Distribution of type 2 biomarkers and association with severity, clinical characteristics and comorbidities in the BREATHE real-life asthma population

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Shareable abstract (@ERSpublications)
T2 inflammation is highly prevalent, with biomarker co-expression a particular trait of severe asthma. The best treatable trait, eosinophilia, is missed in half of patients with mild–moderate and one-third with severe asthma without airway inflammometry. https://bit.ly/3rVPNbv

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Abstract

Background Type 2 (T2) high asthma is recognised as a heterogenous entity consisting of several endotypes; however, the prevalence and distribution of the T2 biomarkers in the general asthma population, across asthma severity, and across compartments is largely unknown. The objective of the present study was to describe expression and overlaps of airway and systemic T2 biomarkers in a clinically representative asthma population.

Methods Patients with asthma from the real-life BREATHE cohort referred to a specialist centre were included and grouped according to T2 biomarkers: blood and sputum eosinophilia ($\geqslant 0.3\times10^9$ cells·L^{-1} and 3% respectively), total IgE ($\geqslant$150 U·mL^{-1}), and fractional exhaled nitric oxide ($\geqslant$25 ppb).

Results Patients with mild-to-moderate asthma were younger (41 *versus* 49 years, p<0.001), had lower body mass index (25.9 *versus* 28.0 kg·m^{-2}, p=0.002) and less atopy (47% *versus* 58%, p=0.05), higher forced expiratory volume in 1 s (3.2 *versus* 2.8 L, p<0.001) and forced vital capacity (4.3 *versus* 3.9 L, p<0.001) compared with patients with severe asthma, who had higher blood (0.22×10^9 *versus* 0.17×10^9 cells·L^{-1}, p=0.01) and sputum (3.0% *versus* 1.5%, p=0.01) eosinophils. Co-expression of all T2 biomarkers was a particular characteristic of severe asthma (p<0.001). In patients with eosinophilia, sputum eosinophilia without blood eosinophilia was present in 45% of patients with mild-to-moderate asthma and 35% with severe asthma.

Conclusion Severe asthma is more commonly associated with activation of several T2 pathways, indicating that treatments targeting severe asthma may need to act more broadly on T2 inflammatory pathways. Implementation of airway inflammometry in clinical care is of paramount importance, as the best treatable trait is otherwise is overlooked in a large proportion of patients irrespective of disease severity.

Introduction

Asthma is increasingly recognised as a heterogeneous disease consisting of several endotypes [1]. The main pathogenic mechanism in the majority of asthma cases is perceived to be type 2 (T2) inflammation, driven by type 2 helper T-cells (Th2) and type 2 innate lymphoid cells (ILC2) and mediated through interleukin (IL)-5 and IL-4/IL-13 signalling pathways.

The introduction of treatments targeting the key T2 cytokines has provided important insights into their relationship with clinically available biomarkers, with cross-sectional data suggesting a marked

heterogeneity within the T2-high entity [2–4]. Peripheral blood eosinophil count (B-EOS) reflects IL-5 production and is reduced by treatments targeting IL-5, IL-5 receptor (IL-5R) and thymic stromal lymphopoietin (TSLP) [5–9], whereas treatments targeting IgE [10] and IL-4 receptor-α (IL-4Rα) [11] do not. Exhaled nitric oxide fraction (F_{eNO}) is induced by IL-13 at the bronchial epithelium, reflecting airway IL-13 activity [12–14], and is reduced by treatments targeting IgE, IL-4, IL-13Rα and TSLP therapy [9–11]. IgE is produced by B-cells in an IL-4-driven process and is gradually decreased by anti-TSLP therapy [1, 9].

Airway sampling using induced sputum is rarely used in routine clinical care and B-EOS is often used as a surrogate marker of eosinophilic airway inflammation [15], despite recent evidence highlighting a marked spatial heterogeneity across compartments, with concordant eosinophilic inflammation present in only half of patients (37–52%) with eosinophilia in blood or sputum [3, 16, 17].

At present, the prevalence and distribution of T2 biomarkers in the general asthma population and across asthma severity are largely unknown. Uncovering the patterns of pathway activity and their consistency across compartments and asthma severity is an important step towards understanding partial or non-response to targeted treatment in patients with an inflammatory phenotype indicative of response.

Here, we report the expression and overlaps of airway and systemic T2 biomarkers in a clinically representative asthma population. We hypothesised that single-pathway activation is a sign of more benign disease and, consequently, that co-activation of inflammatory pathways as well as global eosinophilic inflammation across compartments is more prevalent in patients with severe disease.

