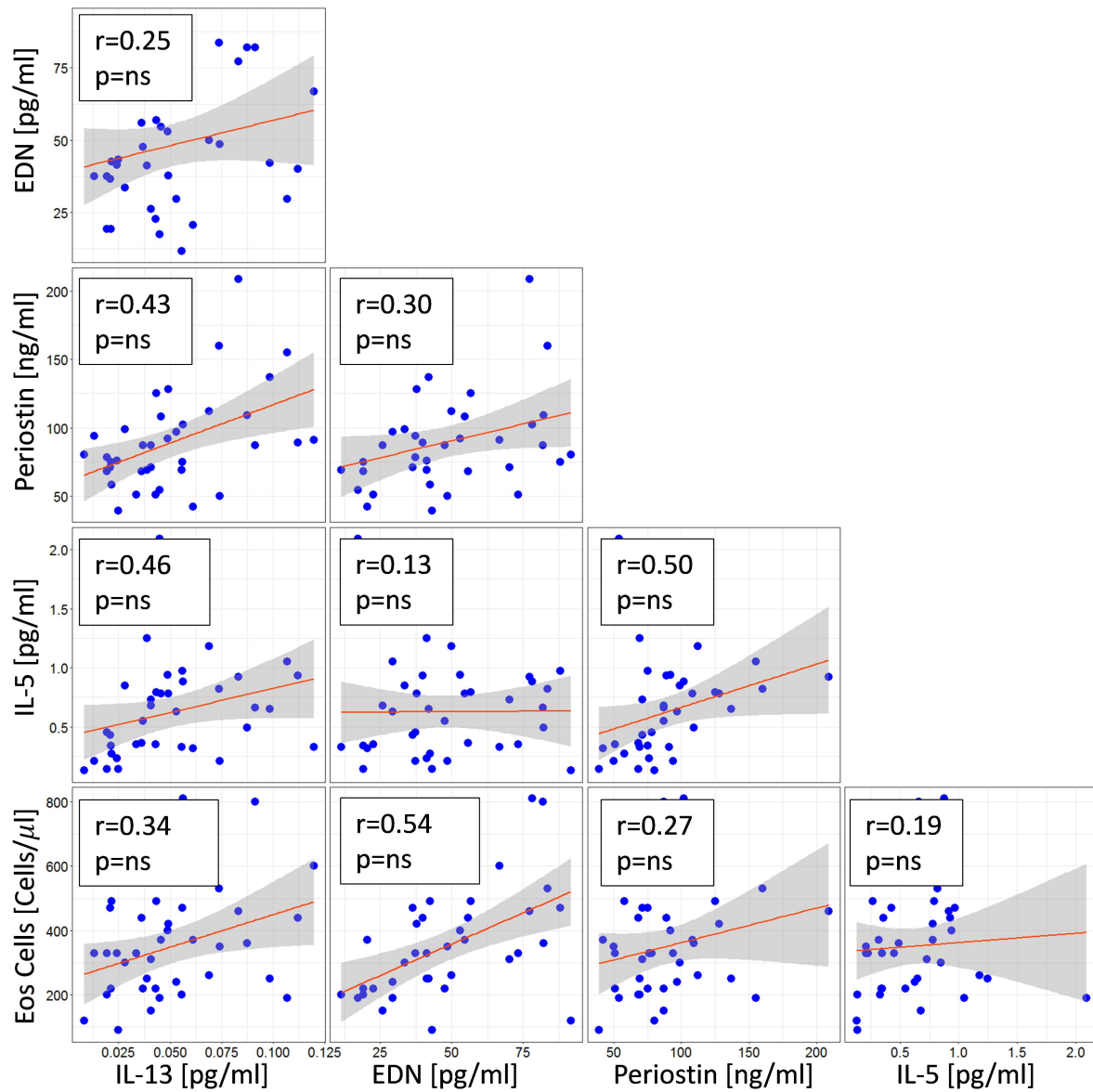
Agache I et al Figure E1.

cluster A and E (all low eosinophilic)

