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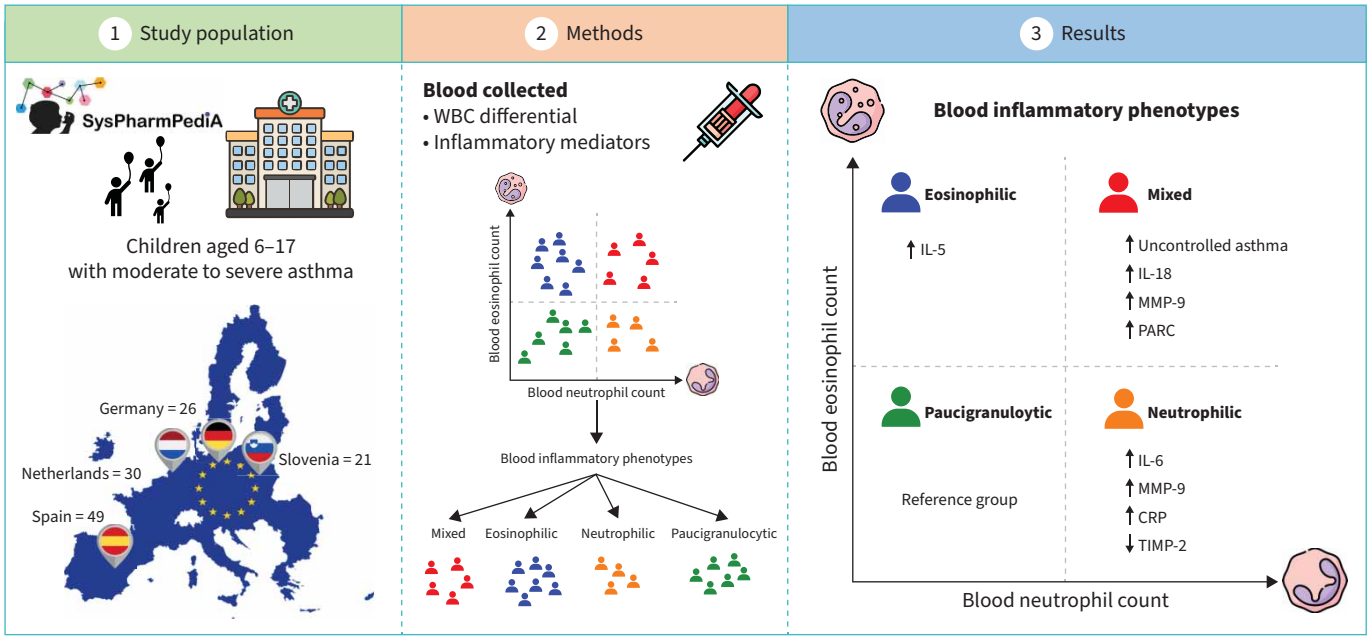

GRAPHICAL ABSTRACT

Association of blood inflammatory phenotypes and asthma burden in children with moderate-to-severe asthma

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Different blood inflammatory phenotypes (mixed, eosinophilic, neutrophilic and paucigranulocytic) with distinct inflammatory mediator profiles and clinical outcomes can be identified in children with moderate-to-severe asthma https://bit.ly/4fbeFTH

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Abstract

Background Underlying immunological mechanisms in children with moderate-to-severe asthma are complex and unclear. We aimed to investigate the association between blood inflammatory parameters and asthma burden in children with moderate-to-severe asthma.

Methods Blood inflammatory parameters (eosinophil and neutrophil counts and inflammatory mediators using multiplex immunoassay technology) were measured in children (6–17 years) with moderate-to-severe asthma from the SysPharmPediA cohort across four European countries. Based upon low/high blood eosinophil (LBE/HBE) counts of $</\geqslant 0.3\times10^{9}\cdot L^{-1}$, respectively and low/high blood neutrophil (LBN/HBN) counts of $</\geqslant 4\times10^{9}\cdot L^{-1}$, respectively, mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN) phenotypes were defined. Inflammatory mediator profiles and burden of disease (asthma control status, exacerbations and school days missed in the past year) were compared between phenotypes using adjusted logistic regression models.

Results Among 126 included children (41% girls and mean (SD) age of 11.94 (2.76)), 22%, 44%, 11% and 23% were classified as mixed, eosinophilic, neutrophilic and paucigranulocytic phenotypes, respectively. Neutrophilic children had the lowest lung function (forced expiratory volume in 1 s % predicted pre-salbutamol) compared with other groups. Children with mixed asthma were most often

uncontrolled and had the highest asthma-related school absence in the past year. Interleukin (IL)-6 and matrix metalloproteinase-9 levels were significantly higher in patients with mixed or neutrophilic asthma, whereas tissue inhibitor of metalloproteinase-2 was lower in patients with neutrophilic asthma compared with eosinophilic or paucigranulocytic asthma. IL-5 was increased in eosinophilic group compared with the neutrophilic and paucigranulocytic groups, irrespective of the chosen cut-off for eosinophilia.
Conclusion Differences in asthma burden-related clinical expression and distinct blood inflammatory mediator profiles were found between phenotypes, highlighting implications for optimising personalised treatment and management strategies in children with moderate-to-severe asthma.

Introduction

Asthma is the most common airway inflammatory disease among children. It is a heterogeneous disease, where different pathophysiological mechanisms are implicated [1]. Moderate-to-severe childhood asthma has a great impact on the quality of life of children and their families [2]. The occurrence and intensity of symptoms vary among patients over time [3]. A better understanding of the heterogeneity of underlying inflammatory mechanisms [4] may help to identify potential biomarkers for better asthma phenotyping. This may improve personalised management and treatment of children with asthma.

Asthma can be classified into clinical phenotypes based on observational clinical characteristics and into inflammatory or molecular endotypes according to inflammatory biomarkers and/or omics analyses, which may reflect underlying biological mechanisms [1]. Type-2 high and type-2 low are the two well-known molecular endotypes for asthma [5]. Eosinophilic, mixed, neutrophilic and paucigranulocytic asthma can be considered inflammatory phenotypes [4, 6].

Clinical asthma phenotypes and treatment outcomes have been linked to distinct inflammatory phenotypes based on measurements of inflammatory cells in sputum and/or peripheral blood [4, 7–9]. However, data on children are limited, especially concerning type-2 low asthma and non-eosinophilic asthma. Characterising inflammatory phenotypes based on induced sputum measurements in children with asthma is complicated due to the invasiveness of measurements. Blood eosinophil and neutrophil count cut-offs for phenotyping patients might be an alternative, and is more affordable and less time-consuming. Furthermore, studies on adult populations have shown that blood inflammatory phenotypes are associated with clinical outcomes such as exacerbation rate, symptom occurrence and severity, unplanned respiratory visits and specific inflammatory mediators [4, 7–9].

To the best of our knowledge, no previous studies have investigated the association of blood inflammatory phenotypes defined by blood eosinophil and neutrophil counts with asthma burden in children with moderate-to-severe asthma on maintenance treatment. Nevertheless, this correlation was only partially explored in a few publications on children. A study among 142 children found higher blood neutrophil levels in those with asthma compared with healthy peers (median (interquartile range) ($\times 10^9 \cdot L^{-1}$) 2.91 (2.42–3.93) and 6.63 (4.36–9.33), respectively) [10]. Another study on 38 children has reported lower lung function and higher sputum levels of interleukin (IL)-17 and IL-8 in the neutrophilic asthma group *versus* other groups. While sputum IL-5 was higher in the eosinophilic group [11].

We hypothesise that different blood inflammatory phenotypes are associated with a different burden of disease and blood inflammatory mediator profiles in children with moderate-to-severe asthma. A better understanding of inflammatory phenotypes can help to improve asthma management and personalise treatment. Hence, in this study, we aim to investigate the association of blood inflammatory phenotypes based on blood eosinophil and neutrophil count cut-offs with asthma burden among children with moderate-to-severe asthma. In addition, we assessed the inflammatory mediator profiles between these four inflammatory phenotypes to gain more insights into the inflammatory pathways underlying these phenotypes.

Methods

Study design and population

A cross-sectional analysis was performed in the SysPharmPediA study, which is a multicentre European observational paediatric asthma cohort. The study population and design were described previously in detail [12]. In summary, children aged 6–17 years with moderate-to-severe doctor-diagnosed asthma under the Global Initiative for Asthma (GINA) step ⩾3 were included in the tertiary care hospitals by a physician or paediatrician from four European countries: The Netherlands, Germany, Spain and Slovenia. This study was carried out in accordance with the Declaration of Helsinki and approved by the local medical review board of all study centres. More details can be found in the supplementary information. The SysPharmPediA study was registered at ClinicalTrials.gov under identifier NCT04865575. All participants/parents gave their informed consent.

