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Title Page

Identification and prospective stability of eNose derived inflammatory phenotypes in severe asthma.

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ABSTRACT

Background

Severe asthma is a heterogeneous condition as shown by independent cluster analyses based on demographic, clinical and inflammatory characteristics. A next step is to identify molecular driven phenotypes using 'omics'-technologies. Molecular fingerprints of exhaled breath are associated with inflammation and may qualify as non-invasive assessment of severe asthma phenotypes.

Objectives

We aimed: 1) to identify severe asthma phenotypes by exhaled metabolomic fingerprints obtained from a composite of electronic noses (eNoses); 2) to assess stability of eNose derived phenotypes in relation to within-patient clinical and inflammatory changes.

Methods

In this longitudinal multicenter study exhaled breath samples were taken from an unselected subset of adult severe asthma subjects from the U-BIOPRED cohort. Exhaled metabolites were centrally analyzed by an assembly of eNoses. Unsupervised Ward clustering enhanced by Similarity Profile Analysis (SPA) together with K-Means clustering was performed. For internal validation Partitioning Around Medoids (PAM) and topological data analysis (TDA) were applied. Samples at 12-18 months of prospective follow-up were used to assess longitudinal within-patient stability.

Results

Data were available for 78 subjects (age 55 [IQR: 45-64] years, 41% male). Three eNose-driven clusters (n=26/33/19) were revealed, showing differences in circulating eosinophil- (p=0.045)

and neutrophil percentages (p=0.017) and ratio of patients using oral corticosteroids (p=0.035). Longitudinal within-patient cluster stability was associated to changes in sputum eosinophils (p=0.045).

Conclusions

We have identified and followed-up exhaled molecular phenotypes of severe asthma, which were associated with changing inflammatory profile and oral steroid usage. This suggests that breath analysis might contribute to the management of severe asthma.

Abstract word count: 250

Key messages:

- Unbiased clustering of exhaled breath profiles captured by eNose technology revealed three phenotypes of severe asthma, which significantly differ with respect to systemic inflammatory markers and usage of anti-inflammatory medication.
- Follow-up at 12-18 months showed a significant difference between 'cluster stable' and 'cluster migrating' patients with regard to their longitudinal changes in sputum eosinophils.

Capsule summary:

We have identified and followed-up exhaled molecular phenotypes of severe asthma, which were associated with changing inflammatory profile and oral steroid usage. This suggests that breath analysis might contribute to the management of severe asthma.

Keywords:

electronic nose technology; exhaled breath; volatile organic compound; follow-up; severe asthma; unbiased clustering; eosinophils; neutrophils; oral corticosteroids.

Abbreviations:

BMI: body mass index

IgE: immunoglobulin E.

IQR: interquartile range

RNA: ribonucleic acid

Th17: T-helper 17 cells

Th2: T-helper 2 cells

INTRODUCTION

From a clinical point of view, asthma is defined as a disease of episodic and recurrent chest symptoms and variable airflow limitation, including features of airways inflammation and structural changes[1]. When considering clinical management, international guidelines are distinguishing different levels of asthma control and severity[2, 3]. Patients with severe asthma form nearly 5% of the total asthma population[4]. It is well recognized that severe asthma is a heterogeneous chronic inflammatory disease, with several clinical presentations, physiological characteristics, inflammatory and structural profiles and outcomes[5, 6]. This heterogeneity of severe asthma shows the need of recognizing distinct phenotypes, which may allow more personalized management.

Disease phenotyping is based on classification of patients into subgroups using clinical and/or biological parameters[7, 8]. Identification of such subgroups can be accomplished through unbiased or hypothesis-driven methods [7, 9, 10], resulting in clusters that may or may not be linked to currently known pathophysiological pathways. Based on clinical, physiological and/or cellular disease features (*e.g.* disease-onset, gender, BMI, lung function, (non-)eosinophilic profile, etc.) independent studies have revealed remarkably similar clusters of severe asthma[11, 12]. This supports the clinical potential of improving long term disease outcomes by tailoring individual treatment[13-15].

A next step towards tailoring asthma management is to phenotype patients based on the multi-molecular profiles. The availability of high-throughput large-scale analytical methods and complex statistical and computational procedures are making the search for new biomarkers at high dimensional 'omics'-level a tangible option[7, 16]. 'Omics' concerns the acquisition and analysis of such large-scale biological datasets with the aim to discover and

identify biomarkers of diseases and new pathophysiological mechanisms. This approach purposely avoids *a priori* assumptions about markers that may be associated with a particular disease or phenotype[8]. The 'omics' field has demonstrated to be valuable in asthma research. A transcriptomics study on airway epithelial brushings identified two asthma subgroups defined by the degree of Th2 inflammation[17], whilst gene expression profiling of induced sputum samples from patients with severe asthma not only showed three distinct phenotypes[9], but also its capability to distinguish Th2-high and Th2-low subtypes of asthma[18]. Furthermore, clustering of sputum cytokine-high profiles revealed five unique asthma molecular phenotypes[19]. Although these data are demonstrating the merit of molecular profiling in asthma, the technologies are analytically demanding and far from being applicable in daily medical care. Therefore, there is a need to bridge the gap between 'omics' technologies and clinical diagnostics and monitoring.

Metabolomics of exhaled air (breathomics) can serve the purpose of molecular profiling in asthma at point-of-care. Exhaled breath contains a complex gas mixture of volatile organic compounds (VOCs), which can be obtained noninvasively. These composite VOC samples can be analysed by detecting individual molecular compounds using gas chromatography–mass spectrometry (GC-MS) or through merely pattern recognition of exhaled VOCs using cross-reactive sensor arrays by electronic nose (eNose) technology[20]. Indeed, GC-MS has been applied in the discovery of biomarkers for the prediction of eosinophilic *versus* non-eosinophilic asthma[21], the discrimination between clinically stable and unstable episodes of asthma[22] and in the inflammatory phenotyping of patients with chronic obstructive pulmonary disease (COPD)[23]. Notably, when using the faster electronic nose (eNose) technology[20], based on a combination of cross-reactive sensors and pattern recognition algorithms, exhaled breath from patients with asthma, COPD and healthy controls could be discriminated in several

independent studies[24-26]. Moreover, cluster analysis by eNose among a study population of both asthma and COPD patients resulted in clusters that are not determined by diagnosis, but rather by clinical and inflammatory characteristics[27]. This raises the questions whether phenotyping of severe asthma patients based on solely exhaled breath profiles is possible and what the within-patient stability of those phenotypes is over time.

We hypothesized that eNose technology is suitable for non-invasive identification of severe asthma phenotypes. Therefore, we our aims were: 1) to identify severe asthma phenotypes by unbiased and benchmarked cluster analysis based on metabolomic fingerprints from exhaled breath by an composite of different brands of electronic noses (U-BIOPRED eNose platform), 2) to assess within-patient stability in eNose derived clusters, in relation to changes in clinical and inflammatory characteristics after 12-18 months of prospective follow-up.

METHODS

Subjects

In this study, an unselected subset of adult subjects (aged ≥18 yr.) from the pan-EUROPEAN U-BIOPRED cohort study was included[28]. All participants were diagnosed with severe asthma according IMI-criteria[5] In short, patients with a prescription for high dose inhaled corticosteroids (ICS) (≥ 1000 μg fluticasone propionate (FP) or equivalent) plus at least one other controller medication and were defined uncontrolled according to Global Initiative for Asthma (GINA) guidelines *and/or* had 2 or more severe exacerbations per year *and/or* required prescription of oral corticosteroids (OCS) minimally daily to achieve asthma control. Subjects were excluded if they changed asthma medication or required high dose OCS or a doubling of maintenance OCS for at least 3 days due to a severe asthma exacerbation in the month prior to the study visits. The study was approved by all local Medical Ethics Committees and all patients gave their written informed consent. The study was registered at ClinicalTrials.gov identifier: NCT01976767.

Design

The U-BIOPRED study in adults consisted of three visits in the severe asthma cohorts[28]. At the first visit participants were screened for eligibility to participate according to the inclusion and exclusion criteria. For the purpose of the present study several measurements were performed: at the baseline visit and at the 12-18 months prospective follow-up visit FE_{NO} was measured, followed by exhaled breath collection. Subsequently, pre- and post-bronchodilator spirometry and a sputum induction were performed. Finally allergy tests, blood and questionnaires were taken as outlined previously[28].

Measurements

Exhaled breath collection. Exhaled breath of severe asthma patients was collected at seven participating sites as previously described[24, 25, 29]. After refraining from eating, drinking and smoking for at least 2 hours, patients breathed for 5 minutes at tidal volume through a three-way non re-breathing valve and an inspiratory carbon VOC-filter (A2, North Safety, Middelburg, NL). Next, the subject exhaled a single vital capacity volume into a 10 L Tedlar bag (SKC Inc, Eighty Four, PA, USA). The VOCs in the Tedlar bag were trapped on thermal desorption tubes containing Tenax (Tenax GR SS 6mm x 7", Gerstel, Mülheim an der Ruhr, Germany), by drawing the air through the Tenax tube using a peristaltic pump at a flow rate of 250 ml/min. Such storage of VOCs preserves the exhaled marker signal[30].

The tubes were sent to the Academic Medical Centre Amsterdam for central analysis. After desorption of VOCs using a thermal desorption oven (TDS 3, Gerstel, Mülheim an der Ruhr, Germany), the stored VOCs were transferred into a Tedlar bag with nitrogen as carrier gas. Subsequent analysis was carried out using the composite U-BIOPRED eNose platform. This eNose platform consists of an assembly of four eNoses all from different developers, using distinct sensor technologies: 1) Cyranose C320 using carbon black-polymer sensors[31] [32 sensors], 2) Tor Vergata eNose using quartz crystal microbalances (QMB) covered with metalloporphyrins[32] [8 sensors], 3) Common Invent eNose using metal oxide semiconductor sensors[33] [8 sensors], and 4) Owlstone Lonestar based on field asymmetric ion mobility spectrometry [110 datapoints][34]. For all samples analysed by the U-BIOPRED eNose platform, the structure of the output is similar: a 158-datapoints counting profile based on responses of all four eNoses.

Lung function. Spirometry was performed before and 10 minutes after inhalation of 400 µg of salbutamol via a spacer according to ERS recommendations using daily-calibrated equipment[35].

Sputum Induction. Sputum was induced by inhalation of hypertonic saline according to the ERS recommendations[36]. Selected samples were processed with 0.1% dithioerithrytol (DTE) and differential cell counts were expressed as percentage of non-squamous cells.

Blood. Blood eosinophils and neutrophils percentages were obtained from standard complete blood counts.

Allergic status. Allergy testing was performed using total and specific serum IgE and skin prick test (SPT) to a panel of common aeroallergens. Atopy was defined as the presence of sensitisation on SPT (wheal ≥ 3 mm) or serum specific IgE (≥ 0.35 kU/L).

FE_{NO}. Fractional exhaled lower respiratory nitric oxide was measured with a portable analyser (NIOX Mino System; Aerocrine, Solna, Sweden) at a constant flow rate of 50 mL/s, according to ATS/ERS recommendations[37].

Questionnaires. Asthma control was assessed by the Juniper asthma control questionnaire (ACQ) that is a validated 5-item questionnaire [38], while the SNOT-20 questionnaire was used as measure for rhinosinusitis status[39].

Statistical analysis

Repeatability testing. For the purpose of repeatability testing, the mean±standard deviation (SD) of the within-subject coefficient of variation (WSCV) among all 158 sensors was calculated based on available duplicate samples from the present study.

Data pre-processing. After ComBat[40] batch correction, a BoxCox[41] power transformation was applied to achieve optimal data distribution. To avoid data from different eNoses being disparate, it was normalised by adjusting the average and standard deviation of each individual eNose sensor to respectively 0 an 1[42].

Principal component analysis. From a total of 158 eNose sensors principal components (PC's) were derived in order: 1) to achieve an optimal eNose platform fingerprint based on individual sensor deflections utilizing a limited number of variables[43], and 2) to suppress noise, because the variance captured by the least important principal components represents noise that should be rejected. Considering the Kaiser Criterion[44], only PC's with eigenvalues greater than 1 were retained for further analysis.

Cluster analysis. This was performed based on a strategy reported by Amelink *et al.*[45]. First hierarchical Ward clustering, combined with Similarity Profile Analysis (SPA) was used to assess the number of significant cluster groups[46]. Then, the actual grouping based on K-Means clustering was performed. Non-hierarchical K-Means clustering partitions the input space (*i.e*. centroid locations are calculated) instead of building a dendogram, which makes it suitable for longitudinal modelling.

Between cluster comparison. Between-cluster comparison and pairwise post-hoc analyses of clinical, physiological and inflammatory variables at baseline was performed by the Kruskal-Wallis test for continuous data and the Pearson's Chi-squared Test for categorical data. Clinical variables were considered statistically significant when $p \leq 0.05$. Furthermore, the distribution of patients from the individual clinical sites among the revealed clusters was evaluated through Pearson's Chi-squared testing.

Cluster Benchmarking. The clustering results of the baseline dataset were benchmarked using two more clustering techniques: partitioning around mediods (PAM) and topological data analysis (TDA) by the Ayasdi Workbench, v7.5.0 (Ayasdi Inc., Menlo Park, CA, USA)[10, 16]. Concordance between Ward clustering and TDA was qualitatively assessed by combining results in a graphical display. Similarity of outcomes between Ward, K-Means and PAM clustering was quantified by Rand Indexing, which results in a value between 0 (complete disagreement) and 1 (complete agreement)[47].

Follow-up. The data workup to appraise the longitudinal behaviour of the clusters existed of three steps: 1) data pre-processing similar to baseline. 2) Calculation of PC's based on the loading factors of the baseline dataset. 3) Cluster membership prediction for each individual patient, using the former K-Means clustering results for modelling.

The baseline and longitudinal clustering outcomes were cross-tabulated. In order to compare the characteristics between 'cluster stable' patients vs. 'cluster migrating' patients, absolute Δ's between baseline and follow-up visits (*i.e.* changes over time) regarding clinical, physiological and inflammatory variables were evaluated using the Mann–Whitney–Wilcoxon test for continuous data or the Pearson's Chi-squared test categorical variables.

Exploratory analyses. A series of exploratory analyses was performed through Partial least squares Discriminant Analysis (PLS-DA) and cross-validated Partial least squares regression (PLS regression).

1) Analysis of correlation between the 158 eNose sensors by PLS-DA at eNose sensor level vs. obtained clusters.
2) Examination of relationship between significantly different inflammatory parameters and VOC patterns through PLS regression.
3) Testing of association between the U-BIOPRED sputum transcriptomic clusters (TAC 1, 2, 3) delineated by Kuo *et al.* [9] and VOC patterns reported here via PLS-DA.

Apart from TDA analysis , all analyses were performed in R studio (v.1.1.383) using R (v.3.3.3) as engine and supported by R packages: caret, clue, clustsig, ConsensusClusterPlus, data.table, fossil, Hmisc, MASS, stringr, sva, tableone, mixOmics, plsdepot and boot

RESULTS

With a success rate of 100%, baseline breath samples of 80 severe asthma patients were available, recruited from seven different sites across five countries in Europe. Two samples were lost due to wet-lab technicalities, resulting in a complete data of 78 patients. Baseline characteristics are summarized in table 1.