Methods

Design

BREATHE was a multicentre, cross-sectional study recruiting patients with asthma and/or chronic obstructive pulmonary disease from five clinical centres: two specialist care units in Eastern Denmark and one specialist and two primary care units in southern Sweden [18]. The recruitment period was 2 years (February 2017–February 2019). Further details have been published previously [18].

Study population

Patients with an asthma diagnosis recruited at a specialist care unit were included in this study because patients from primary care (n=290) did not have sputum collected or IgE measured [18].

Patients without a complete biomarker panel, *i.e.* measurement of F_{eNO} and IgE and an evaluation of eosinophilia (B-EOS and/or sputum eosinophil count (S-EOS)), were excluded.

A diagnosis of asthma was based on a thorough medical history, clinical evaluation, static and dynamic lung function and an indirect bronchial provocation test (mannitol).

Patients were stratified by disease severity into two groups: severe asthma (SA) and mild-to-moderate asthma (MMA) based on the European Respiratory Society/American Thoracic Society criteria for possible SA [19].

Assessments

Sputum was collected following a mannitol provocation test or induction with either isotonic saline (0.9%) or incremental concentrations of NaCl solutions (3%, 4% and 5%), and processed as described [20, 21]. A cut-off of 3% for eosinophils and 61% for neutrophils was used for inflammatory phenotyping [22].

Specific serum IgE tests were performed using a standard panel including pollen from birch, grass and mugwort; dander from horse, cat and dog; house-dust mites *Dermatophagoides pteronyssinus* and *Dermatophagoides farina*; and the fungi *Alternaria alternata/tenuis* and *Cladosporium herbarum*. Allergic sensitisation was defined as elevated specific IgE (>0.35 $kU \cdot L^{-1}$) for a minimum one of the 10 tested aeroallergens.

Statistical analyses

Binary cut-offs for elevated biomarker expression were used: blood eosinophilia was defined as B-EOS $\geqslant 0.3\times10^{9} \cdot L^{-1}$, elevated F_{eNO} as F_{eNO} $\geqslant$25 ppb, elevated IgE as total IgE $\geqslant$150 $U \cdot mL^{-1}$ and sputum eosinophilia as S-EOS $\geqslant$3% [7, 11, 23–28].

A conservative cut-off for IgE was used in an attempt to ensure well-defined groups because median IgE levels in previous severe asthma cohorts have been markedly above the normal range (109–126 $U \cdot mL^{-1}$) [3, 29, 30].

Parametric and non-parametric continuous variables are reported as mean±SD and median (25th and 75th percentiles) and were tested using Welch's ANOVA or Kruskal–Wallis, respectively. Categorical variables were tested using Chi-squared or Fisher's exact test when needed. To correct for multiple testing, a p-value of 0.0025 was considered significant in exploratory analyses.

A multiple linear regression analyses including F_{eNO}, IgE and B-EOS and controlled for age and sex was performed to assess the independent association of individual T2 biomarkers with key clinical characteristics (Asthma Control Questionnaire (ACQ) score, forced expiratory volume in 1 s (FEV_1) and exacerbation rate). Analyses were performed using SAS Studio (SAS Institute, Cary, NC, USA).

Results

A total of 511 out of 569 patients (90%) had a complete biomarker panel available (S-EOS and/or B-EOS, F_{eNO} and IgE): 421 had MMA and 90 had SA.

Patients with MMA were younger (41 years *versus* 49 years, p<0.001), had lower body mass index (BMI) (25.9 $kg \cdot m^{-2}$ *versus* 28.0 $kg \cdot m^{-2}$, p=0.002), less allergic sensitisation (47% *versus* 58%, p=0.05), higher FEV_1 % predicted (95% *versus* 85%, p<0.001) and higher forced vital capacity % predicted (104% *versus* 98%, p=0.01) compared to patients with SA, who had higher levels of blood eosinophils ($0.22 \times 10^9 \cdot L^{-1}$ *versus* $0.17 \times 10^9 \cdot L^{-1}$, p=0.01) and sputum eosinophils (3.0% *versus* 1.5%, p=0.01) as well as total IgE (143 $IU \cdot mL^{-1}$ *versus* 57 $IU \cdot mL^{-1}$, p<0.001) (table 1).

Based on the quality-of-life questionnaires 12-Item Short Form Survey (SF-12) and Mini Asthma Quality of Life Questionnaire (miniAQLQ), patients with MMA reported significantly better physical health (SF-12) and asthma-related quality of life (miniAQLQ) than patients with SA (table 2).

Patients with SA had significantly more exacerbations (during the past year) than patients with MMA (p<0.001). Uncontrolled asthma, defined by either an ACQ5 score >1.5 or an Asthma Control Test (ACT) score ⩽19, was significantly more prevalent in SA (ACQ5: 76% *versus* 51%, p<0.001; ACT: 73% *versus* 56%, p=0.003) (table 2).