Data collection

Briefly, standardised study procedures across countries were used, including clinical evaluation, spirometry before and after the bronchodilator, fractional exhaled nitric oxide testing, blood draw (for differential blood counts), Asthma Control Test (ACT) [13, 14] and assessment of medication use [12]. Additional sociodemographic and clinical information was obtained from hospital patient files, physician reports and questionnaires completed by patients, their parents or caregivers.

Inflammatory phenotypes

Differential blood counts were measured at the baseline visits, which are described in detail in the supplementary information. Two cut-offs ($0.3\times10^9\cdot L^{-1}$ and $0.5\times10^9\cdot L^{-1}$) are commonly used to indicate eosinophilia in previously published clinical studies to distinguish between high and low blood eosinophil levels in healthy and asthma populations and also in adults and children [7, 8, 15–18]. We used $0.3\times10^9\cdot L^{-1}$ as the main cut-off for eosinophilia and $0.5\times10^9\cdot L^{-1}$ was used in the sensitivity analyses (Supplementary methods). Due to the lack of consensus-based cut-off for neutrophils, especially for children with asthma, we evaluated the cut-offs used in clinical studies on adults ($3.42\times10^9\cdot L^{-1}$, $4\times10^9\cdot L^{-1}$, $5\times10^9\cdot L^{-1}$ [8] and $4.4\times10^9\cdot L^{-1}$ [7]) and in a study on children with severe asthma ($7\times10^9\cdot L^{-1}$ and $8\times10^9\cdot L^{-1}$ [6]) and the optimal cut-off ($4.18\times10^9\cdot L^{-1}$) using receiver operating characteristic curves based on Youden's index to choose the best one ($4\times10^9\cdot L^{-1}$) based on the results of adjusted multivariable logistic regression models (supplementary figure 1). Based upon low/high blood eosinophil (LBE/HBE) counts of $</\geqslant0.3\times10^9\cdot L^{-1}$, respectively and low/high blood neutrophil (LBN/HBN) counts of $</\geqslant4\times10^9\cdot L^{-1}$, respectively, participants were categorised into mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN) phenotypes (figure 1). In addition, cut-offs of $0.5\times10^9\cdot L^{-1}$ for eosinophils and $4\times10^9\cdot L^{-1}$ for neutrophils were used to compare four phenotypes as a sensitivity analysis (results are shown in the supplementary information).

Inflammatory mediator assays

To gain more insights into the link between inflammatory phenotypes and inflammatory mediator profiles, serum samples were used to measure levels of 34 inflammatory mediators using Luminex multiplex immunoassays (R&D systems Inc., Minneapolis, MN, USA) and IL-9 and IL-22 (Invitrogen/ProcartaPLex, Waltham, MA, USA) as described previously [19–21]. IL-5 levels were measured using a Luminex high-sensitivity assay (R&D systems). All the plates were analysed using a Bioplex 200 (Bio-Rad, Hercules, CA, USA). Full details of sample collection and inflammatory mediator assay are described in the supplementary material.

Clinical outcomes

The burden of disease was based on asthma control, the history of exacerbations and school days missed in the past 12 months before the baseline visit. The primary outcome of this study was asthma control, where uncontrolled asthma was defined as being on GINA [22] treatment steps three or higher and at least one of the following: 1) $\geqslant1$ exacerbation(s) in the past year requiring oral corticosteroid intake; 2) $\geqslant1$ exacerbation(s) that necessitated hospitalisation or emergency room visit(s) in the past year; and 3) ACT score $\leqslant19$ at the inclusion time [13, 14]. The secondary outcomes of this study were the patient-reported exacerbation rate in the past year and the number of school days missed in the past year.

Statistical analysis

Full details of general statistical and data analysis are described in the supplementary information.

Briefly, demographic and clinical characteristics were compared between four groups (HBE-HBN, HBE-LBN, LBE-HBN and LBE-LBN) using parametric and nonparametric tests (Kruskal–Wallis test followed by a Dunn's *post hoc* test, Pearson chi-square test or Fisher's exact test) as appropriate. Multivariable and univariable logistic regressions were conducted to examine the adjusted (for confounding factors such as age, sex, smoking exposure, body mass index (BMI) z-score, centre of inclusion, antibiotic intake in the past 2 months, inhaled corticosteroid (ICS) daily dose level (low, moderate and high based on GINA guidelines), medication adherence, biological intake and ethnicity (White or non-White) as defined in the directed acyclic graph (DAG), supplementary figure 2) and unadjusted associations between blood inflammatory phenotypes and the burden of disease. The paucigranulocytic group was considered as the reference group. Mediator values below the lowest standard curve point (limit of detection (LOD)) were imputed with the LOD/square root of 2 [23]. As a sensitivity analysis for IL-5, because all the values in one group were below the LOD, the imputed values (LOD/square root of 2) were multiplied with a random factor between 0.75 and 1.25 [24]. The values above the upper LOD were considered as missing values and excluded from the analysis. In addition, inflammatory mediator profiles were compared between the four groups from both univariable (Kruskal–Wallis test

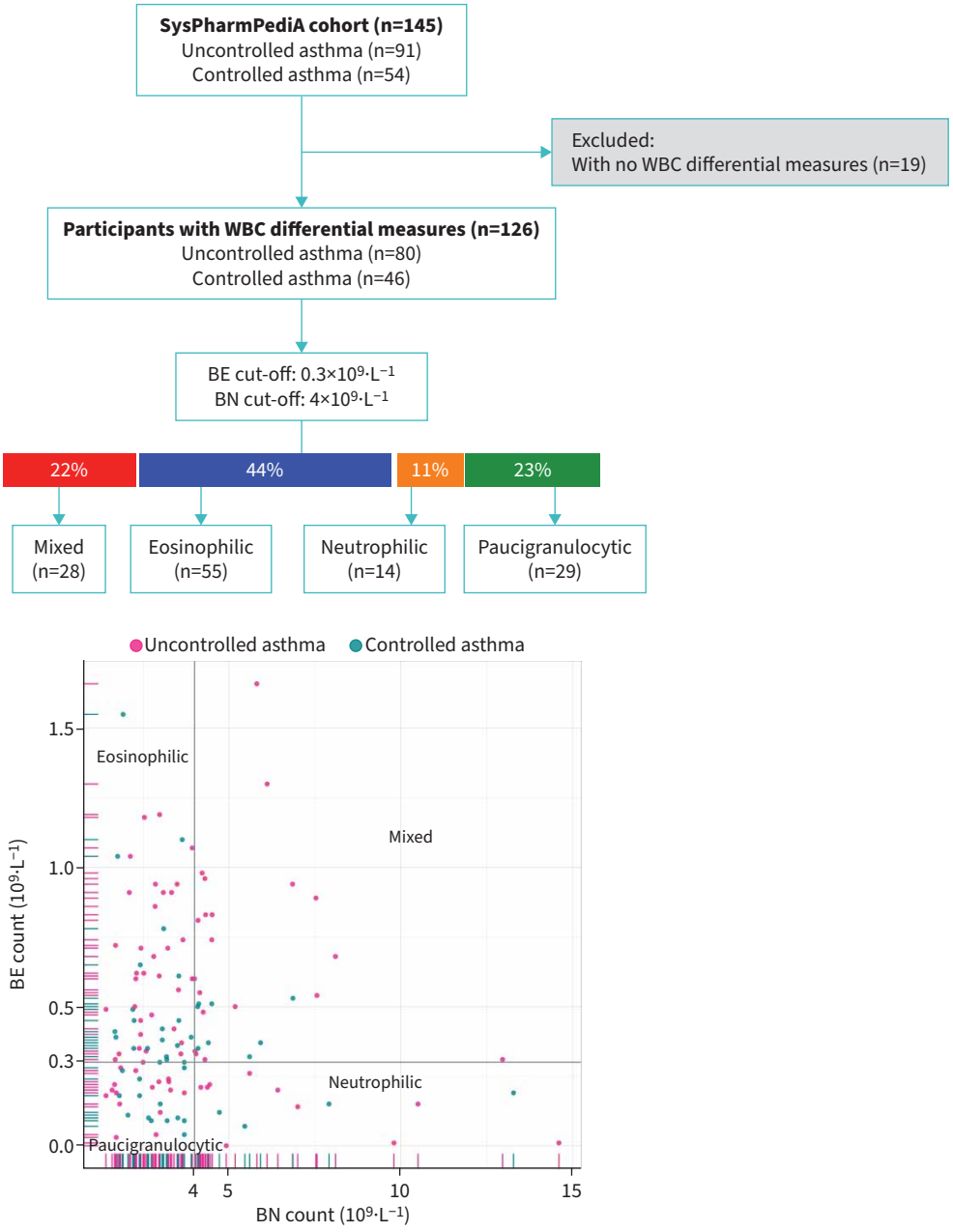

FIGURE 1 Categorising participants into four different groups using a set of cut-offs for blood eosinophil (BE) ($0.3\times10^{9}\cdot L^{-1}$) and blood neutrophil (BN) ($4\times10^{9}\cdot L^{-1}$) counts. WBC: white blood cell.

followed by a Dunn's *post hoc* test and corrected by multiple testing by Benjamini–Hochberg false discovery rate) and multivariable perspectives (supervised sparse partial least squares – discriminant analysis (sPLS-DA) [25] from the mixOmix R package was performed to assess the most discriminative features in inflammatory mediator profiles between groups). An sPLS-DA plot, the strongest contributing variables and sPLS-DA component 1 and 2 comparisons were reported for both the main and sensitivity cut-off sets and for both after and before imputation for the mediator values below the lowest point of the standard curve (LOD) (supplementary methods). All the analyses and data visualisations were conducted using R software (version 4.2.2) and R Studio (version 2023.03.1+ 446).