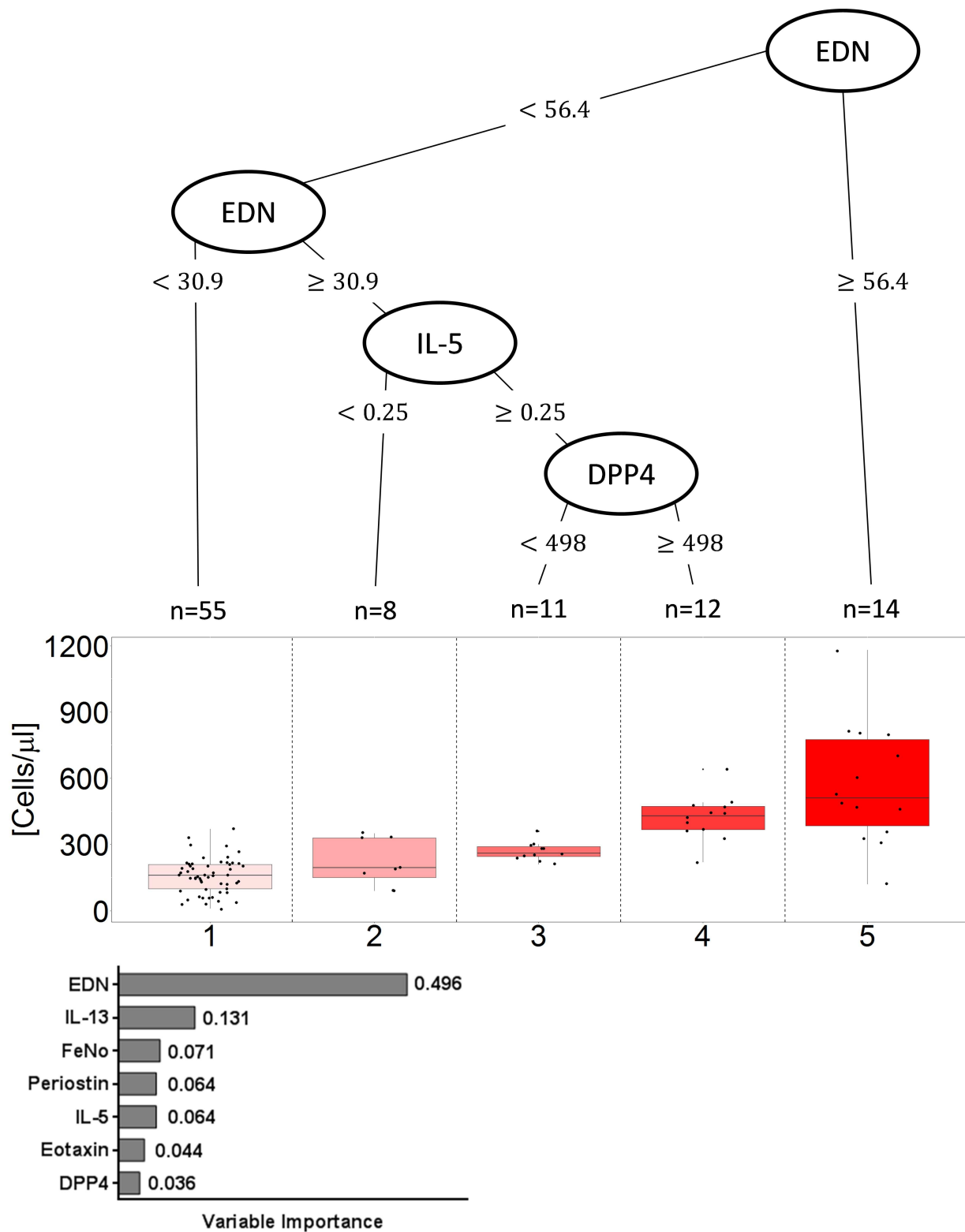


Agache I et al Figure E2.

cluster C and D (medium and high eosinophilic)



Agache I et al Figure E3.



Agache I et al Figure E4.