Unbiased cluster analysis of exhaled breath profiles

From the original dataset based on 158 eNose sensors (mean±standard deviation of WSCV: 6.28±1.70%, *n*=11), thirty four principal components with an eigenvalue of above 1 were derived. By applying K-Means clustering with a predefined number of clusters based on Ward clustering three groups of severe asthma patients were delineated solely on their exhaled breath profiles (Figure 1). Clusters 1 consists of 26 patients, cluster 2 of 33 and cluster 3 includes 19 patients.

Clinical characteristics of clusters

Clinical data for each cluster are shown in table 2 and between-cluster post-hoc analyses are presented in figure E2 (online repository). There were significant differences between the clusters in chronic OCS usage and eosinophil- and neutrophil percentages in blood, as outlined in the following cluster profiles. No significant relation between sample origin (sites) and revealed clusters was found (table E1 (online repository)).

Cluster 1:

The first group includes 33% of the patients (*n* = 26) and is the 'middlemost' cluster. With blood eosinophil percentages of 4.1 (IQR: 1.8-6.9), blood neutrophil percentages of 57.8 (IQR: 54.0-67.1) and a percentage of oral corticosteroids users of 39%, it represents the second highest/lowest levels at all three discriminating variables among the clusters.

Cluster 2:

The second cluster includes 42% (*n* = 33) of the study population and can be described as neutrophilic inflammation-predominant, with the highest blood neutrophil percentage of the three clusters, being 65.6% (IQR: 60.0-76.2). With an oral corticosteroid usage of 58%, this is the cluster with the highest number of patients using OCS maintenance therapy.

Cluster 3:

The final cluster comprises 24% of the patients (*n* = 19). This phenotype differentiates from the other two, since only 4 out of 19 patients (21%) are chronically using OCS and it shows the highest percentages of peripheral blood eosinophilia 4.7% (IQR: 2.64-8.00) among the three groups.

Benchmarking

TDA demonstrated largely similar findings compared to Ward clustering. To demonstrate coherence between both methods data points in figure 2 are colour-coded based on the three significant clusters revealed by K-Means clustering. Quantitative assessment of the similarity between K-Means vs. Ward and PAM clustering resulted in a Rand Indexing scores of respectively 0.82 and 0.74.

Follow-up

Fifty-one out of the 78 patients included in baseline visit provided a breath sample at the 12-18 months' follow-up visit (table 1). When comparing the baseline clustering results with the longitudinal allocation, 21 patients (41%) were 'cluster stable', while 30 patients migrated to another cluster (table 3). There was a significant difference between these two groups in their change (absolute *Δ*) of sputum eosinophil percentages (table 4).

Exploratory analyses

1) SPLS-DA correlation plot (figure E1) indicates how the 158 sensors correlate with each other. Four out of eight sensors from the Tor Vergata eNose have a high correlation with a large number of sensors from of the Cyranose C320.
2) Based on the clustering outcomes (significantly different inflammatory parameters between the eNose clusters) SPLS regression of blood neutrophils (%) and eosinophils (%) at baseline vs. VOC patterns, and absolute delta's (Δ) of sputum eosinophils (%) vs. absolute delta's (Δ) of VOC patterns was performed, resulting in R^2=0.41, 95%CI(0.13 -

0.75), R^2=0.63, 95%CI(0.52 - 0.74) and R^2=0.87, 95%CI(0.76 - 0.99) respectively (figures E4, E5, E6).

3) From a total of 28 patients a complete data concerning their sputum transcriptomic (*TAC)*-profile[9] and an exhaled breath pattern was available. Based on the VOC patterns the three *TAC*-clusters could be discriminated with an accuracy of 0.93 (figure E3).

DISCUSSION

This study shows that unbiased clustering of exhaled breath profiles captured by eNose technology identifies three phenotypes of severe asthma. These clusters significantly differed with respect to systemic inflammatory markers and usage of anti-inflammatory medication. These findings were benchmarked using different clustering techniques. Follow-up at 12-18 months showed a significant difference between 'cluster stable' and 'cluster migrating' patients with regard to their longitudinal changes in sputum eosinophils. Our results support the concept of using exhaled breath analysis by eNose for quick and non-invasive inflammatory phenotyping of severe asthma patients, which can be of clinical value with respect to personalized management or monitoring of these difficult to treat patients.

To our knowledge, this is the first study that establishes clusters of patients with severe asthma based on exhaled breath analysis by eNose technology. The second novelty of this study is the assessment of stability of patients in those clusters during longitudinal follow-up. Previously, clustering techniques have been used to describe and to monitor phenotypes of asthma based on clinical characteristics[12, 48-51] and for eNose technology focused on phenotyping broad cohorts of obstructive pulmonary diseases [27, 52]. The present study however, merged both approaches by using unbiased molecular profiling as starting point for the follow-up of severe asthma phenotypes. At a cross-sectional level this concept is similar to the sputum gene expression profiling by Baines *et al* [53] , Kuo *et al* [9] and Seys *et al*[19] as well as the serum cytokine profile clustering by Liang *et al* [54]or imaging-based cluster analysis by Choi *et al*[55]. These approaches cluster patients solely based on manifest molecular mechanisms and pathophysiology, thereby allowing the biology to drive the classification. In this way 'omics' technologies are employed to generate clusters based on both known and unknown pathophysiological pathways, rather than examining *a priori* defined molecular

mechanisms, such as Th2 or Th17 pathways[9, 10, 17]. The present eNose clusters show that exhaled metabolites yield differential signals mainly related to inflammatory profiles, which extends similar observations based on profiling at RNA levels in sputum[9, 53]. Therefore, our data suggest that 'breathomics' can bridge the gap between clinical and laboratory assessments in the phenotyping of severe asthma.

Our study has a number of strengths. First, exhaled breath samples were analyzed on the U-BIOPRED eNose platform according to the most recent standards for quality control[20], an assembly of electronic noses from four different brands, all driven by different sensor techniques. Using this composite system, we expect to have approximated an optimal dataset from VOC mixtures as based on available electronic nose technology. Second, the participants were carefully characterized and recruited from eight different sites across five European countries in this study. This international and multi-center character strengthens the general validity of the observed eNose-phenotypes of severe asthma. Finally, we used distinct clustering techniques to test the stability of clusters and added similarity profile analysis for determining the number of significant clusters with the assumption of no *a priori* groups. The latter strengthens the validity of our findings.

Our study also has limitations. First, an external validation in a new and independent cohort was lacking. This would have required to repeat the U-BIOPRED study, which understandably is a future goal. The second best option would have been a split into a training and validation set[56], but this resulted in sample sizes too small for adequate cluster analysis, thereby promoting inevitable overfitting. We did, however, validate our findings by benchmarking three specifically different clustering techniques: hierarchical methods vs. partitioning vs. clumping (continuous) variation combined with similarity testing by Rand Indexing. The

consistency of these results adds to the plausibility of our findings. Second, eNose technology seems to be suitable for the non-invasive identification of severe asthma phenotypes, but is principally unable to identify the individual VOCs that are driving the distinction between the subgroups. Specific analysis by GC-MS will then be necessary in order to unravel the identity of the combination of VOCs. Recent GC-MS studies have shown that asthma particularly features exhaled VOCs that are associated with lipid peroxidation and inflammatory phenotypes[21, 57], the distribution of which is likely to drive the distinction between the three clusters. Finally, given the nature of the project there is an inevitable influence of medication and smoking on top of endogenous biological mechanisms. Inhaled and systemic corticosteroids as well as short-acting beta2-agonists are likely to affect metabolomics fingerprints[58, 59] and thereby patients' breathprints. Notably, the present analysis picked this up by showing that systemic steroid usage was one of the distinguishing features. Therefore, we believe that including real life patients with varying levels of treatment is not a major limitation of the present study.

How can we interpret these results? It appears that the eNose platform predominantly captured features of inflammation and anti-inflammatory treatment, such as eosinophils and neutrophils and oral steroid usage. Similar to several previous studies on exhaled VOC analysis in obstructive pulmonary diseases [21-23, 27, 52, 60], our data confirms that exhaled VOCs appears to be associated with both systemic and local airway eosinophilic inflammation. Apparently, both types of inflammation express themselves through a different set of exhaled VOCs since there is little to no agreement in outcomes between the two inflammatory features among our reported clusters. This might be caused by the distinct molecular pathways underlying eosinophilic inflammation[61]. Furthermore, differences in metabolic profiles between cluster 2 and 3 might particularly be linked to a combination of blood eosinophil percentages and OCS

usage (see table 2), whereas cluster 1 doesn't show specific distinctive characteristics as measured by the available clinical and inflammatory features. Nevertheless, the latter group does differ with regard to its exhaled VOC driven profile, which points towards a complementary value of molecular assessment on top of what we know from our patients by commonly used assessment. Considering the aims of the U-BIOPRED consortium, such discovery of previously unknown molecular phenotypes is key to increased understanding of severe asthma. Additional explorative analysis concerning identification of sputum transcriptomic phenotypes (TAC 1, 2, 3) based on exhaled breath patterns resulted in an accuracy of 0.93. This identification of the sputum transcriptomics clusters is driven by different PCs than the ones most relevant for the primary clusters reported in this manuscript. We believe this underlines the wealth of information that is available in exhaled breath.

What are the clinical implications of our data? When eNose technology is capable of identifying clinically revealed and unrevealed asthma phenotypes, it can serve a transitional role between 'omics' technologies and clinical medicine. Transcriptomics, proteomics and metabolomics in tissue, sputum or blood have demonstrated their value by identification of distinct phenotypes in asthma[7, 9, 10, 16, 17, 19, 23, 53, 62]. Since pathophysiological mechanisms have been linked to these phenotypes, new specifically targeted treatments are currently being developed. This leads to an increasing need for point-of-care biomarkers to predict and to monitor the responsiveness of patients to particular interventions. Whilst most 'omics' technologies are difficult to implement in daily medical care, gas sensor based exhaled breath analysis is developing towards clinical implication[27, 63], thereby having the potential to fulfil this increasing clinical need. In addition, even though biomarkers for capturing the eosinophilic phenotype and predicting responsiveness to common therapies are available, these are either not widely used because of laborious procedures (sputum eosinophilia) or

exhibit insufficient accuracy (exhaled nitric oxide)[64, 65]. We believe that the present study adds to strengthening the clinical utility of eNose measurements by showing that migration of patients between the clusters is associated with changes in sputum eosinophilia whilst we observed relatively high correlations between the exhaled breath profiles and eosinophilic and neutrophilic inflammation. This fits in with the predictive capacity of eNose assessment for steroid responsiveness[66] and its potential to discriminate between clinically stable and unstable episodes of asthma[22]. It will require longerterm follow-up studies to examine the clinical course of the cluster-stable and cluster-unstable patients as identified by eNose.

In conclusion, this study reveals unbiased clusters of severe asthma patients based on exhaled breath profiles captured by the U-BIOPRED eNose platform. Three significant clusters with differences regarding eosinophilic/neutrophilic inflammation and systemic steroid usage were delineated. Notably, after 12-18 months of follow-up 'cluster stable' and 'cluster migrating' patients differed with regard to their longitudinal changes in sputum eosinophils. These results warrant prospective studies on the potential of exhaled breath fingerprinting by eNose as point-of-care procedure for (therapeutic) management of patients with severe asthma.

483 References

484 1. Holgate ST. Asthma: a simple concept but in reality a complex disease. *Eur J Clin*
485 *Invest* 2011;**41**(12):1339-1352.

486 2. Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and
487 Prevention. 2017 [cited; Available from: http://ginasthma.org/

488 3. Taylor DR, Bateman ED, Boulet LP, Boushey HA, Busse WW, Casale TB, et al. A new
489 perspective on concepts of asthma severity and control. *Eur Respir J* 2008;**32**(3):545-554.

490 4. Hekking PP, Wener RR, Amelink M, Zwinderman AH, Bouvy ML, Bel EH. The
491 prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015;**135**(4):896-902.

492 5. Bel EH, Sousa A, Fleming L, Bush A, Chung KF, Versnel J, et al. Diagnosis and definition
493 of severe refractory asthma: an international consensus statement from the Innovative
494 Medicine Initiative (IMI). *Thorax* 2011;**66**(10):910-917.

495 6. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International
496 ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. *Eur Respir J*
497 2014;**43**(2):343-373.

498 7. Wheelock CE, Goss VM, Balgoma D, Nicholas B, Brandsma J, Skipp PJ, et al.
499 Application of 'omics technologies to biomarker discovery in inflammatory lung diseases. *Eur*
500 *Respir J* 2013;**42**(3):802-825.

501 8. Auffray C, Adcock IM, Chung KF, Djukanovic R, Pison C, Sterk PJ. An integrative
502 systems biology approach to understanding pulmonary diseases. *Chest* 2010;**137**(6):1410-
503 1416.

504 9. Kuo CS, Pavlidis S, Loza M, Baribaud F, Rowe A, Pandis I, et al. T-helper cell type 2
505 (Th2) and non-Th2 molecular phenotypes of asthma using sputum transcriptomics in U-
506 BIOPRED. *Eur Respir J* 2017;**49**(2).

10. Hinks TS, Zhou X, Staples KJ, Dimitrov BD, Manta A, Petrossian T, et al. Innate and adaptive T cells in asthmatic patients: Relationship to severity and disease mechanisms. *J Allergy Clin Immunol* 2015;**136**(2):323-333.

11. Newby C, Heaney LG, Menzies-Gow A, Niven RM, Mansur A, Bucknall C, et al. Statistical cluster analysis of the british thoracic society severe refractory asthma registry: clinical outcomes and phenotype stability. *PLoS One* 2014;**9**(7):e102987.

12. Moore WC, Meyers DA, Wenzel SE, Teague WG, Li H, Li X, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. *Am J Respir Crit Care Med* 2010;**181**(4):315-323.

13. Bel EH, Wenzel SE, Thompson PJ, Prazma CM, Keene ON, Yancey SW, et al. Oral glucocorticoid-sparing effect of mepolizumab in eosinophilic asthma. *N Engl J Med* 2014;**371**(13):1189-1197.

14. Honkoop PJ, Loijmans RJ, Termeer EH, Snoeck-Stroband JB, van den Hout WB, Bakker MJ, et al. Symptom- and fraction of exhaled nitric oxide-driven strategies for asthma control: A cluster-randomized trial in primary care. *J Allergy Clin Immunol* 2015;**135**(3):682-688.e611.

15. Petsky HL, Li A, Chang AB. Tailored interventions based on sputum eosinophils versus clinical symptoms for asthma in children and adults. *Cochrane Database Syst Rev* 2017;**8**:Cd005603.

16. Siddiqui S, Shikotra A, Richardson M, Doran E, Choy D, Bell A, et al. Airway pathological heterogeneity in asthma: Visualization of disease microclusters using topological data analysis. *J Allergy Clin Immunol* 2018.

17. Woodruff PG, Modrek B, Choy DF, Jia G, Abbas AR, Ellwanger A, et al. T-helper type 2-driven inflammation defines major subphenotypes of asthma. *Am J Respir Crit Care Med* 2009;**180**(5):388-395.

18. Peters MC, Mekonnen ZK, Yuan S, Bhakta NR, Woodruff PG, Fahy JV. Measures of gene expression in sputum cells can identify TH2-high and TH2-low subtypes of asthma. *J Allergy Clin Immunol* 2014;**133**(2):388-394.