Results

Participant characteristics

Among the 145 participants included in the SysPharmPediA cohort, differential blood counts were available for 126 participants. The mean age of these participants was 11.9 (±2.8) years and 41% were

female. All participants were on GINA treatment step 3 or higher and took regular daily ICSs. Based on the GINA guideline classification [22], 44%, 33% and 22% were on low, medium and high daily ICS dosages, respectively. 80 out of 126 participants (63%) were classified as having uncontrolled asthma. The 19 excluded participants with no differential blood counts were younger (mean age 10.1 years compared with 11.9 years) than the 126 included children and were mostly from Germany (68%). No other differences were observed between the included and excluded participants (supplementary table 1). The mean (SD, minimum–maximum) ($10^9 \cdot L^{-1}$) for blood eosinophil and neutrophil counts were 0.46 (0.33, 0–1.66) and 3.83 (2.26, 1.42–14.6), respectively. There was a significant negative correlation between eosinophils and age (correlation coefficient −0.29, 95% CI −0.44– −0.12). A significant association between the country of inclusion and both eosinophil and neutrophil counts was observed (supplementary table 2). Patients who were under biological treatments (omalizumab or mepolizumab) had a lower eosinophil level compared with children who were not taking biologics (mean (SD) 0.22 (0.17) $\times 10^9 \cdot L^{-1}$ *versus* 0.50 (0.33) $\times 10^9 \cdot L^{-1}$).

Clinical characteristics of the blood inflammatory phenotypes

Table 1 and supplementary table 3 show the demographic and clinical characteristics of participants in the main analyses using the main cut-off set ($0.3\times10^9 \cdot L^{-1}$ for eosinophils and $4\times10^9 \cdot L^{-1}$ for neutrophils). 22% of children were classified as mixed, 44% as eosinophilic, 11% as neutrophilic and 23% as paucigranulocytic phenotypes (figure 1). The neutrophilic group had the highest mean age (13.9±3.19 years) and proportion of girls (71%), were more exposed to smoking (64%), were more often treated with higher GINA treatment steps and still showed lowest lung function (forced expiratory volume in 1 s (FEV_1) % predicted before salbutamol, 83.1). In addition, the neutrophilic group had the lowest percentage of history of doctor-diagnosed atopic dermatitis (8%) and allergic rhinitis (46%). By using a higher eosinophil cut-off ($0.5\times10^9 \cdot L^{-1}$) in the sensitivity analysis (supplementary results and supplementary tables 4 and 5) most of the children in the mixed and eosinophilic groups had uncontrolled asthma (79%), and about two-thirds of patients (65%) in the mixed asthma group had more than five asthma-related school days missed in the past year.

Association of blood inflammatory phenotypes with asthma burden

Children with high eosinophil levels ($\geqslant0.3\times10^9 \cdot L^{-1}$) were younger (11.41 (2.6) years old) than their peers with low eosinophils (12.96 (2.8) years old) (supplementary table 6). There were no differences in terms of uncontrolled asthma, exacerbations >3 and school days missed >5 between low and high blood eosinophil groups (supplementary figures 3 and 4). Whereas children with a high blood eosinophil count ($\geqslant0.5\times10^9 \cdot L^{-1}$) demonstrated a significantly higher risk of uncontrolled asthma and a trend of having more than five school days missed compared with their peers with low eosinophil counts (adjusted OR (95% CI) 4.03 (1.15–12.25) and 2.74 (0.97–8.24)) (supplementary figure 3 and supplementary table 7). Participants with high neutrophil levels ($\geqslant4\times10^9 \cdot L^{-1}$) were more often female (55% *versus* 35%), had a higher BMI z-score (0.88 (1.37) *versus* 0.29 (1.24)), were more exposed to smoking (46% *versus* 27%) and had less history of doctor-diagnosed allergic rhinitis (59% *versus* 85%) compared with children with low neutrophils (supplementary table 8). Participants with high neutrophil levels showed a significantly higher risk of uncontrolled asthma and >5 asthma-related school days missed than patients with low neutrophil levels (adjusted OR (95% CI) 3.44 (1.11–11.83) and 3.41 (1.03–12.35), respectively) (supplementary figure 3).

The risk of uncontrolled asthma was significantly higher in the mixed group compared with the paucigranulocytic group (adjusted OR (95% CI) 4.47 (1.05–21.03)). Compared with the paucigranulocytic group, the eosinophilic group had lower probabilities of >3 exacerbations during the previous year (adjusted OR (95% CI) 0.30 (0.09–0.98)). Figure 2 and supplementary figure 5 show the results of adjusted and unadjusted logistic regression models, respectively. The outcomes of the adjusted and unadjusted logistic regression models in the sensitivity analysis are displayed in supplementary figures 6 and 7, respectively.

Linking blood inflammatory phenotypes with inflammatory mediators

The inflammatory mediator profiles differed between inflammatory groups irrespective of the chosen cut-off for eosinophilia in univariable (table 2, figure 3 and supplementary figure 8) and multivariable (figure 4 and supplementary figure 9) analyses. In both the main and sensitivity analysis, children in the neutrophilic group showed significantly higher levels of IL-6, matrix metalloproteinase (MMP)-9, C-reactive protein (CRP), and lower levels of tissue inhibitor of metalloproteinase (TIMP)-2 compared with the eosinophilic and paucigranulocytic groups. IL-5 was significantly more detectable in the mixed and eosinophilic phenotypes (56% and 60%, respectively). All IL-5 values in the neutrophilic group and 71% in the paucigranulocytic group were below the lower LOD (supplementary table 10). After imputation for the values below the LOD, IL-5 was significantly higher in the eosinophilic group compared with the

TABLE 1 Demographic and clinical characteristics of the inflammatory phenotypes. Phenotypes were defined based upon low/high blood eosinophil (LBE/HBE) counts of </⩾0.3×10^9·L^{-1}, respectively and low/high blood neutrophil (LBN/HBN) counts of </⩾4×10^9·L^{-1}, respectively, mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN)