19. Seys SF, Scheers H, Van den Brande P, Marijsse G, Dilissen E, Van Den Bergh A, et al. Cluster analysis of sputum cytokine-high profiles reveals diversity in T(h)2-high asthma patients. *Respir Res* 2017;**18**(1):39.

20. Bos LD, Sterk PJ, Fowler SJ. Breathomics in the setting of asthma and chronic obstructive pulmonary disease. *J Allergy Clin Immunol* 2016;**138**(4):970-976.

21. Ibrahim B, Basanta M, Cadden P, Singh D, Douce D, Woodcock A, et al. Non-invasive phenotyping using exhaled volatile organic compounds in asthma. *Thorax* 2011;**66**(9):804-809.

22. Brinkman P, van de Pol MA, Gerritsen MG, Bos LD, Dekker T, Smids BS, et al. Exhaled breath profiles in the monitoring of loss of control and clinical recovery in asthma. *Clin Exp Allergy* 2017;**47**(9):1159-1169.

23. Fens N, de Nijs SB, Peters S, Dekker T, Knobel HH, Vink TJ, et al. Exhaled air molecular profiling in relation to inflammatory subtype and activity in COPD. *Eur Respir J* 2011;**38**(6):1301-1309.

24. Dragonieri S, Schot R, Mertens BJ, Le Cessie S, Gauw SA, Spanevello A, et al. An electronic nose in the discrimination of patients with asthma and controls. *J Allergy Clin Immunol* 2007;**120**(4):856-862.

25. Fens N, Zwinderman AH, van der Schee MP, de Nijs SB, Dijkers E, Roldaan AC, et al. Exhaled breath profiling enables discrimination of chronic obstructive pulmonary disease and asthma. *Am J Respir Crit Care Med* 2009;**180**(11):1076-1082.

554 26. Montuschi P, Santonico M, Mondino C, Pennazza G, Mantini G, Martinelli E, et al.
555 Diagnostic performance of an electronic nose, fractional exhaled nitric oxide, and lung
556 function testing in asthma. *Chest* 2010;**137**(4):790-796.
557 27. de Vries R, Dagelet YWF, Spoor P, Snoey E, Jak PMC, Brinkman P, et al. Clinical and
558 inflammatory phenotyping by breathomics in chronic airway diseases irrespective of the
559 diagnostic label. *Eur Respir J* 2018;**51**(1).
560 28. Shaw DE, Sousa AR, Fowler SJ, Fleming LJ, Roberts G, Corfield J, et al. Clinical and
561 inflammatory characteristics of the European U-BIOPRED adult severe asthma cohort. *Eur*
562 *Respir J* 2015;**46**(5):1308-1321.
563 29. Ahmed W, Brinkman P, Weda H, Knobel H, Xu Y, Nijsen TME, et al. Methodological
564 considerations for large-scale breath analysis studies: lessons from the U-BIOPRED severe
565 asthma project. *J Breath Res* 2018.
566 30. van der Schee MP, Fens N, Brinkman P, Bos LD, Angelo MD, Nijsen TM, et al. Effect of
567 transportation and storage using sorbent tubes of exhaled breath samples on diagnostic
568 accuracy of electronic nose analysis. *J Breath Res* 2013;**7**(1):016002.
569 31. Lewis NS. Comparisons between mammalian and artificial olfaction based on arrays
570 of carbon black-polymer composite vapor detectors. *Accounts of chemical research*
571 2004;**37**(9):663-672.
572 32. Di Natale C, Paolesse R, D'Amico A. Metalloporphyrins based artificial olfactory
573 receptors. *Sensors and Actuators B: Chemical* 2007;**121**(1):238-246.
574 33. Bos LD, van Walree IC, Kolk AH, Janssen HG, Sterk PJ, Schultz MJ. Alterations in
575 exhaled breath metabolite-mixtures in two rat models of lipopolysaccharide-induced lung
576 injury. *J Appl Physiol (1985)* 2013;**115**(10):1487-1495.

577 34. Arasaradnam RP, McFarlane MJ, Ryan-Fisher C, Westenbrink E, Hodges P, Thomas
578 MG, et al. Detection of colorectal cancer (CRC) by urinary volatile organic compound
579 analysis. *PLoS One* 2014;**9**(9):e108750.
580 35. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al.
581 Standardisation of spirometry. *Eur Respir J* 2005;**26**(2):319-338.
582 36. Paggiaro PL, Chanez P, Holz O, Ind PW, Djukanovic R, Maestrelli P, et al. Sputum
583 induction. *Eur Respir J Suppl* 2002;**37**:3s-8s.
584 37. American Thoracic S, European Respiratory S. ATS/ERS recommendations for
585 standardized procedures for the online and offline measurement of exhaled lower
586 respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med*
587 2005;**171**(8):912-930.
588 38. Juniper EF, Svensson K, Mörk A-C, Ståhl E. Measurement properties and
589 interpretation of three shortened versions of the asthma control questionnaire. *Respiratory*
590 *Medicine* 2005;**99**(5):553-558.
591 39. Piccirillo JF, Merritt MG, Jr., Richards ML. Psychometric and clinimetric validity of the
592 20-Item Sino-Nasal Outcome Test (SNOT-20). *Otolaryngol Head Neck Surg* 2002;**126**(1):41-
593 47.
594 40. Johnson WE, Li C, Rabinovic A. Adjusting batch effects in microarray expression data
595 using empirical Bayes methods. *Biostatistics* 2007;**8**(1):118-127.
596 41. Box GEP, Cox DR. An Analysis of Transformations. *Journal of the Royal Statistical*
597 *Society. Series B Methodological* 1964;**26**(2):pp. 211-252.
598 42. van den Berg RA, Hoefsloot HC, Westerhuis JA, Smilde AK, van der Werf MJ.
599 Centering, scaling, and transformations: improving the biological information content of
600 metabolomics data. *BMC Genomics* 2006;**7**:142.

601 43. Lever J, Krzywinski M, Altman N. Principal component analysis. *Nature Methods*
602 2017;**14**:641.
603 44. Yeomans KA, Golder PA. The Guttman-Kaiser Criterion as a Predictor of the Number
604 of Common Factors. *Journal of the Royal Statistical Society. Series D (The Statistician)*
605 1982;**31**(3):221-229.
606 45. Amelink M, de Groot JC, de Nijs SB, Lutter R, Zwinderman AH, Sterk PJ, et al. Severe
607 adult-onset asthma: A distinct phenotype. *J Allergy Clin Immunol* 2013;**132**(2):336-341.
608 46. Clarke KR, Somerfield PJ, Gorley RN. Testing of null hypotheses in exploratory
609 community analyses: similarity profiles and biota-environment linkage. *Journal of*
610 *Experimental Marine Biology and Ecology* 2008;**366**(1–2):56-69.
611 47. Rand WM. Objective Criteria for the Evaluation of Clustering Methods. *Journal of the*
612 *American Statistical Association* 1971;**66**(336):846-850.
613 48. Bourdin A, Molinari N, Vachier I, Varrin M, Marin G, Gamez AS, et al. Prognostic value
614 of cluster analysis of severe asthma phenotypes. *J Allergy Clin Immunol* 2014;**134**(5):1043-
615 1050.
616 49. Zaihra T, Walsh CJ, Ahmed S, Fugere C, Hamid QA, Olivenstein R, et al. Phenotyping of
617 difficult asthma using longitudinal physiological and biomarker measurements reveals
618 significant differences in stability between clusters. *BMC Pulm Med* 2016;**16**(1):74.
619 50. Moore WC, Hastie AT, Li X, Li H, Busse WW, Jarjour NN, et al. Sputum neutrophil
620 counts are associated with more severe asthma phenotypes using cluster analysis. *J Allergy*
621 *Clin Immunol* 2014;**133**(6):1557-1563.e1555.
622 51. Konno S, Taniguchi N, Makita H, Nakamaru Y, Shimizu K, Shijubo N, et al. Distinct
623 Phenotypes of Smokers with Fixed Airflow Limitation Identified by Cluster Analysis of Severe
624 Asthma. *Ann Am Thorac Soc* 2018;**15**(1):33-41.

625 52. Plaza V, Crespo A, Giner J, Merino JL, Ramos-Barbon D, Mateus EF, et al.
626 Inflammatory Asthma Phenotype Discrimination Using an Electronic Nose Breath Analyzer. *J*
627 *Investig Allergol Clin Immunol* 2015;**25**(6):431-437.
628 53. Baines KJ, Simpson JL, Wood LG, Scott RJ, Gibson PG. Transcriptional phenotypes of
629 asthma defined by gene expression profiling of induced sputum samples. *J Allergy Clin*
630 *Immunol* 2011;**127**(1):153-160, 160.e151-159.
631 54. Liang Z, Liu L, Zhao H, Xia Y, Zhang W, Ye Y, et al. A Systemic Inflammatory Endotype
632 of Asthma With More Severe Disease Identified by Unbiased Clustering of the Serum
633 Cytokine Profile. *Medicine (Baltimore)* 2016;**95**(25):e3774.
634 55. Choi S, Hoffman EA, Wenzel SE, Castro M, Fain S, Jarjour N, et al. Quantitative
635 computed tomographic imaging-based clustering differentiates asthmatic subgroups with
636 distinctive clinical phenotypes. *J Allergy Clin Immunol* 2017;**140**(3):690-700.e698.
637 56. McShane LM, Cavenagh MM, Lively TG, Eberhard DA, Bigbee WL, Williams PM, et al.
638 Criteria for the use of omics-based predictors in clinical trials: explanation and elaboration.
639 *BMC Med* 2013;**11**:220.
640 57. Meyer N, Dallinga JW, Nuss S, Moonen E, van Berkel J, Akdis C, et al. Defining adult
641 asthma endotypes by clinical features and patterns of volatile organic compounds in exhaled
642 air. *Respir Res* 2014;**15**(1):136.
643 58. Beauchamp J. Inhaled today, not gone tomorrow: pharmacokinetics and
644 environmental exposure of volatiles in exhaled breath. *J Breath Res* 2011;**5**(3):037103.
645 59. Gaugg MT, Engler A, Nussbaumer-Ochsner Y, Bregy L, Stoberl AS, Gaisl T, et al.
646 Metabolic effects of inhaled salbutamol determined by exhaled breath analysis. *J Breath Res*
647 2017;**11**(4):046004.

60. Basanta M, Ibrahim B, Dockry R, Douce D, Morris M, Singh D, et al. Exhaled volatile organic compounds for phenotyping chronic obstructive pulmonary disease: a cross-sectional study. *Respir Res* 2012;**13**:72.

61. Coumou H, Bel EH. Improving the diagnosis of eosinophilic asthma. *Expert Rev Respir Med* 2016;**10**(10):1093-1103.

62. Bigler J, Boedigheimer M, Schofield JP, Skipp PJ, Corfield J, Rowe A, et al. A Severe Asthma Disease Signature from Gene Expression Profiling of Peripheral Blood from U-BIOPRED Cohorts. *Am J Respir Crit Care Med* 2016.

63. de Vries R, Brinkman P, van der Schee MP, Fens N, Dijkers E, Bootsma SK, et al. Integration of electronic nose technology with spirometry: validation of a new approach for exhaled breath analysis. *J Breath Res* 2015;**9**(4):046001.

64. Petsky HL, Cates CJ, Lasserson TJ, Li AM, Turner C, Kynaston JA, et al. A systematic review and meta-analysis: tailoring asthma treatment on eosinophilic markers (exhaled nitric oxide or sputum eosinophils). *Thorax* 2012;**67**(3):199-208.

65. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;**70**(2):115-120.

66. van der Schee MP, Palmay R, Cowan JO, Taylor DR. Predicting steroid responsiveness in patients with asthma using exhaled breath profiling. *Clin Exp Allergy* 2013;**43**(11):1217-1225.

670 Tables

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Table 1: Demographic data an baseline and longitudinal characteristics of study population

	Baseline	Longitudinal
Subjects; *n*	78	51
Age; years [median | IQR]	55.5 | 45.0 - 64.0	57.0 | 49.5 - 64.5
Gender; male [%]	41.0	43.1
Body Mass Index (kg/m2) [median | IQR]	27.8 | 24.4 - 32.6	27.8 | 25.1 - 32.3
Age of onset [median | IQR]	27.5 |7.0 - 40.5	30.0 | 10.5 - 41.5
Smoking status; current / ex- / non-smoker [%]	12.8 / 33.3 / 53.8	15.7 / 35.3 / 49.0
Pack years [median | IQR]	14.5 | 7.8 - 21.3	13.9 | 4.6 - 22.3
OCS use [%]	42.3	36.7
Total daily OCS dose [median | IQR]	10.0 | 10.0 - 12.5	10.0 | 10.0 - 12.5
Exacerbations/year [median | IQR]	1.0 | 0.0 - 2.8	1.0 | 0.0 - 2.0
Atopy; postive [%]	67.9	62.7
ACQ5; Juniper [median | IQR]	2.5 | 1.4 - 3.4	1.6 | 0.7 - 3.0
$PbFEV_1$ % predicted [median | IQR]	76.5 | 60.9 - 88.5	71.8 | 60.6 - 86.2
$PbFEV_1$/FVC actual ratio [median | IQR]	80.3 | 69.7 - 89.8	77.8 | 70.6 - 87.5
FE_{NO}; ppb [median | IQR]	28.5 | 14.4 - 49.0	29.0 | 17.5 - 52.0
SNOT-20 [median | IQR]	1.60 | 1.10 - 2.25	1.58 | 1.05 - 2.05
Sputum eosinophils; % [median | IQR]	5.0 | 1.3 - 19.4	2.0 | 0.8 - 17.4
Sputum neutrophils; % [median | IQR]	52.2 | 37.2 - 69.6	51.9 | 34.5 - 75.0
Blood eosinophils; % [median | IQR]	3.1 | 1.4 - 6.6	3.7 | 1.1 - 5.5
Blood neutrophils; % [median | IQR]	61.6 | 54.3 - 73.2	60.7 | 53.8 - 68.4

OCS use, regular daily usage of oral corticosteroids; Total daily OCS dose, daily OCS dose normalized to prednisolone, amongst OCS users; atopy, presence of sensitization on SPT (wheal ≥ 3mm) or serum specific IgE (≥ 0.35 kU/L); ACQ5, Juniper – 5 items Asthma Control Questionnaire; $PbFEV_1$, Post-bronchodilator Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; FE_{NO}, Fraction of Exhaled Nitric Oxide in parts per billion; SNOT-20, 20 items Sino-Nasal Outcome Test Questionnaire.

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Table 2: Characteristics of baseline clusters.

Cluster number	Cluster 1	Cluster 2	Cluster 3	*p*-value
Subjects *n*	26	33	19	
Age; years [median | IQR]	59.0 | 50.3 - 63.8	55.0 | 43.0 - 62.0	56.0 | 44.5 - 68.5	0.405
Gender; male [%]	42	46	32	0.640
Body Mass Index (kg/m2) [median | IQR]	28.1 | 25.2 - 32.3	28.2 | 24.9 - 33.4	25.8 | 23.6 - 33.0	0.618
Age of onset [median | IQR]	30.0 | 15.0 - 44.0	18.0 | 6.0 - 39.0	27.0 | 17.5 - 34.5	0.702
Smoking status; current / ex- / none smoker [%]	11.5 / 46.2 / 42.3	9.1 / 33.3 / 57.6	21.1 / 15.8 / 63.2	0.233

Pack years [median | IQR]	10.0 | 5.8 - 20.5	13.8 | 4.1 - 23.8	20.5 | 11.4 - 31.3	0.479
OCS use [%]	38.5	57.6	21.1	**0.035**
Total daily OCS dose [median | IQR]	3.0 | 1.0 - 11.0	4.0 | 2.0 - 14.5	10.0 | 4.5 - 19.5	0.428
Exacerbations/year [median | IQR]	1.5 | 0.0 - 3.0	1.0 | 1.0 - 2.0	1.0 | 0.0 - 2.0	0.785
Atopy; postive [%]	57.7	72.7	73.7	0.393
ACQ5; Juniper [median | IQR]	2.5 | 1.4 - 3.4	2.2 | 1.4 - 3.4	2.8 | 1.1 - 3.6	0.958
$PbFEV_1$ % predicted [median | IQR]	74.7 | 52.0 - 84.2	76.6 | 62.5 - 92.1	75.4 | 66.9 - 87.7	0.531
$PbFEV_1$/FVC actual ratio [median | IQR]	75.8 | 66.9 - 85.9	81.5 | 70.1 - 90.4	82.7 | 77.7 - 94.9	0.146
FE_{NO}; ppb [median | IQR]	25.5 | 14.0 - 53.0	33.0 | 16.9 - 46.5	28.0 | 14.5 - 49.5	0.840
SNOT-20 [median | IQR]	1.7 | 1.1 - 2.0	1.5 | 1.1 - 2.2	1.9 | 1.2 - 2.5	0.550
Sputum eosinophils; % [median | IQR]	4.8 | 1.6 - 15.9	5.3 | 2.1 - 16.5	2.2 | 1.0 - 29.5	0.915
Sputum neutrophils; % [median | IQR]	52.2 | 39.0 - 65.4	52.8 | 45.0 - 65.8	44.2 | 9.4 - 85.8	0.733
Blood eosinophils; % [median | IQR]	4.1 | 1.8 - 6.9	2.3 | 1.0 - 4.4	4.7 | 2.6 - 8.0	**0.045**
Blood neutrophils; % [median | IQR]	57.8 | 54.0 - 67.1	65.6 | 60.0 - 76.2	55.5 | 52.3 - 64.0	**0.017**

OCS use, regular daily usage of oral corticosteroids; Total daily OCS dose, daily OCS dose normalized to prednisolone, amongst OCS users; atopy, presence of sensitization on SPT (wheal ≥3mm) or serum specific IgE (≥0.35 kU/L); ACQ5, Juniper – 5 items Asthma Control Questionnaire; $PbFEV_1$, Post-bronchodilator Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; FE_{NO}, Fraction of Exhaled Nitric Oxide in parts per billion; SNOT-20, 20 items Sino-Nasal Outcome Test Questionnaire. Differences between clusters were tested using Kruskal-Wallis testing for continuous data and the Pearson's Chi-squared tests for categorical data.

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Table 3: Cross-table of clustering results among 51 patients with both a baseline and follow-up visit based on exhaled breath profiles: 21 patients are 'cluster stable' (grey cells), while 30 patients migrate between clusters.

		Baseline clusters			
		1	2	3	
Longitudinal clusters	1	6	2	1	9
	2	12	10	4	26
	3	4	7	5	16
		22	19	10	

Baseline clusters, cluster allocation of patients at baseline visits; Longitudinal clusters, cluster allocation of patients at 12-18 months follow-up visit.

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681 Table 4: 'Cluster stable' vs. 'cluster migrated' patients: delta (Δ) [abs(baseline – longitudinal)].

	'custer stable'	'cluster migrated'	*p*-value
Subjects; *n*	21	30	
OCS use Yes / No [%]			0.477
No / No	10	17	
Yes / No	4	2	
Yes / yes	7	11	
Total daily OCS dose [median | IQR]	1.0 | 1.0 - 1.8	1.0 | 0.0 - 1.0	0.283
Exacerbations/year [median | IQR]	1.0 | 0.0 - 2.0	1.0 | 0.0 - 2.0	0.418
ACQ5; Juniper [median | IQR]	0.8 | 0.4 - 1.2	0.6 | 0.3 - 1.0	0.430
$PbFEV_1$ % predicted [median | IQR]	9.0 | 4.3 - 11.9	6.0 | 2.3 - 12.8	0.752
$PbFEV_1$/FVC actual ratio [median | IQR]	5.4 | 2.0 - 8.7	5.4 | 2.3 - 9.6	0.863
FE_{NO}; ppb [median | IQR]	10 | 6.8 - 18.5	7.8 | 4.0 - 14.4	0.181
SNOT-20 [median | IQR]	0.49 | 0.19 - 0.81	0.30 | 0.13 - 0.50	0.204
Sputum eosinophils; % [median | IQR]	2.2 | 1.0 - 3.8	7.2 | 3.3 - 12.3	**0.046**
Sputum neutrophils; % [median | IQR]	16.5 | 12.4 - 26.1	12.3 | 4.0 - 20.0	0.166
Blood eosinophils; % [median | IQR]]	1.5 | 0.8 - 4.5	1.2 | 0.7 - 2.5	0.803
Blood neutrophils; % [median | IQR]	4.4 | 1.9 - 11.1	7.9 | 2.8 - 13.7	0.216

OCS use, regular daily usage of oral corticosteroids at Baseline / Follow-up; Total daily OCS dose, daily OCS dose normalized to prednisolone, amongst OCS users; ACQ5, Juniper – 5 items Asthma Control Questionnaire; $PbFEV_1$, Post-bronchodilator Forced Expiratory Volume in one second; FVC, Forced Vital Capacity; FE_{NO}, Fraction of Exhaled Nitric Oxide in parts per billion.

682 Figures

683 Figure 1: Upper panel - Three delineated clusters (red, green, blue) during Ward clustering
684 combined with similarity profile analysis (SPA) based on exhaled breath samples. Lower
685 panel - K-Means clustering results with a predefined number of clusters based on Ward
686 clustering combined with SPA. Rand Indexing scoring resulted in a similarity measure
687 between K-Means vs. Ward clustering of 0.82. Colouring for both panels is according to
688 included legend.

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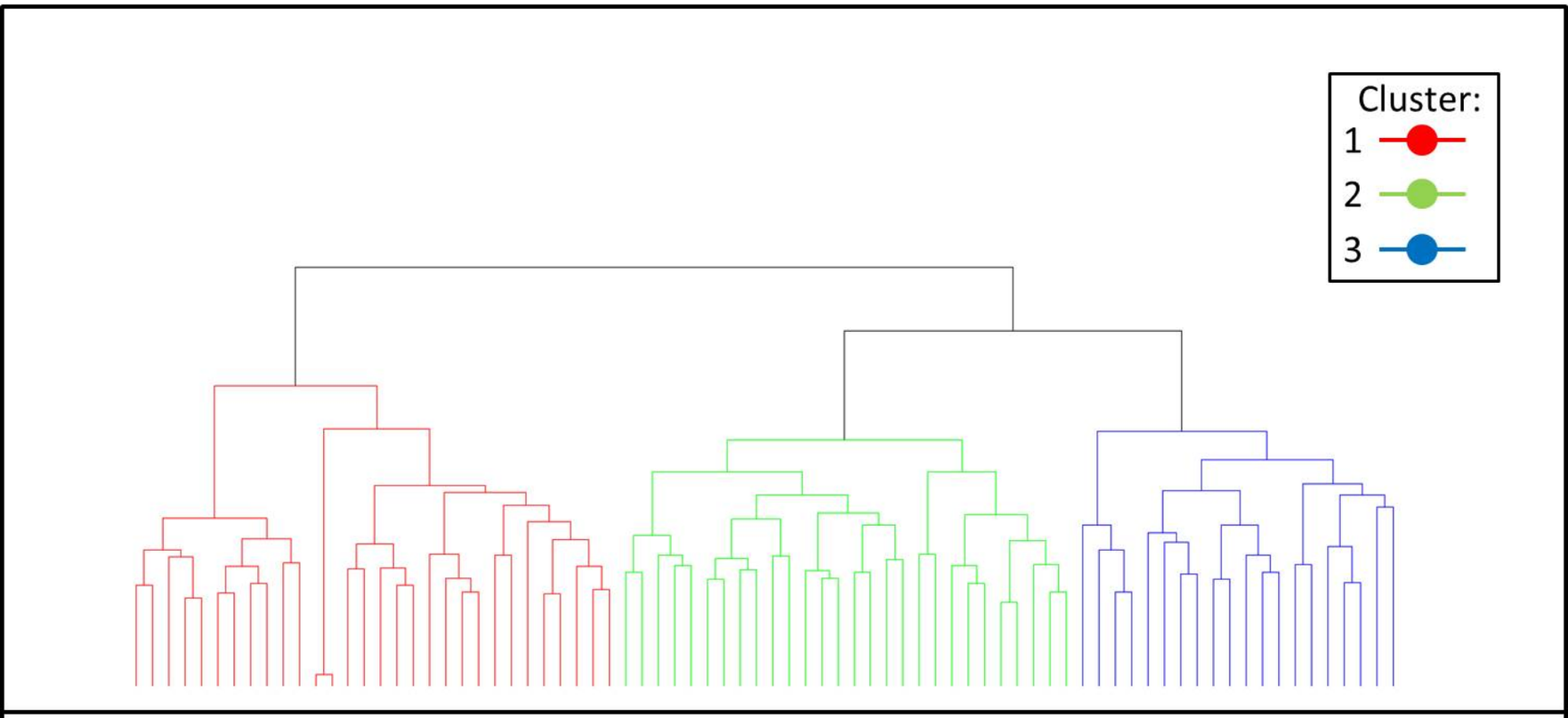
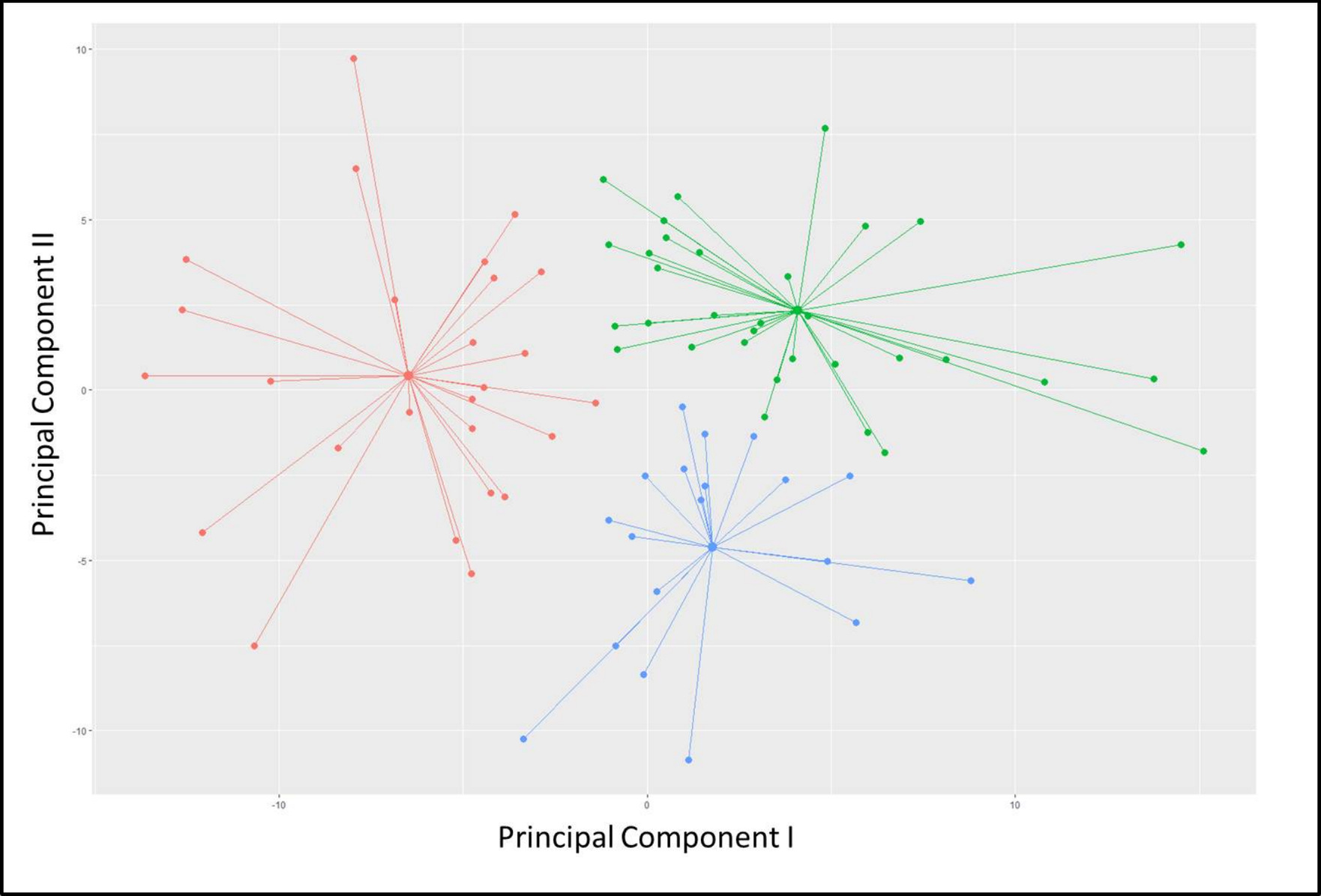
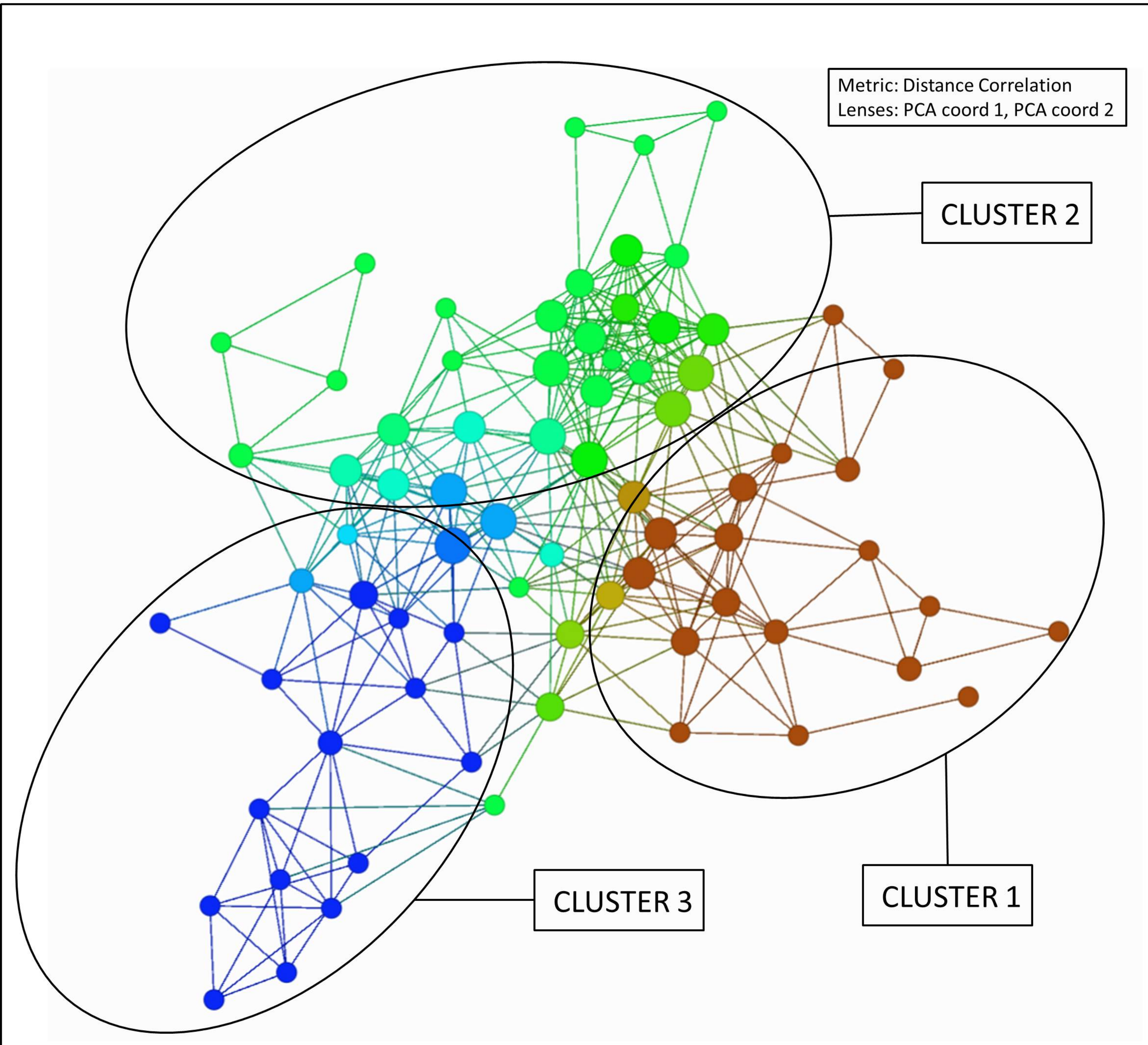
Figure 2: TDA network clustering results. Data points are coloured in accordance with K-Means clustering outcomes.