Characteristics	Mixed (n=28)	Eosinophilic (n=55)	Neutrophilic (n=14)	Paucigranulocytic (n=29)	p-value	Total (n=126)
Demographics						
Age, years, mean±SD	11.08±2.84 (n=28)	11.58±2.48 (n=55)	13.90±3.19 (n=14)	12.50±2.53 (n=29)	0.007	11.94±2.76 (n=126)
Female	13/28 (46)	21/55 (38)	10/14 (71)	8/29 (28)	0.046	52/126 (41)
Ethnicity						
White	23/28 (82)	44/55 (80)	9/14 (64)	19/29 (66)	0.287	95/126 (75)
Non-White	5/28 (18)	11/55 (20)	5/14 (36)	10/29 (34)		31/126 (25)
Body mass index z-score, mean±SD	0.70±1.28 (n=28)	0.26±1.39 (n=54)	1.25±1.53 (n=14)	0.35±1.39 (n=29)	0.058	0.49±1.31 (n=125)
Smoking exposure	10/26 (38)	17/55 (31)	7/11 (64)	5/28 (18)	0.048	39/120 (32)
Country of inclusion						
Spain	6/28 (21)	30/55 (55)	1/14 (7)	12/29 (41)	0.001	49/126 (39)
Germany	7/28 (25)	8/55 (15)	3/14 (21)	8/29 (28)		26/126 (21)
The Netherlands	5/28 (18)	10/55 (18)	7/14 (50)	8/29 (28)		30/126 (24)
Slovenia	10/28 (36)	7/55 (13)	3/14 (21)	1/29 (3)		21/126 (17)
Clinical characteristics						
Asthma control status						
Uncontrolled	20/28 (71)	34/55 (62)	10/14 (71)	16/29 (55)	0.557	80/126 (63)
Number of school days missed because of asthma in the last year						
⩽5	13/26 (50)	39/54 (72)	7/13 (54)	21/29 (72)	0.163	80/122 (66)
>5	13/26 (50)	15/54 (28)	6/13 (46)	8/29 (28)		42/122 (34)
Number of exacerbations in the past year						
⩽3	14/26 (54)	37/54 (69)	8/13 (62)	17/29 (59)	0.588	76/122 (62)
>3	12/26 (46)	17/54 (31)	5/13 (38)	12/29 (41)		46/122 (38)
Asthma severity						
Moderate	14/28 (50)	25/55 (45)	4/14 (29)	13/29 (45)	0.614	56/126 (44)
Severe	14/28 (50)	30/55 (55)	10/14 (71)	16/29 (55)		70/126 (56)
GINA steps						
Step 3	14/28 (50)	25/55 (45)	4/14 (29)	13/29 (45)	0.006	56/126 (44)
Step 4	13/28 (46)	29/55 (53)	5/14 (36)	10/29 (34)		57/126 (45)
Step 5	1/28 (4)	1/55 (2)	5/14 (36)	6/29 (21)		13/126 (10)
ACT score, median (IQR)	22.5 (18.0–25.0) (n=26)	23.0 (21.0–25.0) (n=54)	20.0 (16.0–23.0) (n=13)	23.0 (19.0–24.0) (n=29)	0.226	23.0 (19.2–25.0) (n=122)
Lung function test, median (IQR)						
FEV_1 % predicted pre-salbutamol	95.2 (86.4–106.4) (n=27)	91.3 (82.1–100.4) (n=54)	83.1 (70.3–92.6) (n=13)	97.6 (90.7–103.3) (n=29)	0.016	93.4 (82.6–102.7) (n=123)
FEV_1 % predicted post-salbutamol	103.0 (94.8–115.7) (n=27)	98.2 (87.3–103.9) (n=54)	96.8 (88.5–100.0) (n=13)	103.1 (92.0–110.1) (n=29)	0.097	99.5 (90.2–108.1) (n=123)
History of doctor-diagnosed allergic disorders (ever)						
Atopic dermatitis	12/27 (44)	23/54 (43)	1/12 (8)	12/28 (43)	0.123	48/121 (40)
Allergic rhinitis	18/28 (64)	48/53 (91)	6/13 (46)	21/28 (75)	0.002	93/122 (76)

Data are presented as n (%) except where indicated. FEV_1: forced expiratory volume in 1 s; IQR: interquartile range; GINA: Global Initiative for Asthma; ACT: Asthma Control Test.

neutrophilic and paucigranulocytic groups (adjusted p-values for both the main imputation method and the sensitivity analysis for IL-5, p<0.05 corrected for the Benjamini–Hochberg false discovery rate). The eosinophilic group had lower IL-18 levels than the mixed group. Using the main cut-off set, the pulmonary and activation-regulated chemokine (PARC) level was higher in the mixed group, periostin (OSF2) was higher in the paucigranulocytic group and macrophage-derived chemokine (MDC) was lower in the neutrophilic group than other groups (adjusted p<0.05 corrected for the Benjamini–Hochberg false discovery rate). IL-17A, IL-22, IL-33 and thymic stromal lymphopoietin (TSLP) were not detectable due to the high number of values below the lower limit of quantitation (n=104 (83%), 88 (70%), 91 (72%) and 94 (75%), respectively).

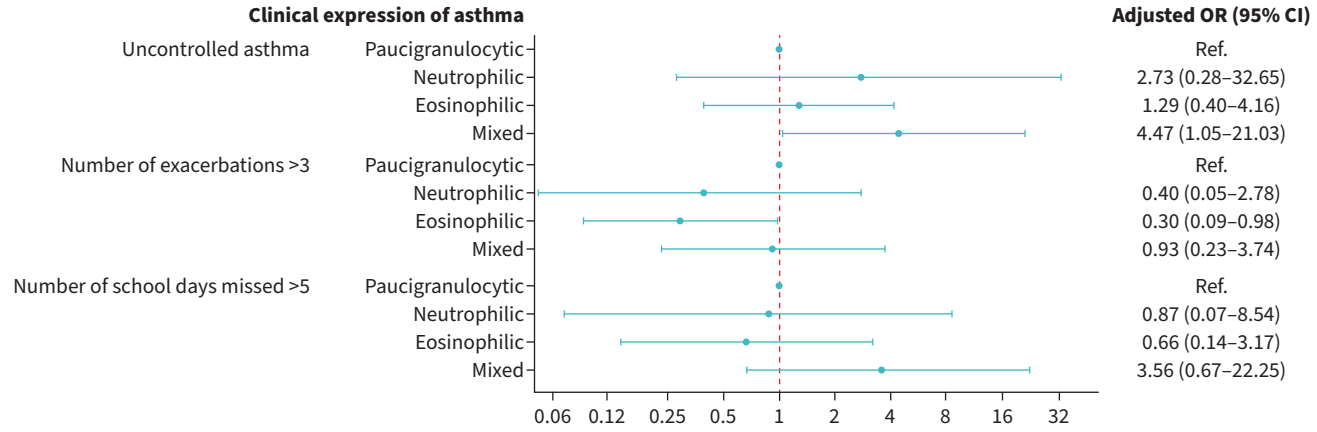

FIGURE 2 Adjusted association of blood inflammatory phenotypes defined by high and low blood eosinophil and neutrophil levels with asthma burden. Phenotypes were defined based upon low/high blood eosinophil (LBE/HBE) counts of </⩾0.3×10^{9}·L^{-1}, respectively, and low/high blood neutrophil (LBN/HBN) counts of </⩾4×10^{9}·L^{-1}, respectively; mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN). The models were adjusted for confounding factors such as age, sex, smoking exposure, BMI z-score, centre of inclusion, antibiotic intake in the past 2 months, inhaled corticosteroid daily dose levels (low, moderate and high based on GINA guidelines), medication adherence, biological intake and ethnicity (White and non-White). The x axis shows the OR values. The red dashed line indicates the significance level threshold. The paucigranulocytic group was considered as the reference group. OR: odds ratio; CI: confidence interval.

Supervised s-PLS-DA showed that MMP-9, MMP-3, IL-7, IL-5, PARC and MDC are the strongest contributing mediators to differentiate groups. Summary findings combining the inflammatory phenotype distinction, inflammatory parameters and asthma burden are depicted in figure 5.

Discussion

This study showed that distinct inflammatory asthma phenotypes exist in children with moderate-to-severe asthma despite high treatment doses. Children with a mixed blood inflammatory phenotype had a higher asthma burden compared with the paucigranulocytic group irrespective of the chosen cut-off for eosinophilia. In addition, distinct inflammatory mediator profiles were shown for each inflammatory phenotype from both univariable and multivariable (supervised sPLS-DA) perspectives, indicating that various inflammatory mechanisms may contribute to each phenotype.

Our findings are in line with recent studies in adult populations. Vedel-Krogh *et al.* [9] showed the association of higher blood eosinophil and neutrophil counts with higher asthma exacerbations in a Danish general population aged 20–100 years. In a large cohort of 15 019 adults with asthma, Tsiavia *et al.* [8] found that blood inflammatory phenotypes were linked with the occurrence and intensity of symptoms as well as asthma attacks. According to a 12-year follow-up study conducted in Finland on 203 individuals with asthma, neutrophilic and mixed inflammatory phenotypes (classified by 0.3×10^{9}·L^{-1} and 4.4×10^{9}·L^{-1} cut-offs for blood eosinophils and neutrophils) were associated with more unscheduled respiratory visits and increased asthma severity [7].

Although this study is the first study to investigate the associations between four inflammatory phenotypes (based on blood eosinophil and neutrophil levels) and asthma burden and inflammatory mediators, a few studies on children have partly investigated this association. A study on 38 children has reported a significantly lower lung function FEV_1 % predicted and higher sputum IL-17 and IL-8 levels in the neutrophilic asthma group compared with eosinophilic, paucigranulocytic and healthy children, whereas, the level of sputum IL-5 was significantly higher in the eosinophilic group compared with the neutrophilic and paucigranulocytic groups [11]. These findings are consistent with ours.