Ward and K-Means clustering outcomes





TDA clustering results, coloured according to K-Means outcomes



Online Repository

Identification and prospective stability of eNose derived inflammatory phenotypes in severe asthma.

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Table E1: Distribution of patients from the same centers among the three clusters. A chi-square analysis resulted in a *p*-value of 0.079.

p = 0.079	Site 1	Site 2	Site 3	Site 4	Site 5	Site 6	Site 7
Cluster 1	2	3	3	6	1	0	4
Cluster 2	3	2	15	6	2	4	1
Cluster 3	3	1	10	5	3	0	2

Figure E1: Left panel - PLS-DA results based on 78 exhaled breath profiles of severe asthma patients by the U-BIOPRED eNose platform and the three revealed clusters. (blue circles = cluster 1, orange triangles = cluster 2, grey plus = cluster 3). x-axis: PLS-DA component I, y-axis: PLS-DA component II. Right panel – Correlation plot based on components I (x-axis) & II (y-axis) of the U-BIOPRED eNose platforms outcomes by PLS-DA. Strongly associated (or correlated) variables are projected in the same direction from the origin. The greater distance from the origin the stronger the association. [Green] CYRA - Cyranose 320); [Black] TOR - Tor Vergata eNose; [Red] CI - Comon Invent eNose; [Blue] LONE - Owlstone Lonestar.

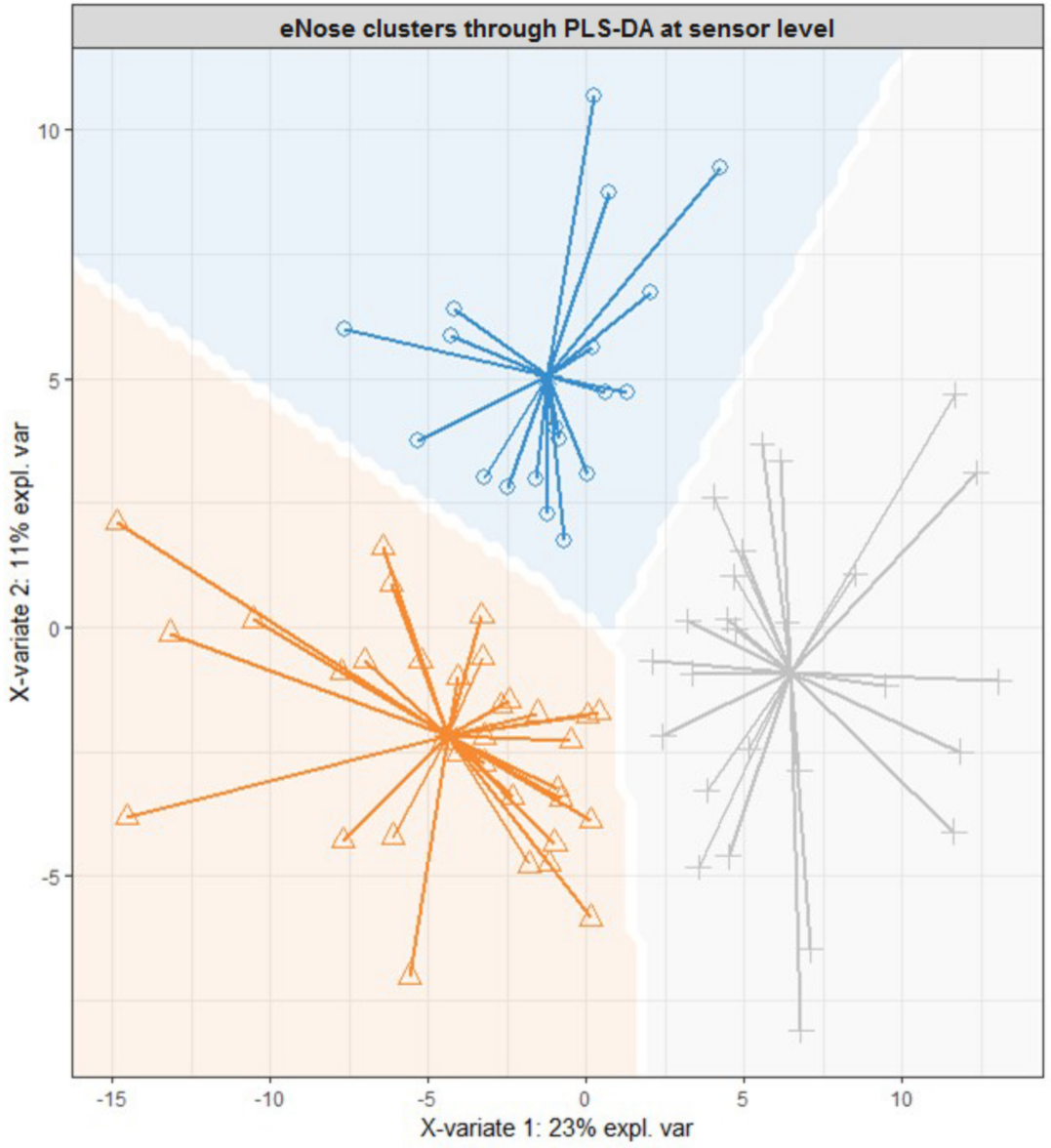
Figure E2: Box plots of all variables listed in table 2 of the main document. Between-cluster comparison of clinical, physiological and inflammatory variables at baseline was performed by the Kruskal-Wallis test for continuous data and the Pearson's Chi-squared Test for categorical data.

Figure E3: PLS-DA results based on 28 exhaled breath profiles of severe asthma patients by the U-BIOPRED eNose platform at PC level and their sputum transcriptomics phenotypes by Kuo *et al.* ERJ 2017. (blue circles = TAC1, orange triangles = TAC2, grey plus = TAC3). x-axis: PLS-DA component I, y-axis: PLS-DA component II. Right panel – Correlation plot based on components I (x-axis) & II (y-axis) of the U-BIOPRED eNose platforms outcomes by PLS-DA. Strongly associated (or correlated) PCs are projected in the same direction from the origin. The greater the distance from the origin the stronger the association.
note: colorization of PC labels 1 to 34 [red, green, blue, black] serves no other goal than improvement of readability.

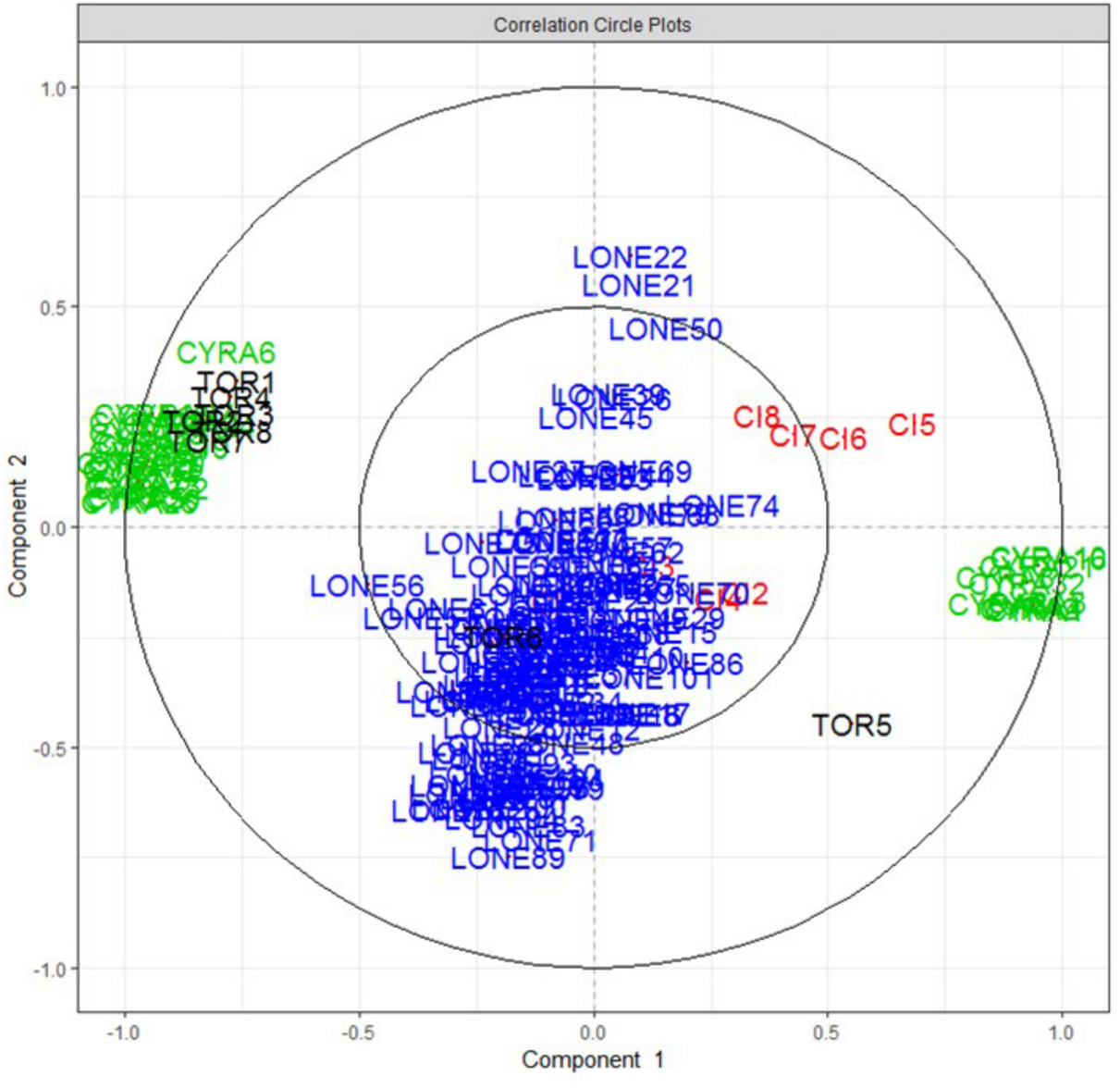
Figure E4: Associations between exhaled markers by the U-BIOPRED eNose platform vs. blood neutrophils (%) by Partial Least Squares Regression analysis R^2=0.41, 95%CI(0.13 - 0.75). Grey area = 95%CI, dashed lines = 95% prediction interval.

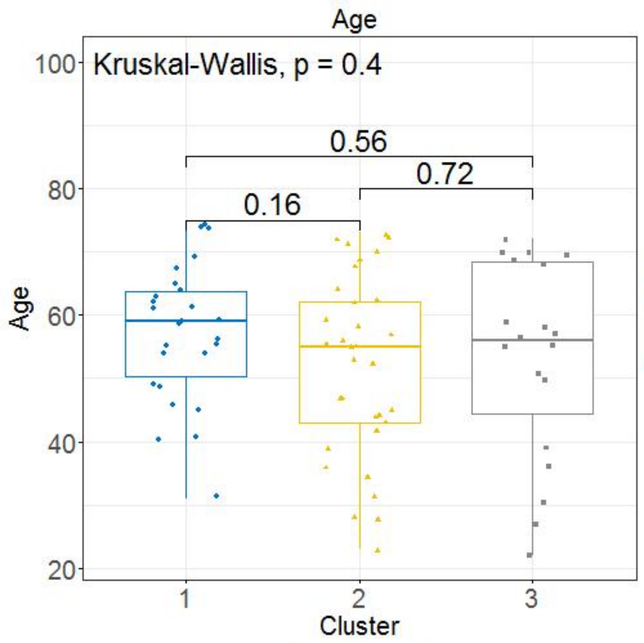
Figure E5: Associations between exhaled markers by the U-BIOPRED eNose platform vs. blood eosinophils (%) by Partial Least Squares Regression analysis R^2=0.63, 95%CI(0.52 - 0.74). Grey area = 95%CI, dashed lines = 95% prediction interval.

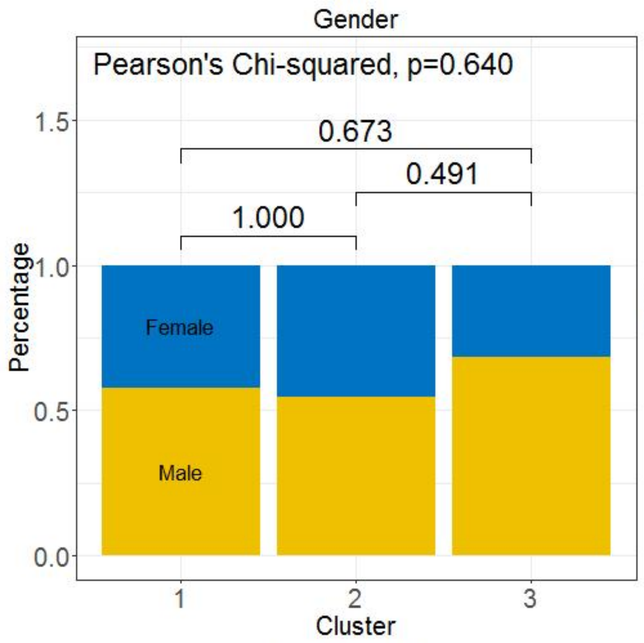
Figure E6: Associations between absolute delta's of exhaled breath profiles by the U-BIOPRED eNose platform vs. absolute delta's of sputum eosinophils (%) by Partial Least Squares Regression analysis R^2=0.63, 95%CI(0.52 - 0.74). Grey area = 95%CI, dashed lines = 95% prediction interval.

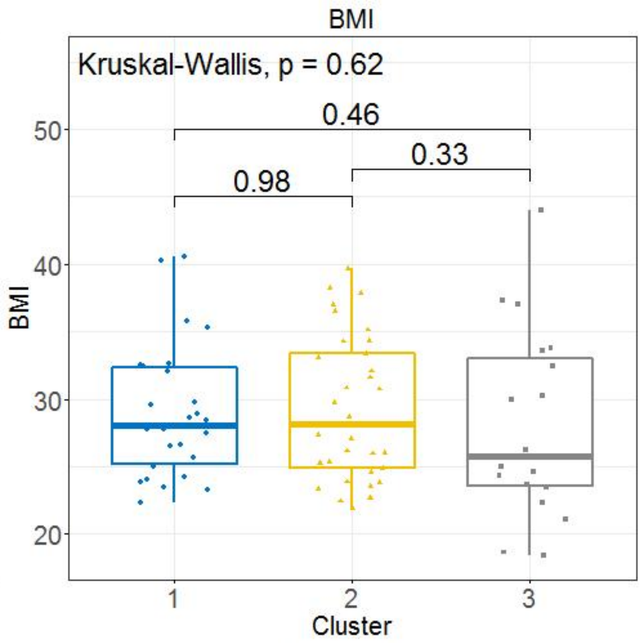

eNose clusters through PLS-DA at sensor level
X-variate 2: 11% expl. var
X-variate 1: 23% expl. var
10
5
0
-5
-15
-10
-5
0
5
10

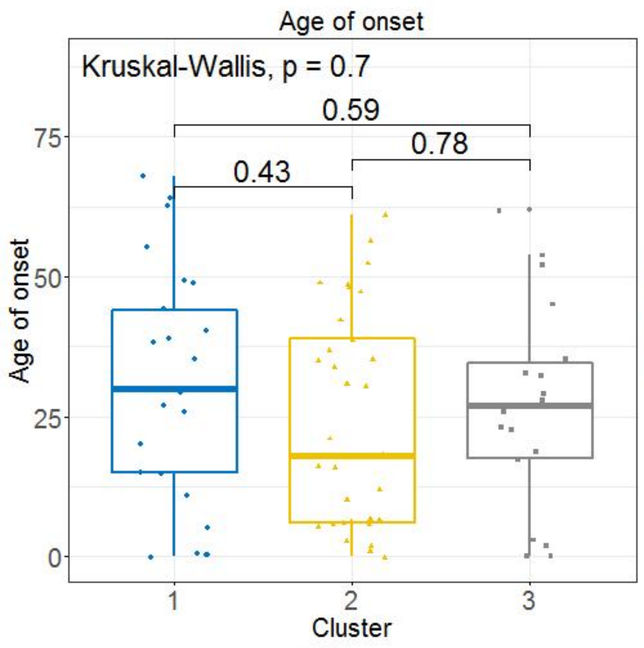

Legend
1
2
3

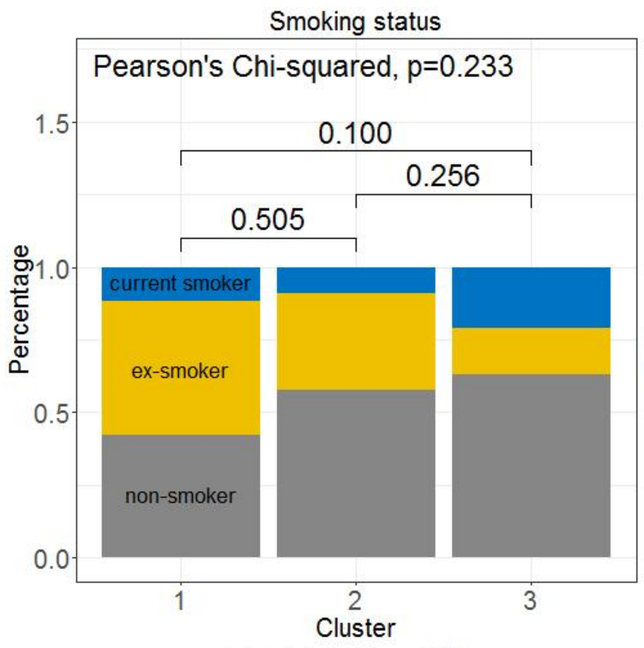

Correlation Circle Plots
Component 2
Component 1
1.0
0.5
0.0
-0.5
-1.0
LONE22
LONE21
LONE50
CYRA6
TOR1
LONE45
CI8
CI7
CI6
CI5
LONE74
LONE56
TOR5
LONE89

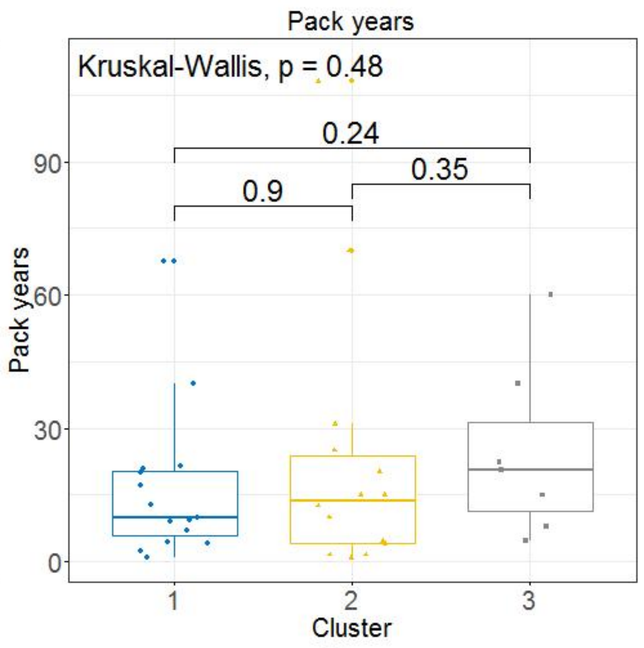

Age
Kruskal-Wallis, p = 0.4
0.56
0.72
0.16
Age
Cluster

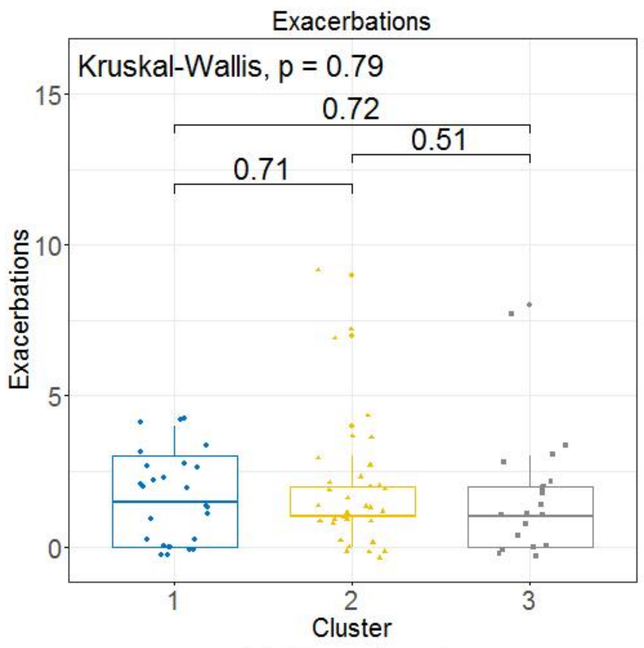

Gender
Pearson's Chi-squared, p=0.640
0.673
0.491
1.000
Female
Male
Percentage
Cluster

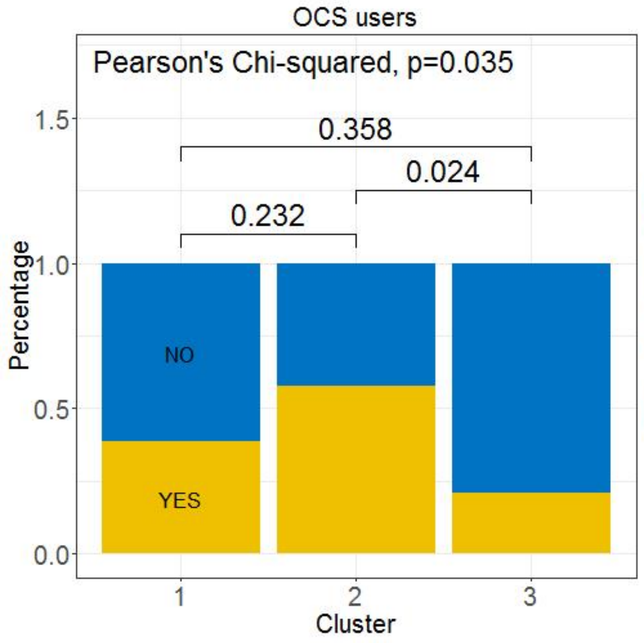

BMI
Kruskal-Wallis, p = 0.62
0.46
0.33
0.98
BMI
Cluster

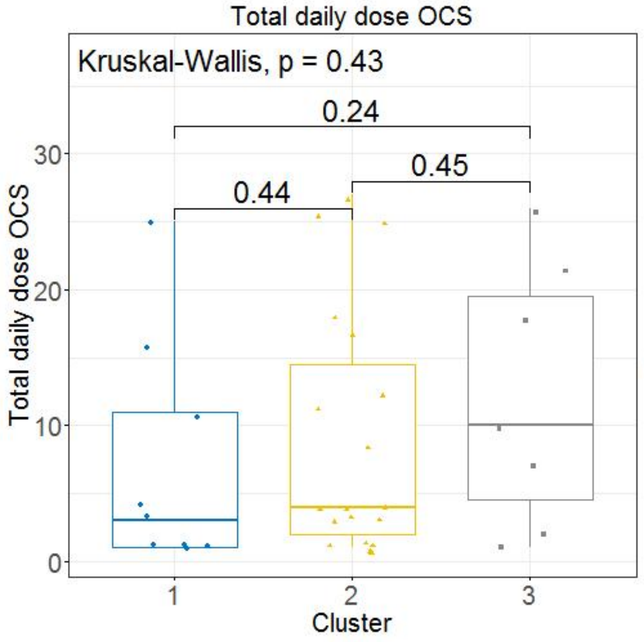

Age of onset
Kruskal-Wallis, p = 0.7
0.59
0.78
0.43
Age of onset
Cluster

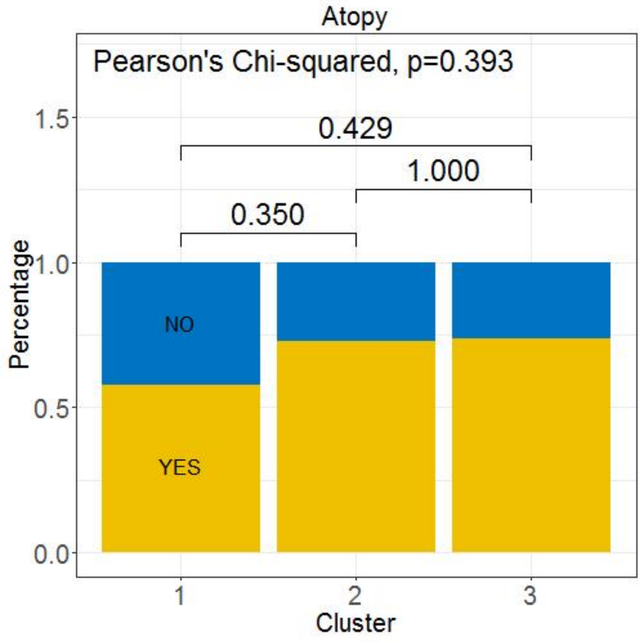

Smoking status
Pearson's Chi-squared, p=0.233
0.100
0.256
0.505
current smoker
ex-smoker
non-smoker
Percentage
Cluster

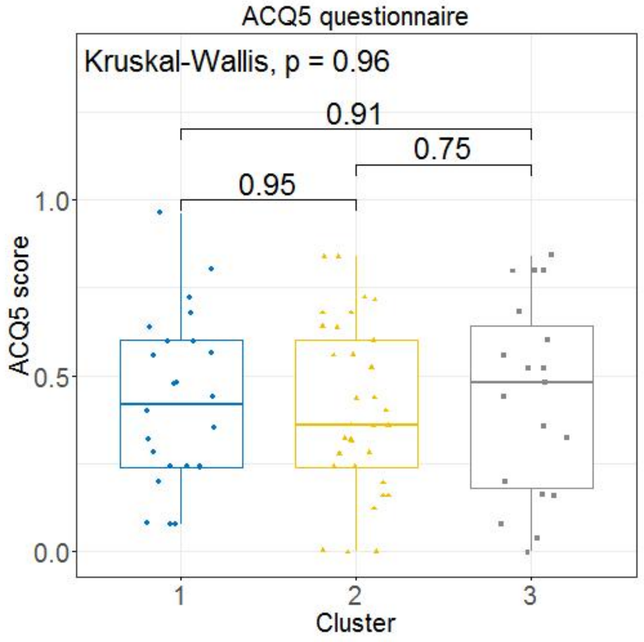

Pack years
Kruskal-Wallis, p = 0.48
0.24
0.35
0.9
Pack years
Cluster

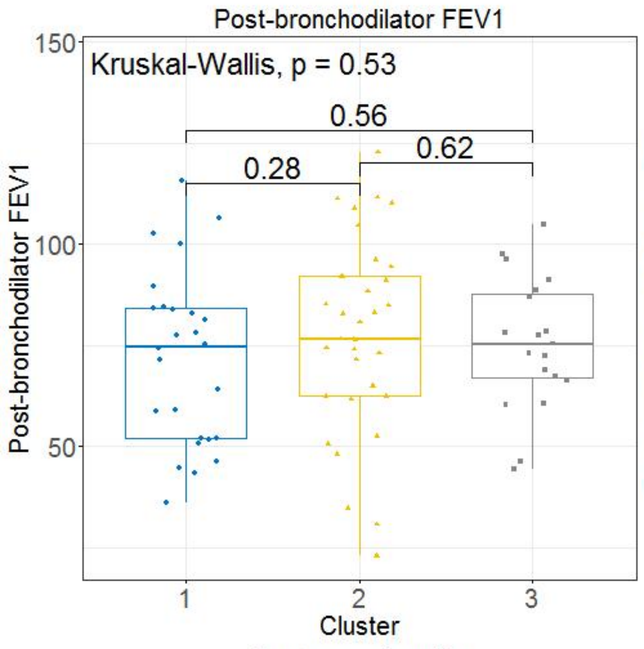

Exacerbations
Kruskal-Wallis, p = 0.79
0.72
0.51
0.71
Exacerbations
Cluster

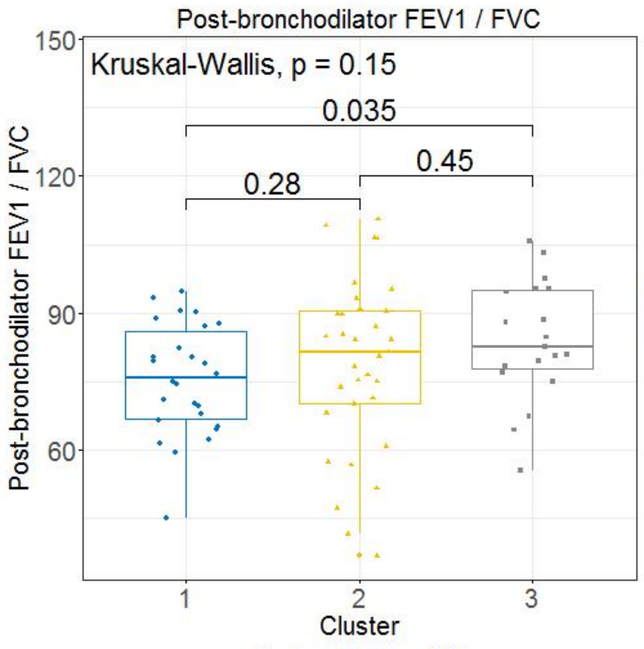

OCS users
Pearson's Chi-squared, p=0.035
0.358
0.024
0.232
NO
YES
Percentage
Cluster