There is no consensus on a cut-off value for blood eosinophilia and neutrophilia in children. Therefore, in this study, we utilised 4×10^{9}·L^{-1} as a cut-off for neutrophils, which was selected based on the outcomes of adjusted multivariable logistic regression models for various cut-offs that were used in adult populations. In addition, we also estimated the optimal cut-off for neutrophils in our population based on Youden's index (4.18×10^{9}·L^{-1}). However, due to the low sensitivity of the test, we did not consider this cut-off in our analysis. Two common cut-offs for eosinophils (0.3×10^{9}·L^{-1} and 0.5×10^{9}·L^{-1}), which are used in many clinical studies [7, 8, 15–18] to classify patients into four groups and are described in detail in the methods

TABLE 2 Unadjusted and adjusted (corrected by multiple testing by Benjamini–Hochberg false discovery rate) p-values of the mediator levels comparison between inflammatory phenotypes in both main and sensitivity analysis. Due to a high number of values below the lower limit of detection, we were not able to study some of the mediators, such as IL-17a, IL-22, IL-33 and TSLP

Mediator name	n	p-value			
		Main analysis cut-offs: BE, $0.3\times10^{9}\cdot L^{-1}$ BN, $4\times10^{9}\cdot L^{-1}$		Sensitivity analysis cut-offs: BE, $0.5\times10^{9}\cdot L^{-1}$ BN, $4\times10^{9}\cdot L^{-1}$	
		Unadjusted	FDR adjusted	Unadjusted	FDR adjusted
IL-1β	119	**0.046**	0.112	**0.032**	0.092
IL-4	119	0.203	0.310	0.207	0.334
IL-5	117	**0.002**	**0.010**	**<0.001**	**0.001**
IL-6	119	**<0.001**	**0.003**	**<0.001**	**0.001**
IL-7	119	**0.017**	0.050	**0.030**	0.092
IL-8	119	0.330	0.479	0.512	0.593
IL-9	119	0.676	0.784	0.791	0.820
IL-10	119	0.780	0.838	0.319	0.488
IL-13	94	0.452	0.572	0.443	0.557
IL-18	119	**0.005**	**0.017**	**0.006**	**0.032**
MMP-1	119	0.704	0.786	0.443	0.557
MMP-3	119	**0.033**	0.087	0.058	0.140
MMP-9	119	**<0.001**	**<0.001**	**<0.001**	**<0.001**
TNF-α	119	0.094	0.209	0.115	0.223
TARC	119	0.101	0.209	**0.007**	**0.032**
PARC	119	**0.003**	**0.012**	**0.013**	**0.049**
GRO-α	119	0.975	0.975	0.874	0.874
TIMP-1	119	0.454	0.572	0.445	0.557
TIMP-2	119	**0.001**	**0.010**	**0.002**	**0.012**
TIMP-4	119	0.541	0.654	0.655	0.703
MCP-4	119	0.119	0.225	0.110	0.223
CD14	119	0.124	0.225	0.103	0.223
VEGF	119	0.857	0.888	0.052	0.136
MIP-3β	119	0.157	0.253	0.461	0.557
IP10	119	0.378	0.522	0.160	0.290
OSF2	119	**0.015**	**0.049**	0.575	0.641
MDC	114	**<0.001**	**0.003**	0.202	0.334
RAGE	119	0.132	0.225	0.390	0.557
CRP	119	**0.002**	**0.010**	**0.008**	**0.032**

IL: interleukin; TSLP: thymic stromal lymphopoietin; BE: blood eosinophils; BN: blood neutrophils; FDR: false discovery rate; MMP: matrix metalloproteinase; TNF: tumour necrosis factor; TARC: thymus and activation-regulated chemokine; PARC: pulmonary and activation-regulated chemokine; GRO-α: growth-regulated peptide; TIMP: tissue inhibitor of metalloproteinase; MCP-4: monocyte chemoattractant protein 4; CD14: cluster of differentiation 14; VEGF: vascular endothelial growth factor; MIP-3β: macrophage inflammatory protein-3β; IP10: interferon gamma-induced protein 10; OSF2: periostin; MDC: macrophage-derived chemokine; RAGE: receptor for advanced glycation end-products; CRP: C-reactive protein. Bold p-values are significant.

section, were used ($0.3\times10^{9}\cdot L^{-1}$ considered in the main analysis and $0.5\times10^{9}\cdot L^{-1}$ in the sensitivity analysis). Choosing a different eosinophil cut-off had a large impact on the different group sizes. Children with a mixed asthma phenotype showed a higher risk of uncontrolled asthma in both the main and sensitivity analysis. Whereas, by choosing a higher cut-off for eosinophils ($0.5\times10^{9}\cdot L^{-1}$), the odds of uncontrolled asthma and school days missed are increased, indicating that in this moderate-to-severe asthma population, where all patients were on maintenance GINA treatment steps 3 or higher, the higher eosinophil cut-off ($0.5\times10^{9}\cdot L^{-1}$) could better distinguish between the inflammatory phenotypes regarding asthma burden-related clinical outcomes than the commonly used cut-off of $0.3\times10^{9}\cdot L^{-1}$.

So far, there has been limited research on blood biomarkers that could identify inflammatory phenotypes related to type-2 low asthma, especially in paediatric asthma. In this study, the neutrophilic phenotype, compared with the eosinophilic and paucigranulocytic phenotypes, had significantly higher IL-6, MMP-9 and CRP and lower TIMP-2 levels. IL-6 and MMP-9 are known to play a role in non-type-2 inflammation and neutrophil-related pathways [5, 26]. MMP-9 is a proinflammatory mediator secreted from neutrophils

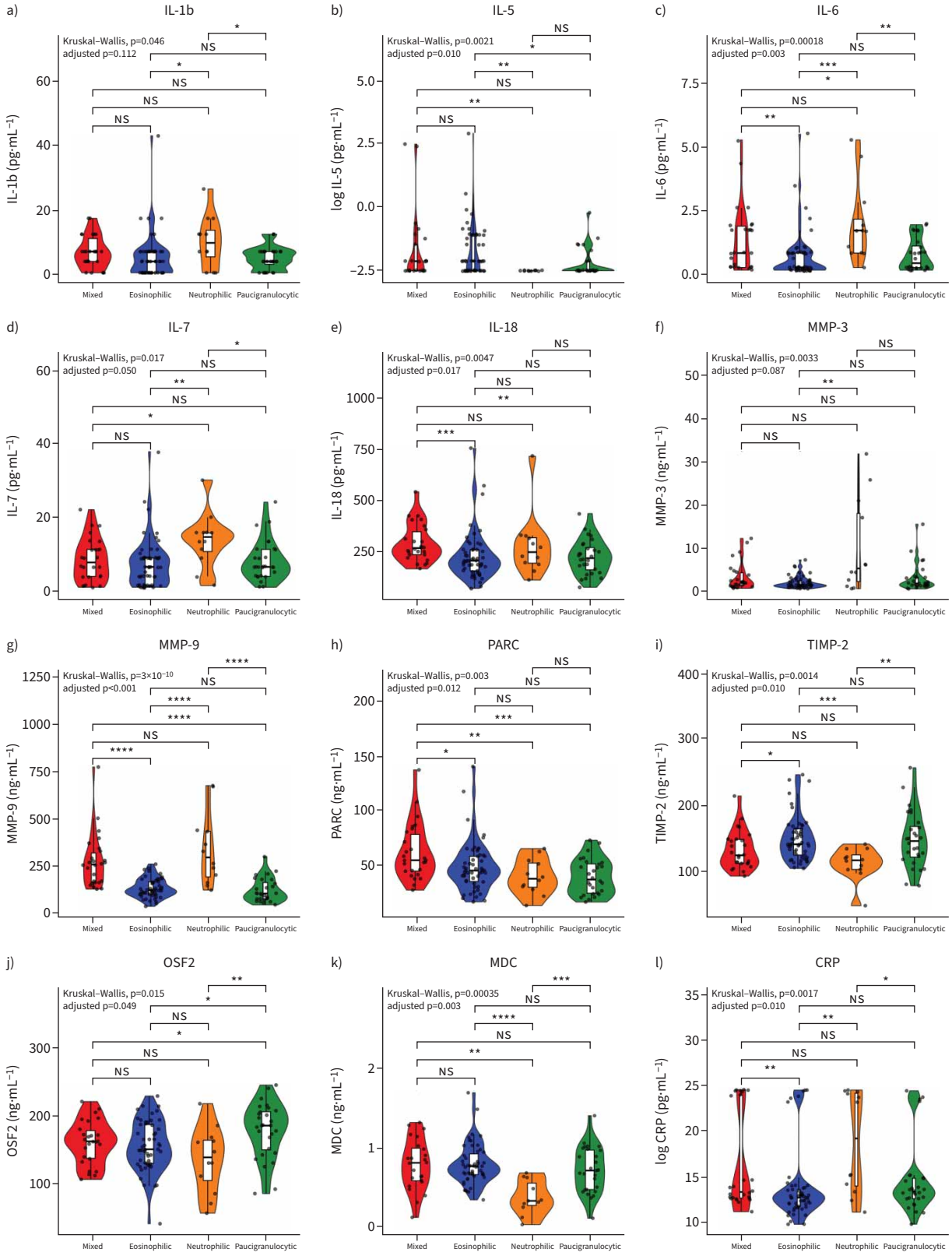

FIGURE 3 Cytokine and mediator levels for each inflammatory phenotype. Phenotypes were defined based upon low/high blood eosinophil (LBE/HBE) counts of </⩾0.3×10^{9}·L^{-1}, respectively and low/high blood neutrophil (LBN/HBN) counts of </⩾4×10^{9}·L^{-1}, respectively), mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN). The adjusted p-values shown in the figures are the corrected p-values by multiple testing by Benjamini–Hochberg false discovery rate. IL: interleukin; MMP: matrix metalloproteinase; PARC: pulmonary and activation-regulated chemokine; TIMP: tissue inhibitor of metalloproteinase; OSF2: periostin; MDC: macrophage-derived chemokine; CRP: C-reactive protein; NS: p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.