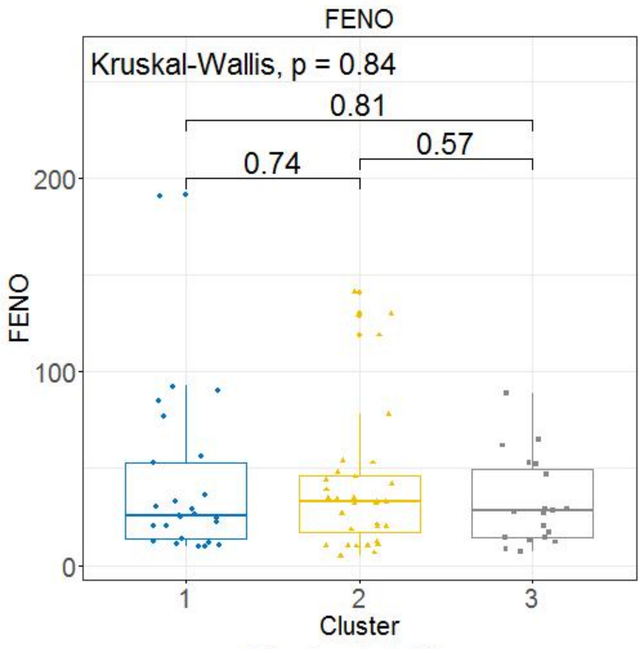

Total daily dose OCS
Kruskal-Wallis, p = 0.43
0.24
0.45
0.44
Total daily dose OCS
Cluster

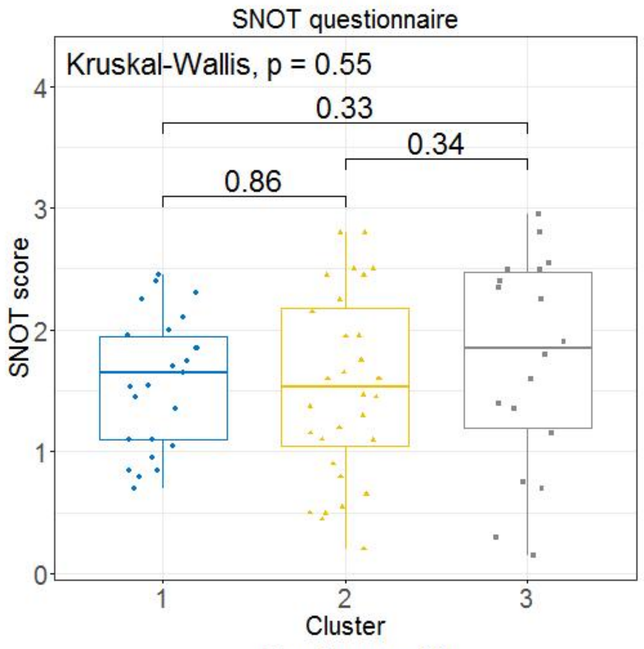

Atopy
Pearson's Chi-squared, p=0.393
0.429
1.000
0.350
NO
YES
Percentage
Cluster

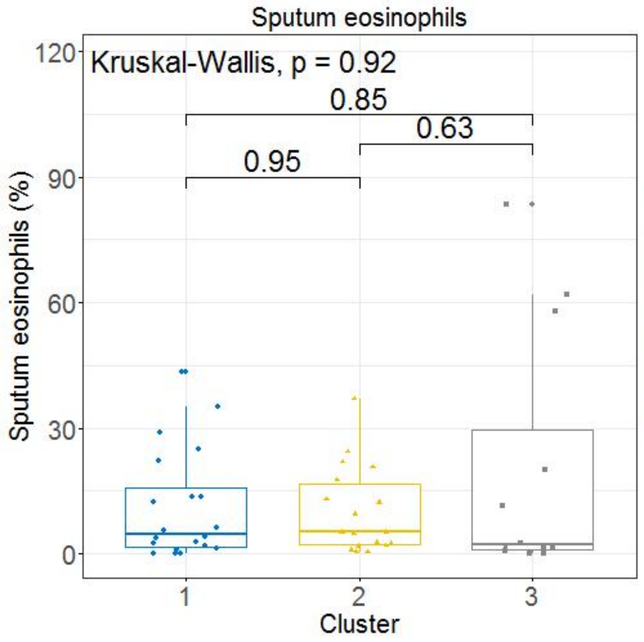

ACQ5 questionnaire
Kruskal-Wallis, p = 0.96
0.91
0.75
0.95
ACQ5 score
Cluster

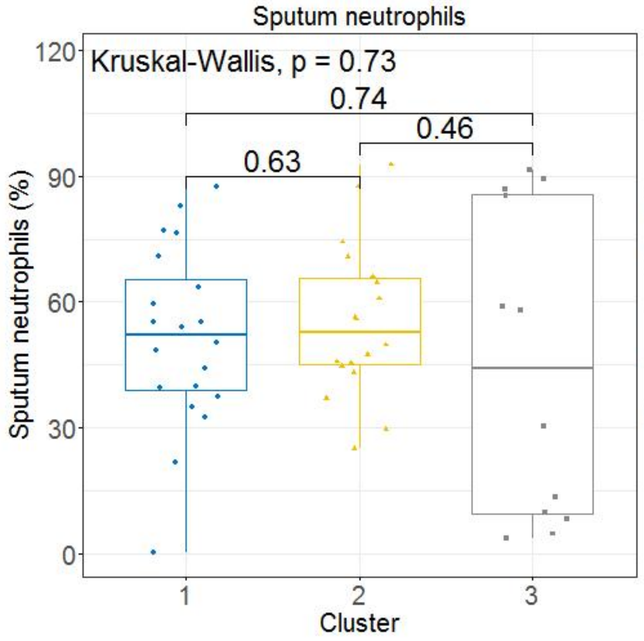

Post-bronchodilator FEV1
Kruskal-Wallis, p = 0.53
0.56
0.62
0.28
Post-bronchodilator FEV1
Cluster

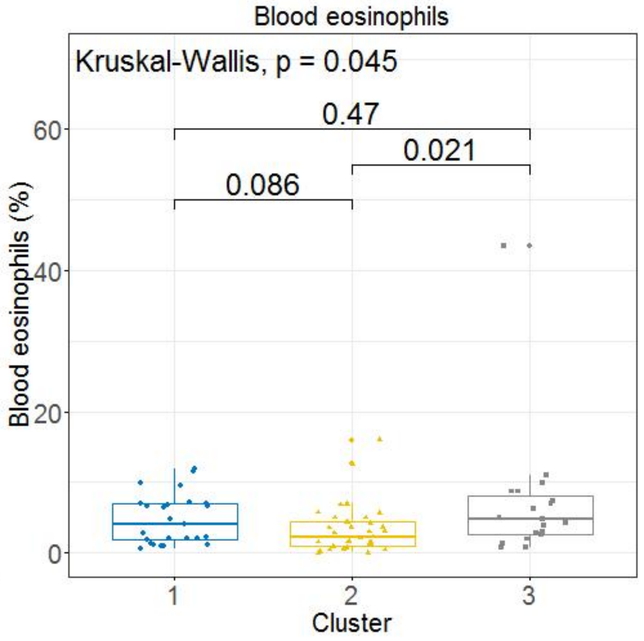

Post-bronchodilator FEV1 / FVC
Kruskal-Wallis, p = 0.15
0.035
0.45
0.28
Post-bronchodilator FEV1 / FVC
Cluster

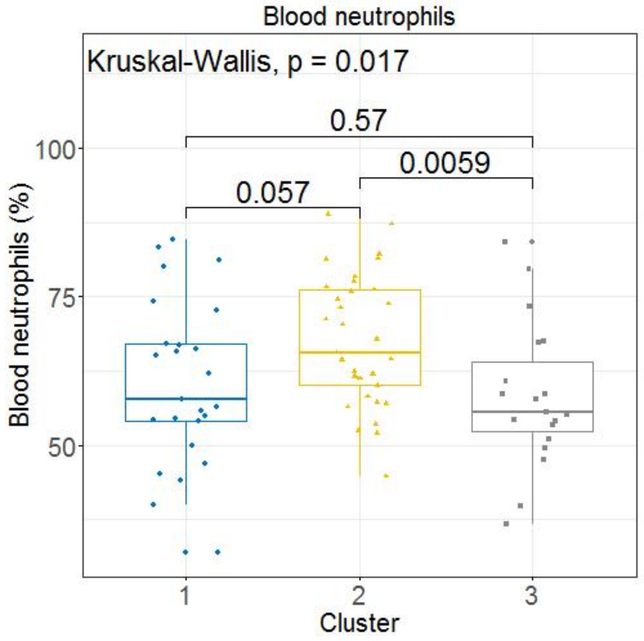

FENO
Kruskal-Wallis, p = 0.84
0.81
0.57
0.74
FENO
Cluster

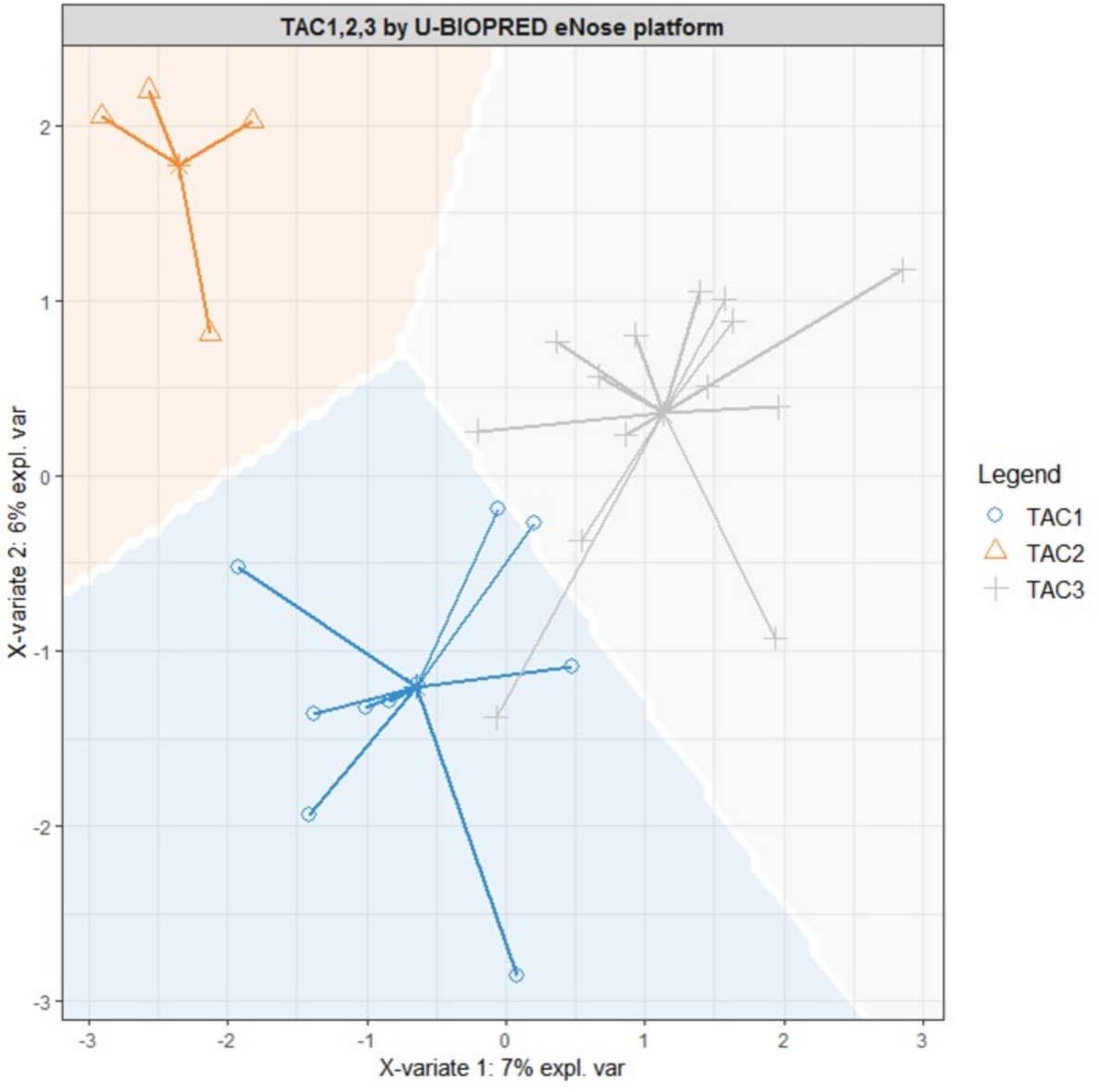

SNOT questionnaire
Kruskal-Wallis, p = 0.55
0.33
0.34
0.86
SNOT score
Cluster

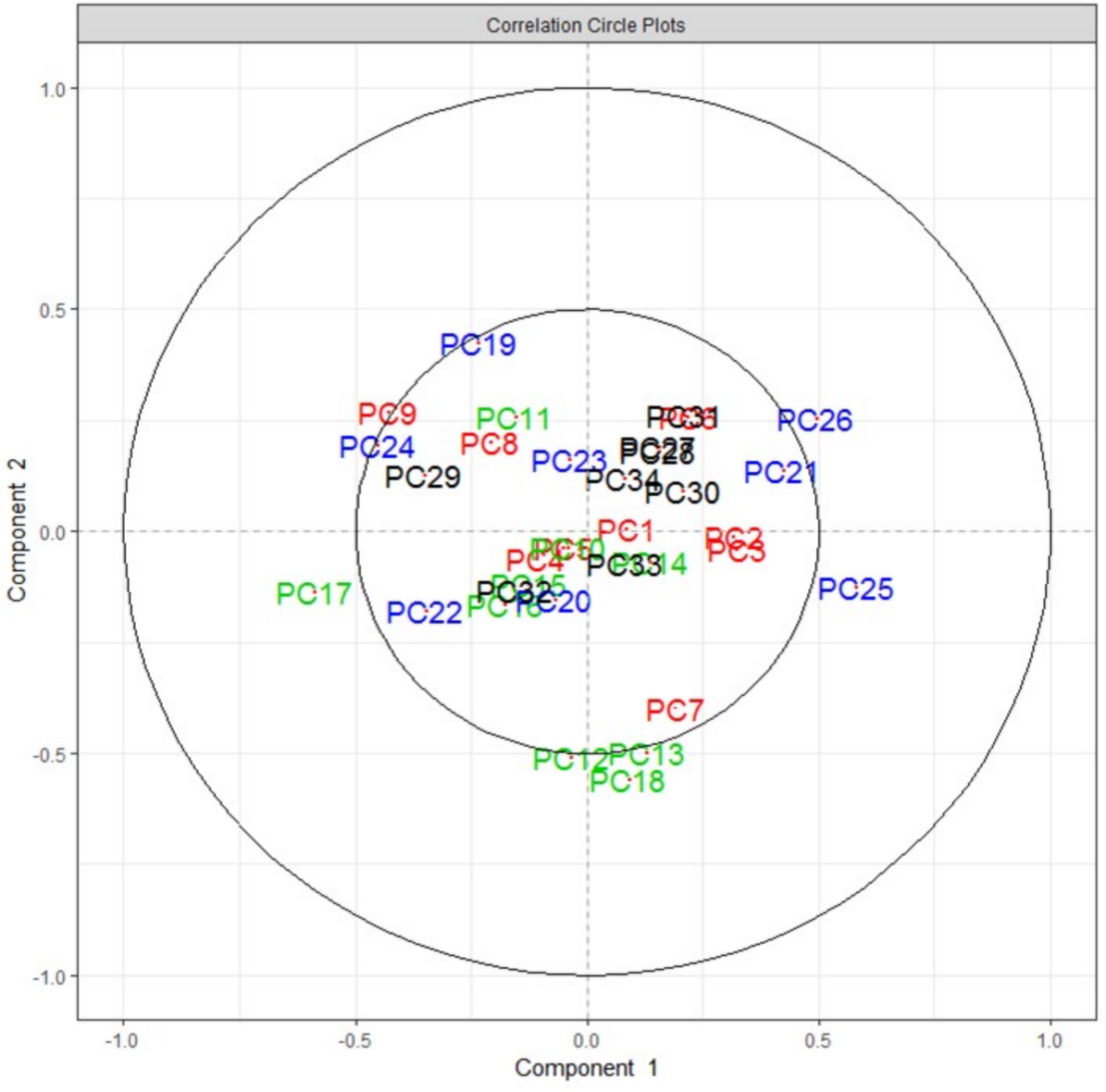

Sputum eosinophils
Kruskal-Wallis, p = 0.92
0.85
0.63
0.95
Sputum eosinophils (%)
Cluster

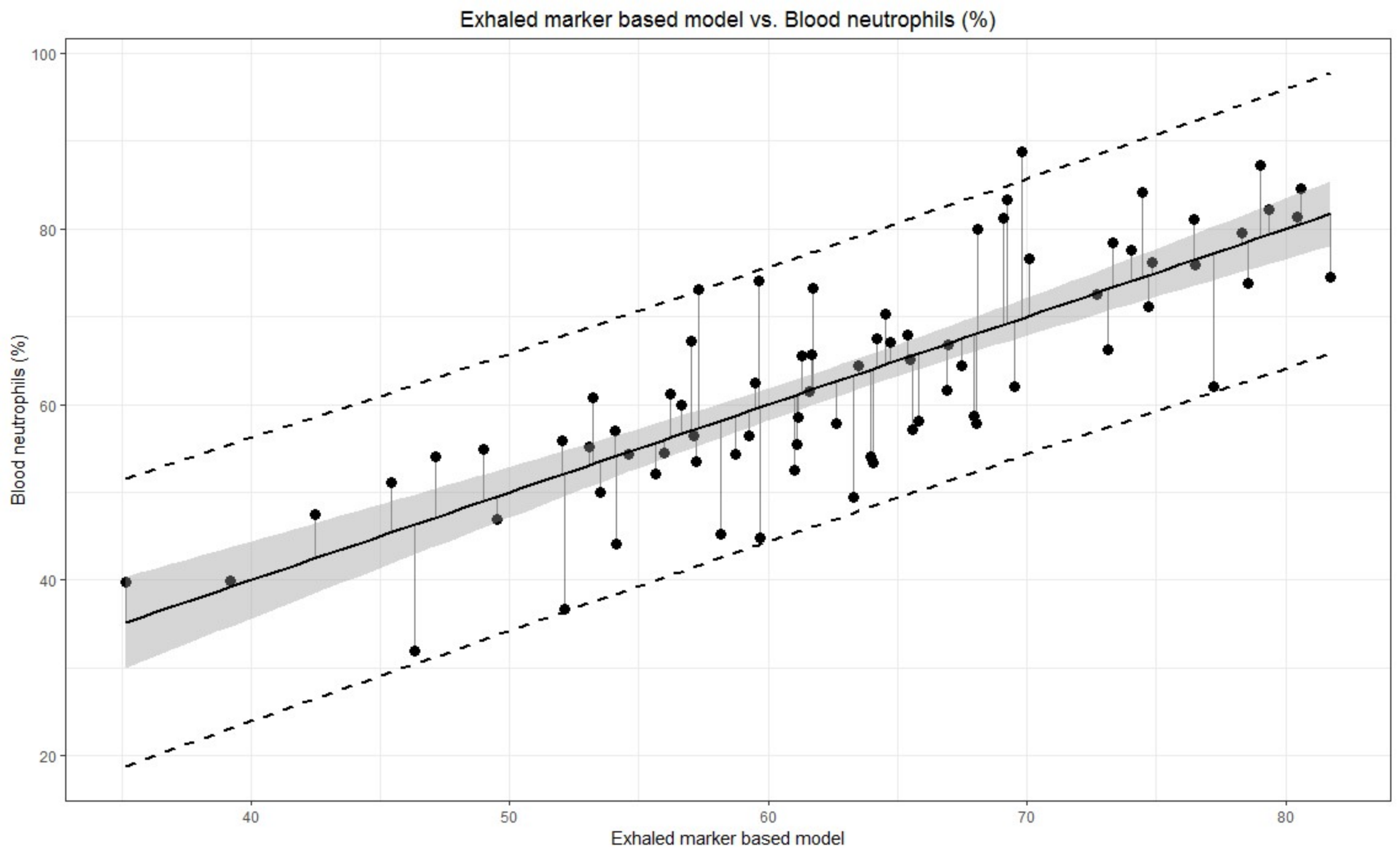

Sputum neutrophils
Kruskal-Wallis, p = 0.73
0.74
0.46
0.63
Sputum neutrophils (%)
Cluster

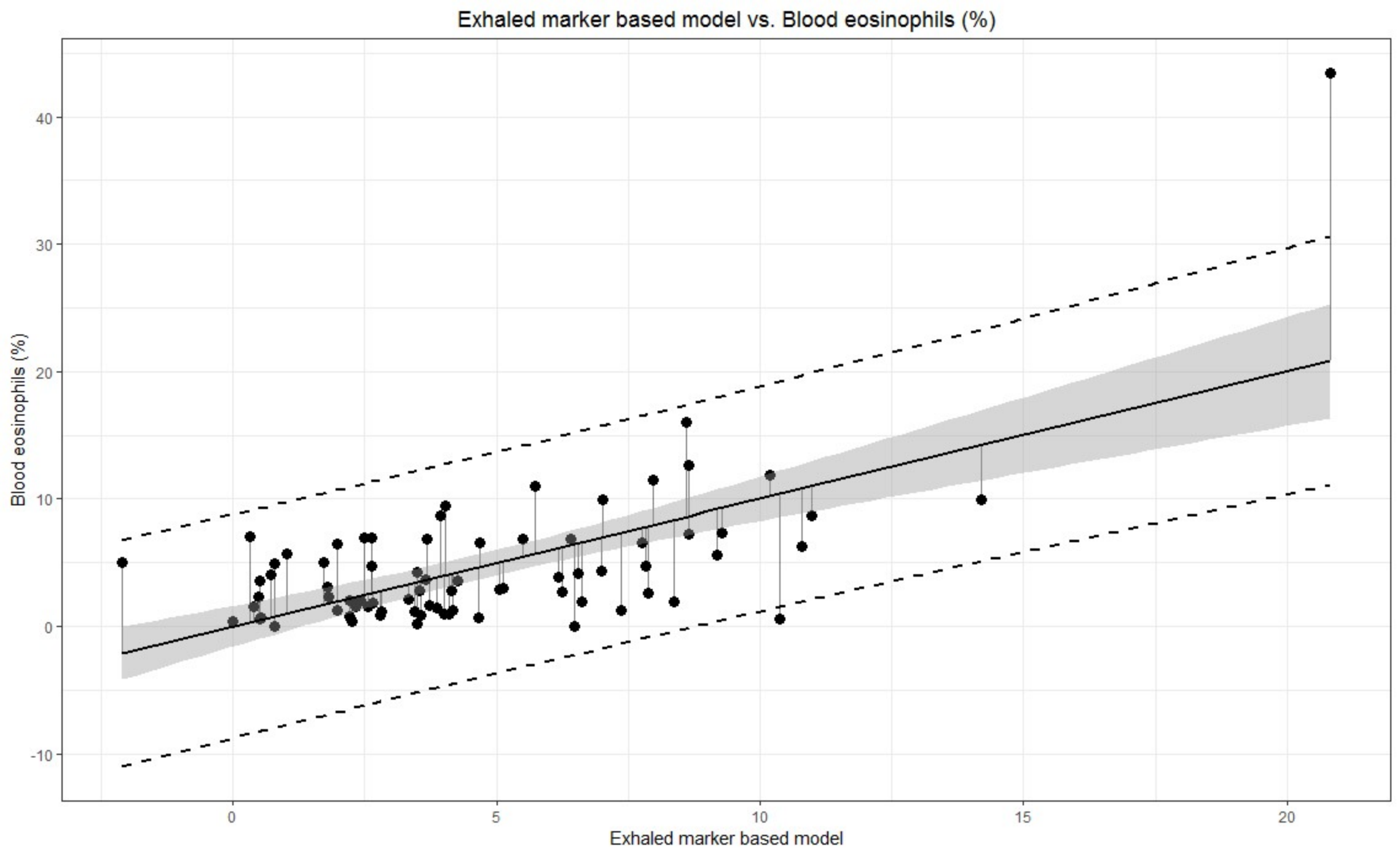

Blood eosinophils
Kruskal-Wallis, p = 0.045
0.47
0.021
0.086
Blood eosinophils (%)
Cluster

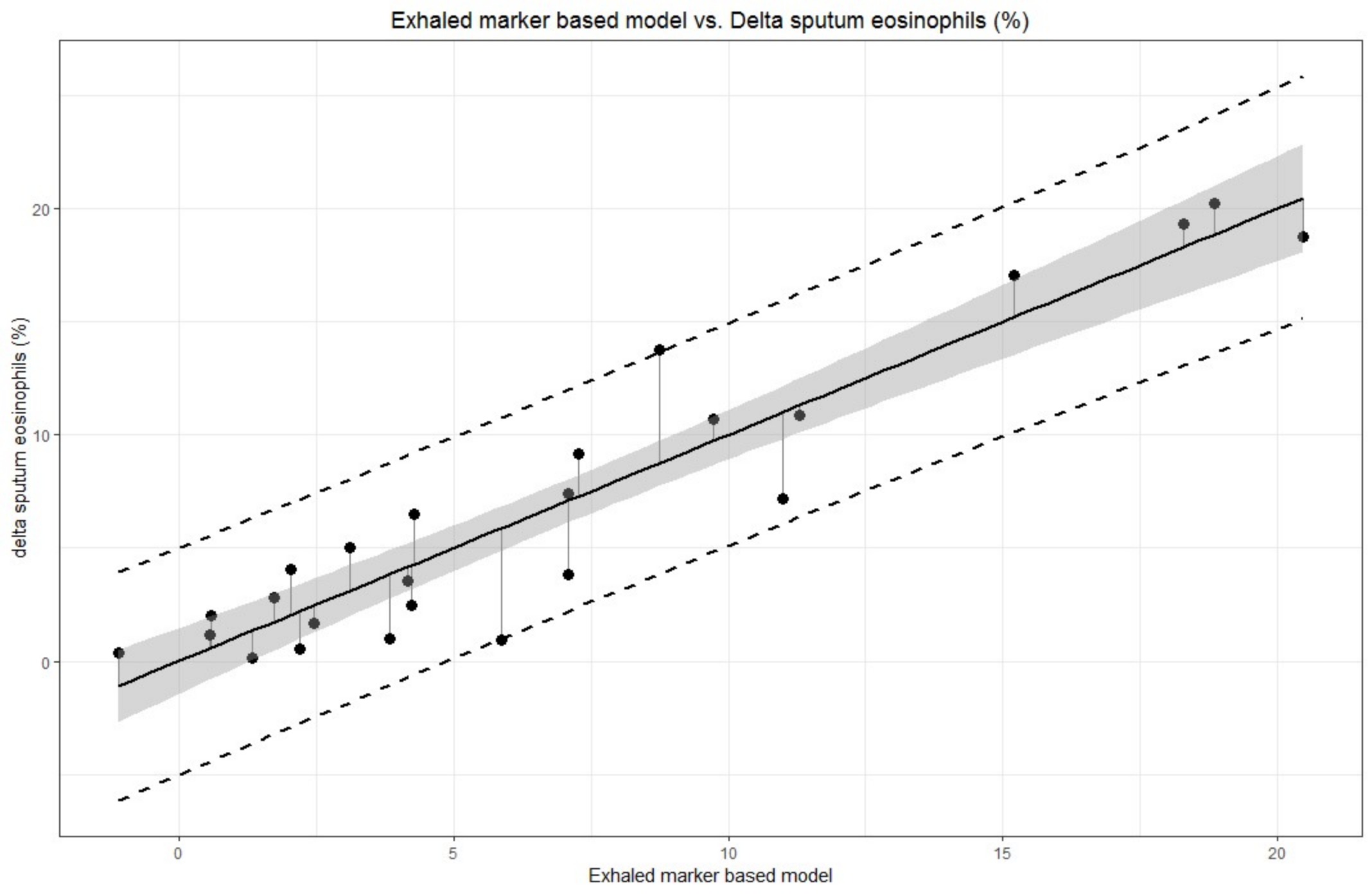

Blood neutrophils
Kruskal-Wallis, p = 0.017
0.57
0.0059
0.057
Blood neutrophils (%)
Cluster


TAC1,2,3 by U-BIOPRED eNose platform
X-variate 2: 6% expl. var
X-variate 1: 7% expl. var
Legend
TAC1
TAC2
TAC3


Correlation Circle Plots
Component 2
Component 1
PC19
PC9
PC11
PC26
PC24
PC8
PC23
PC21
PC29
PC34
PC30
PC1
PC3
PC17
PC22
PC25
PC7
PC12
PC13
PC18


Exhaled marker based model vs. Blood neutrophils (%)
Blood neutrophils (%)
100
80
60
40
20
40
50
60
70
80
Exhaled marker based model


Exhaled marker based model vs. Blood eosinophils (%)
Blood eosinophils (%)
40
30
20
10
0
-10
0
5
10
15
20
Exhaled marker based model


Exhaled marker based model vs. Delta sputum eosinophils (%)
delta sputum eosinophils (%)
20
10
0
0
5
10
15
20
Exhaled marker based model

U-BIOPRED

The U-BIOPRED consortium wishes to acknowledge the help and expertise of the following individuals and groups without whom, the study would not have been possible:

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Data cleaning team
Data analysis team
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Core project management staff

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