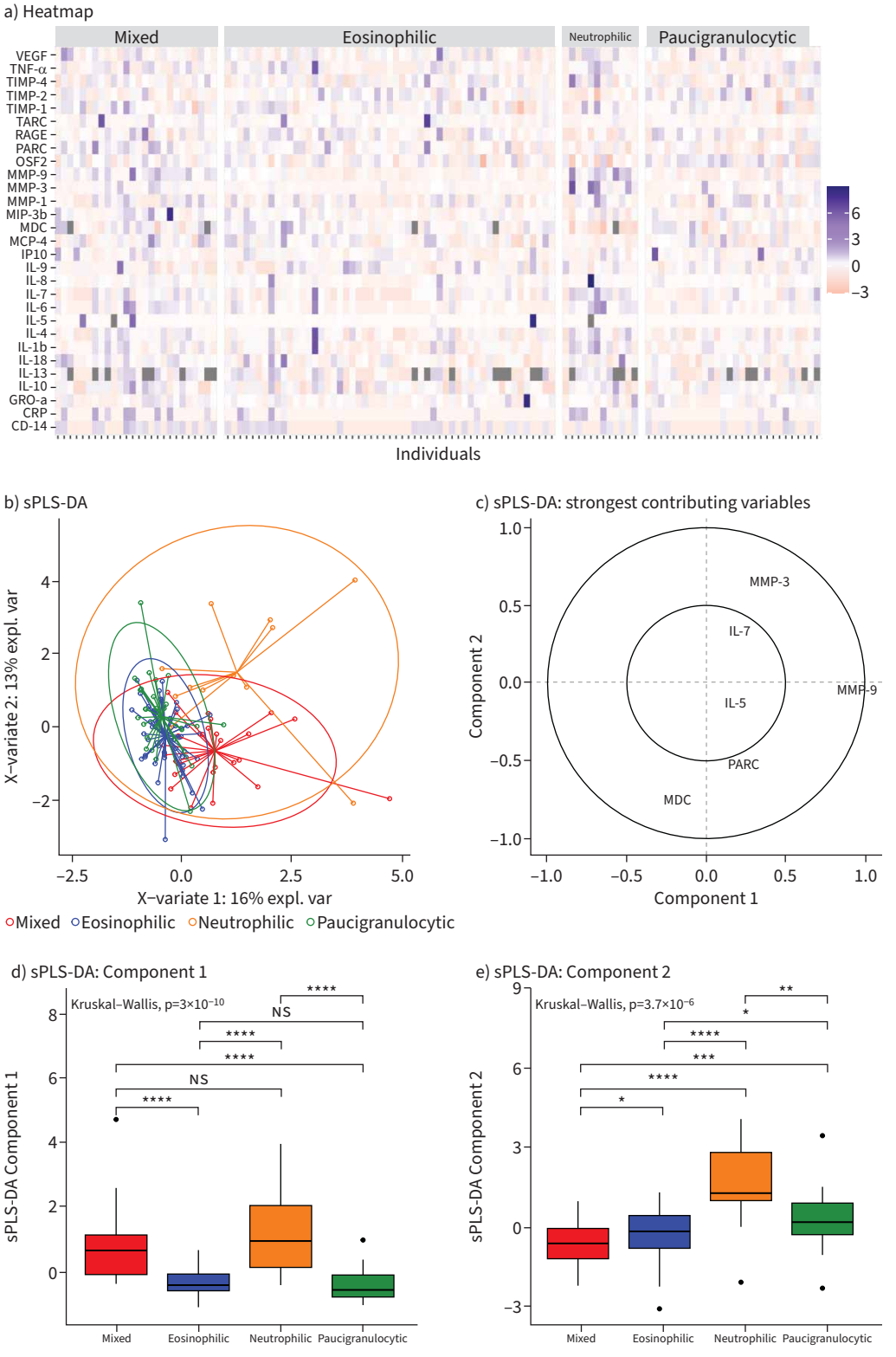

FIGURE 4 Heatmap and cluster analysis of the inflammatory mediator profiles per patient between each phenotype. Phenotypes were defined based upon low/high blood eosinophil (LBE/HBE) counts of </⩾0.3×10^{9}·L^{-1}, respectively and low/high blood neutrophil counts (LBN/HBN) of </⩾4×10^{9}·L^{-1}, respectively, mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN). a) Heatmap of blood inflammatory mediators. Each column represents an individual participant and each row shows the abundance of the mentioned mediator (z-score). b) Sparse partial least squares discriminant

analysis (sPLS-DA) scores plot for each phenotype. c) Strongest contributing variables (mediators) to sPLS-DA components. d,e) Boxplots of sPLS-DA components 1 (d) and 2 (e) for each phenotype, including Kruskal–Wallis and pairwise Wilcoxon test results (false discovery rate adjusted). IL: interleukin; MMP: matrix metalloproteinase; PARC: pulmonary and activation-regulated chemokine; TIMP: tissue inhibitor of metalloproteinase; OSF2: periostin; MDC: macrophage-derived chemokine; CRP: C-reactive protein; NS: p>0.05; *: p<0.05; **: p<0.01; ***: p<0.001; ****: p<0.0001.

[27], which plays a role in airway inflammation and promotes angiogenesis in asthma [28]. TIMP-2 is an inhibitor of MMP activity [29]. Low TIMP-2, together with high MMP-9, which we found to be increased in the neutrophilic phenotype, have previously been linked to the pulmonary structural remodelling [29]. Flinkman *et al.* [7] also showed that IL-6, MMP-9 and CRP levels were higher in the neutrophilic phenotype in adults. By applying a higher eosinophil cut-off ($0.5\times10^{9}\cdot L^{-1}$), IL-6 and MMP-9 levels were also significantly higher in the neutrophilic group compared with the mixed group. This means that using a higher eosinophilic cut-off, IL-6 and MMP-9 can be used as potential blood biomarkers to distinguish the neutrophilic phenotype from other inflammatory phenotypes in paediatric asthma. IL-6 and MMP-9 may be probable targets for this phenotype in terms of asthma management and targeted treatment. In addition, MMP-9 was one of the strongest contributing factors in supervised sPLS-DA, which plays a remarkable role in differentiating inflammatory phenotypes.

The eosinophilic phenotype is mainly characterised by high T helper 2 cell-associated cytokines such as IL-4, IL-5 and IL-13. Among these three cytokines, we found that only IL-5 was higher in the high eosinophilic groups (eosinophilic and mixed phenotypes), and groups were better differentiated by using the higher eosinophil cut-off compared with the lower one. Not showing differences in IL-4 and IL-13 levels and no detectability of IL-17a, IL-33 and TSLP due to the high number of values below the LOD may be because of the suppressing effects of treatments, especially corticosteroids. Corticosteroid intake

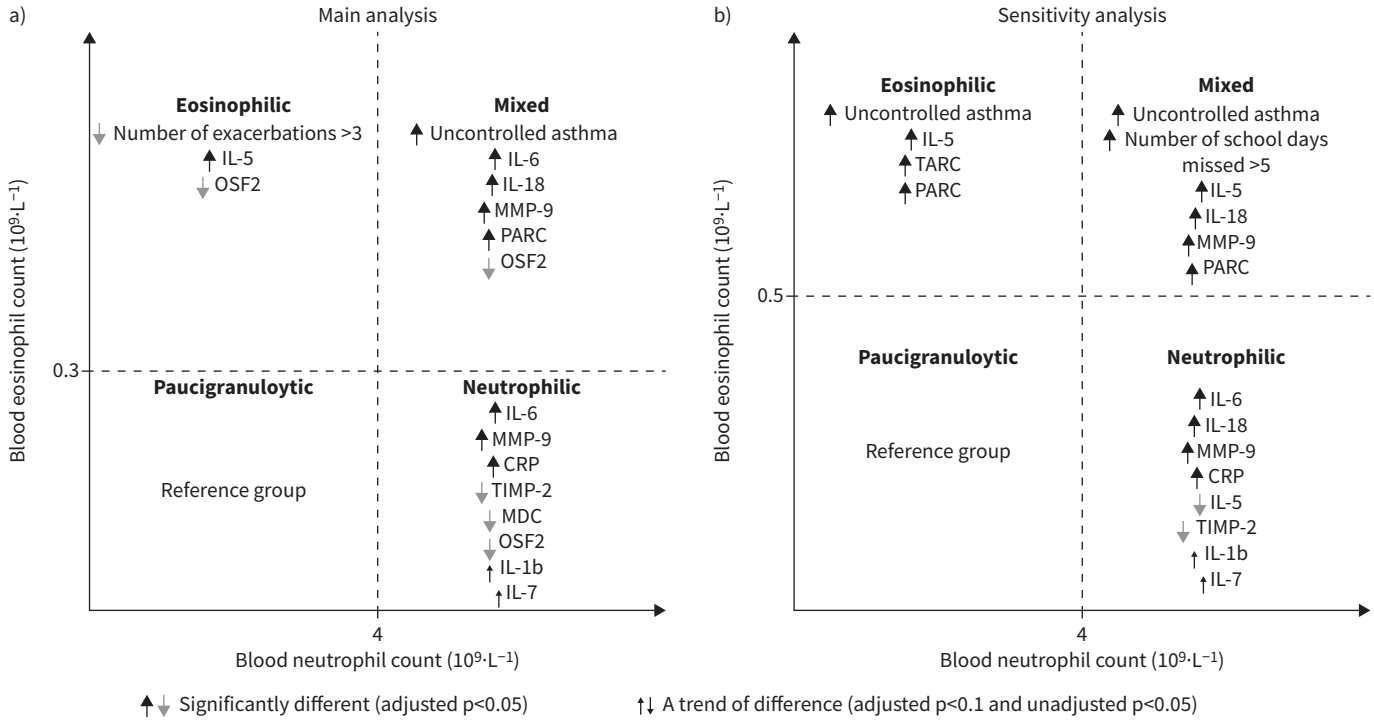

FIGURE 5 Summary of findings regarding asthma burden and inflammatory mediators. The paucigranulocytic group was considered as the reference group. a) Main analysis. b) Sensitivity analysis. Phenotypes were defined based upon low/high blood eosinophil (LBE/HBE) counts of </⩾$0.3\times10^{9}\cdot L^{-1}$ (main analysis) or $0.5\times10^{9}\cdot L^{-1}$ (sensitivity analysis), respectively and low/high blood neutrophil (LBN/HBN) counts of </⩾$4\times10^{9}\cdot L^{-1}$, respectively, mixed (HBE-HBN), eosinophilic (HBE-LBN), neutrophilic (LBE-HBN) and paucigranulocytic (LBE-LBN). IL: interleukin; MMP: matrix metalloproteinase; TARC: thymus and activation-regulated chemokine; PARC: pulmonary and activation-regulated chemokine; TIMP: tissue inhibitor of metalloproteinase; CRP: C-reactive protein.

can downregulate type-2 cytokines such as IL-4, IL-5 and IL-13 and the number of airway eosinophils, which may lead to mis-phenotyping. However, in this study, all participants were taking regular ICSs and there were no differences in terms of ICS dosage, medication adherence and inhaler techniques between patients with different inflammatory phenotypes or with asthma control status [30]. Supervised sPLS-DA can better differentiate groups with high neutrophil counts (neutrophilic or mixed) from groups with low neutrophil counts (eosinophilic and paucigranulocytic) because more non-type-2-related mediators differed between phenotypes. We found a moderate negative correlation between age and blood eosinophils counts in this population (correlation coefficient −0.29, 95% CI −0.44– −0.12). The country of inclusion was associated with both eosinophil and neutrophil levels. Females had a higher neutrophil level than males (4.30 (2.50) *versus* 3.50 (2.03)×10^9·L^{-1}). Hence, we included age, sex, country of inclusion, as well as smoking exposure, BMI z-score, antibiotic intake in the past 2 months, ICS daily dose level (low, moderate and high based on GINA guidelines), medication adherence, biological intake and ethnicity (White and non-White) among confounding factors as defined in the DAG. The multivariable models were corrected for the mentioned confounding factors. However, more research, and specifically longitudinal studies, are needed to investigate the age-related changes in blood and airway inflammatory markers, as well as their correlation, to further refine our understanding of these relationships and their implications for asthma management in paediatric populations.

This study had multiple strengths. This is the first study on paediatric asthma that focused on phenotyping patients based on blood eosinophil and neutrophil counts, assessing different cut-off values and incorporating blood inflammatory mediators. Even though the prevalence of uncontrolled moderate-to-severe asthma in children is low (∼2% [31]), we included patients from four European countries. While the numbers in each country may not be large, this multinational approach enhances the potential generalisability of our findings compared with single-centre studies.

There are also limitations within this study. Residual confounding factors, such as air pollution, occupational exposure and diet were not available. Moreover, due to a high number of values below the lower LOD, we were not able to study some of the important inflammatory mediators, such as IL-17a, IL-33 and TSLP. Low sample size and adjustment for multiple confounding factors caused some reported confidence intervals to be wide. Moreover, we could not assess the temporal stability of the blood inflammatory phenotypes in this study.

Our findings contribute to a better understanding of the complexity of childhood asthma. Increased levels of type-2 biomarkers such as IL-5 detected in the eosinophilic and mixed phenotypes may indicate eligibility for biologics treatment (anti-IL-5). This may help to identify children with type-2-driven inflammation more accurately. However, more insights into the stability of these inflammatory phenotypes and mediator profiles are needed.

Conclusion

This first study on a moderate-to-severe paediatric asthma population indicates that blood eosinophil and neutrophil counts can lead us to identify inflammatory phenotypes with distinct clinical outcomes and inflammatory mediator profiles. Better identifying inflammatory phenotypes in advance can improve the management and treatment of childhood asthma. However, more research is required to evaluate the stability of these phenotypes in paediatric asthma over time.

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Data availability: For clinical and other data generated within the SysPharmPediA study, the authors will make them available upon specific request subject to the requestor obtaining ethical, research, data access and collaboration approvals from the SysPharmPediA study management board. Requests can be sent to a.h.maitland@amsterdamumc.nl.

The SysPharmPediA study is registered at www.clinicaltrials.gov with identifier number NCT04865575.

Ethical statement: This study was carried out in accordance with the Declaration of Helsinki and approved by the local medical review boards of all study centres: the Medical Ethics Committee of the University Medical Center

Utrecht, Utrecht, The Netherlands (NL55788.041.15); the ethics committee of University Regensburg, Germany (18–1034–101); the Clinical Research Ethics Committee of the Basque Country, Spain (PI2015075 (SO)); and the National Medical Ethics Committee, Slovenia (0120–569/2017/4)). All participants/parents gave their informed consent.

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Conflict of interest: A.H. Alizadeh Bahrani, S.J.H. Vijverberg, S. Hashimoto, C. Wolff, C. Almqvist, S. Brandstetter, P. Corcuera-Elosegui, S. Harner, A.M. Hedman, L. López-Fernández, A.D. Kraneveld, O. Sardón-Prado, B.S. Dierdop, T. Dekker, N.K.A. Metwally, R. Lutter and P. Brinkman have no conflicts of interest to disclose. L.D. Bloemsma received funding from partners in the Precision Medicine for More Oxygen (P4O2) consortium, which are the Amsterdam UMC, Leiden University Medical Center, Maastricht UMC+, Maastricht University, UMC Groningen, UMC Utrecht, Utrecht University, TNO, Aparito, Boehringer Ingelheim, Breathomix, Clear, Danone Nutricia Research, Fluidda, MonitAir, Ncardia, Ortec Logiqcare, Philips, Proefdiervrij, Quantib-U, RespiQ, Roche, Smartfish, SODAQ, Thirona, TopMD, Lung Alliance Netherlands, the Lung Foundation Netherlands (Longfonds), PPP Allowance made available by Health~Holland, and Top Sector Life Sciences and Health (LSHM20104 and LSHM20068), to stimulate public–private partnerships and by Novartis. M. Gorenjak received SysPharmPediA grant, cofinanced by the Ministry of Education, Science and Sport Slovenia (MIZS) (contract number C3330-16-500106) and funded by the Slovenian Research Agency (research core funding number P3-0427), and by the Ministry of Education, Science and Sport of the Republic of Slovenia grant PERMEABLE (contract number C3330-19-252012). M. Kabesch received funding from Bundesministerium für Bildung und Forschung (BMFB) grant SysPharmPediA, European Union, BMFB, German Research Foundation, Infectopharm, Bavarian Minsitry of Education and Research, and Bavarian Ministry of Health. He received consulting fees from Bionorica, Sanofi, Novartis and Bencard; he also received honoraria from ERS, EAACI, ATS, Novartis, Glaxo, Chiesi, Sanofi, Nutricia, Hipp and Allergopharma. A.H. Neerincx has received ERANET Systems Medicine and ZonMW grants (project number 9003035001). M. Pino-Yanes received funding from Instituto de Salud Carlos III (AC15/00015) as part of the SysPharmPediA consortium, and grants from MCIN/AEI/10.13039/501100011033, GlaxoSmithKline Spain and CSL Berhing outside of the submitted work. U. Potočnik received SysPharmPediA grant, cofinanced by the Ministry of Higher Education, Science and Innovation Slovenia (MVZI) (contract number C3330-16-500106) and Slovenian Research Agency (research core funding number P3-0427 and research grant number J3-4497). J.W. Duitman received grants from Abbvie and Boehringer Ingelheim in the past 36 months. M.I. Abdel-Aziz was funded by a full PhD scholarship from the Ministry of Higher Education of the Arab Republic of Egypt during the conduct of the study. A.H. Maitland-van der Zee is the principal investigator of a public–private consortium (P4O2) sponsored by Health Holland involving many private partners that contribute in cash and/or in kind (AbbVie, Boehringer Ingelheim, Breathomix, Clear, Fluidda, Ortec Logiqcare, Olive, Philips, Quantib-U, Smartfish, Clear, SODAQ, Thirona, Roche, TopMD, Novartis and RespiQ); she received unrestricted research grants from GSK and Boehringer Ingelheim, and a Vertex Innovation Award grant; and she has received honoraria from Boehringer Ingelheim, GSK and AstraZeneca.

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References

1 Foppiano F, Schaub B. Childhood asthma phenotypes and endotypes: a glance into the mosaic. *Mol Cell Pediatr* 2023; 10: 9.
2 Håkansson KEJ, Guerrero SC, Backer V, *et al.* Burden and unmet need for specialist care in poorly controlled and severe childhood asthma in a Danish nationwide cohort. *Respir Res* 2023; 24: 173.
3 Asher MI, Rutter CE, Bissell K, *et al.* Worldwide trends in the burden of asthma symptoms in school-aged children: Global Asthma Network Phase I cross-sectional study. *Lancet* 2021; 398: 1569–1580.
4 Nadif R, Siroux V, Oryszczyn MP, *et al.* Heterogeneity of asthma according to blood inflammatory patterns. *Thorax* 2009; 64: 374–380.
5 Alizadeh Bahmani AH, Abdel-Aziz MI, Maitland-van der Zee AH, *et al.* Recent advances in the treatment of childhood asthma: a clinical pharmacology perspective. *Expert Rev Clin Pharmacol* 2022; 15: 1165–1176.
6 Ullmann N, Bossley CJ, Fleming L, *et al.* Blood eosinophil counts rarely reflect airway eosinophilia in children with severe asthma. *Allergy* 2013; 68: 402–406.

7 Flinkman E, Vähätalo I, Tuomisto LE, *et al.* Association between blood eosinophils and neutrophils with clinical features in adult-onset asthma. *J Allergy Clin Immunol Pract* 2023; 11: 811–821.
8 Tsiavia T, Henny J, Goldberg M, *et al.* Blood inflammatory phenotypes were associated with distinct clinical expressions of asthma in adults from a large population-based cohort. *EBioMedicine* 2022; 76: 103875.
9 Vedel-Krogh S, Fallgaard Nielsen S, Lange P, *et al.* Association of blood eosinophil and blood neutrophil counts with asthma exacerbations in the Copenhagen general population study. *Clin Chem* 2017; 63: 823–832.
10 Pan R, Ren Y, Li Q, *et al.* Neutrophil-lymphocyte ratios in blood to distinguish children with asthma exacerbation from healthy subjects. *Int J Immunopathol Pharmacol* 2023; 37: 3946320221149849.
11 Wei Q, Liao J, Jiang M, *et al.* Relationship between Th17-mediated immunity and airway inflammation in childhood neutrophilic asthma. *Allergy Asthma Clin Immunol* 2021; 17: 4.
12 Abdel-Aziz MI, Neerincx AH, Vijverberg SJH, *et al.* A system pharmacology multi-omics approach toward uncontrolled pediatric asthma. *J Pers Med* 2021; 11: 484.
13 Liu AH, Zeiger R, Sorkness C, *et al.* Development and cross-sectional validation of the Childhood Asthma Control Test. *J Allergy Clin Immunol* 2007; 119: 817–825.
14 Nathan RA, Sorkness CA, Kosinski M, *et al.* Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004; 113: 59–65.
15 Jesenak M, Diamant Z, Simon D, *et al.* Eosinophils – from cradle to grave an EAACI Task Force paper on new molecular insights and clinical functions of eosinophils and the clinical effects of targeted eosinophil depletion. *Allergy* 2023; 78: 3077–3102.
16 Gupta A, Pouliquen I, Austin D, *et al.* Subcutaneous mepolizumab in children aged 6 to 11 years with severe eosinophilic asthma. *Pediatr Pulmonol* 2019; 54: 1957–1967.
17 Williams KW, Ware J, Abiodun A, *et al.* Hypereosinophilia in children and adults: a retrospective comparison. *J Allergy Clin Immunol Pract* 2016; 4: 941–947.
18 Valent P, Klion AD, Horny HP, *et al.* Contemporary consensus proposal on criteria and classification of eosinophilic disorders and related syndromes. *J Allergy Clin Immunol* 2012; 130: 607–612.
19 de Brabander J, Duijvelaar E, Schippers JR, *et al.* Immunomodulation and endothelial barrier protection mediate the association between oral imatinib and mortality in hospitalised COVID-19 patients. *Eur Respir J* 2022; 60: 2200780.
20 de Brabander J, Boers LS, Kullberg RFJ, *et al.* Persistent alveolar inflammatory response in critically ill patients with COVID-19 is associated with mortality. *Thorax* 2023; 78: 912–921.
21 Zhang S, Boers LS, de Brabander J, *et al.* The alveolar fibroproliferative response in moderate to severe COVID-19-related acute respiratory distress syndrome and 1-yr follow-up. *Am J Physiol Lung Cell Mol Physiol* 2024; 326: L7–L18.
22 Global Initiative for Asthma. Global strategy for asthma management and prevention, 2016. Date last accessed: 2016. Date last updated: 2016. https://ginasthma.org/wp-content/uploads/2019/01/2016-GINA.pdf
23 Duan W, Cen Y, Lin C, *et al.* Inflammatory epithelial cytokines after in vitro respiratory syncytial viral infection are associated with reduced lung function. *ERJ Open Res* 2021; 7: 00365-2021.
24 Schader JF, Haid M, Cecil A, *et al.* Metabolite shifts induced by marathon race competition differ between athletes based on level of fitness and performance: a substudy of the Enzy-MagIC study. *Metabolites* 2020; 10: 87.
25 Chung D, Keles S. Sparse partial least squares classification for high dimensional data. *Stat Appl Genet Mol Biol* 2010; 9: Article17.
26 Israel E, Reddel HK. Severe and difficult-to-treat asthma in adults. *N Engl J Med* 2017; 377: 965–976.
27 Yabluchanskiy A, Ma Y, Iyer RP, *et al.* Matrix metalloproteinase-9: many shades of function in cardiovascular disease. *Physiology (Bethesda)* 2013; 28: 391–403.
28 Varricchi G, Modestino L, Poto R, *et al.* Neutrophil extracellular traps and neutrophil-derived mediators as possible biomarkers in bronchial asthma. *Clin Exp Med* 2022; 22: 285–300.
29 Costanzo L, Soto B, Meier R, *et al.* The Biology and function of tissue inhibitor of metalloproteinase 2 in the lungs. *Pulm Med* 2022; 2022: 3632764.
30 Alizadeh Bahmani AH, Slob EMA, Bloemsma LD, *et al.* Medication use in uncontrolled pediatric asthma: results from the SysPharmPediA study. *Eur J Pharm Sci* 2023; 181: 106360.
31 Nordlund B, Melén E, Schultz ES, *et al.* Prevalence of severe childhood asthma according to the WHO. *Respir Med* 2014; 108: 1234–1237.