No differences in symptom burden, exacerbations or quality of life were observed in patients with SA based on the presence of blood eosinophilia, whereas patients with sputum eosinophilia had more exacerbations than those without, although this did not remain significant when correcting for multiple comparisons (p=0.05) (supplementary table S1).

After correction for multiple comparisons, no significant within-group (*i.e.* SA and MMA, respectively) differences in biomarker expression were identified (supplementary table S2).

Overlapping biomarker expression

Figure 1 illustrates the expression of T2 biomarkers and figure 2 presents the number of elevated biomarkers across asthma severity.

Eosinophilia defined by blood eosinophil count alone

A complete biomarker panel was available in 498 out of 542 patients (92%), of which 413 had MMA and 85 had SA.

The most prevalent T2 biomarker in patients with MMA was elevated F_{eNO} (33%), while elevated IgE (30%) and B-EOS (27%) were almost as frequent (figure 1a). In patients with SA, elevated IgE (49%) was more prevalent than elevated F_{eNO} (39%) or B-EOS (38%).

Among patients with elevated T2 biomarkers, elevated expression of all three biomarkers was markedly more pronounced in patients with SA than MMA (18.8% *versus* 6.3%, p=0.00001) (figure 2a), while elevated expression of one or two T2 biomarkers was not (66% *versus* 56%, p=0.12; 21% *versus* 21%, p=0.93, respectively).

TABLE 1 Baseline characteristics and biomarkers in patients with mild-to-moderate *versus* severe asthma

Variable	Mild-to-moderate asthma	Severe asthma	p-value
Patients total (n)	421	90	
Age (years)	41±17	49±14	<0.0001[¶]
Sex (female)	294 (59%)	51 (52%)	0.3[+]
BMI (kg·m^{-2})	25.9±5.3	28.0±5.8	0.002[¶]
Smoking (pack-years)	0 (0–7)	0 (0–8)	0.4[§]
Allergic sensitisation	196 (47%)	52 (58%)	0.05[+]
Medication			
ICS, budesonide equivalents (µg)	753±318	1797±435	NA
OCS for asthma	-	11 (12%)	NA
OCS for asthma (mg)	-	10 (5–15)	NA
Lung function			
FEV_1 (L)	3.2±0.94	2.8±0.81	<0.0001[¶]
FEV_1 % predicted	93±16	85±22	0.0007[¶]
FVC (L)	4.3±1.2	3.9±1.0	0.0004[¶]
FVC % predicted	104±16.2	98±20.6	0.01[¶]
AHR to mannitol[#]	206/365 (56%)	30/52 (58%)	0.9[+]
PD15 to mannitol	244 (72–395)	233 (73–380)	0.8[§]
Biomarkers			
Blood eosinophils (cells×10^9·L^{-1})	0.17 (0.10–0.30)	0.22 (0.12–0.41)	0.01[§]
Blood eosinophils ⩾0.3 cells×10^9·L^{-1}	113 (27%)	32 (36%)	0.06[+]
Blood eosinophils ⩾0.15 cells×10^9·L^{-1}	237 (57%)	56 (62%)	0.2[+]
Sputum eosinophils (%)	1.5 (0.25–4.5)	3.0 (0.75–7.4)	0.01[§]
Sputum eosinophils ⩾3%	110/303 (36%)	40/79 (51%)	0.02[+]
Sputum neutrophils (%)	37.6 (15.3–63.8)	44.4 (23.8–70.8)	0.06[§]
Sputum neutrophils ⩾61%	84/303 (28%)	25/79 (32%)	0.5[+]
IgE total (IU·mL^{-1})	57 (20–193)	143 (48–347)	<0.0001[§]
IgE total ⩾150 IU·mL^{-1}	125 (30%)	43/90 (48%)	0.0009[¶]
IgE total 75–150 IU·mL^{-1}	59/421 (14%)	17/90 (18%)	0.2[¶]
F_{eNO} (ppb)	17 (10–31)	18 (10–39)	0.6[§]
F_{eNO} ⩾25 ppb	139 (33%)	34 (38%)	0.4[+]
F_{eNO} ⩾50 ppb	62 (15%)	16 (18%)	0.5[+]

Data are presented as n (%), mean±SD or median (interquartile range), unless otherwise stated. BMI: body mass index; ICS: inhaled corticosteroids; OCS: oral corticosteroids; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; AHR: airway hyperresponsiveness; PD15: provocative dose causing a 15% drop in FEV_1; IgE: immunoglobulin E; F_{eNO}: exhaled nitric oxide fraction; NA: not applicable. [#]: proportion of performed mannitol challenge tests that were positive (test performed if FEV_1 ⩾70% predicted); [¶]: Welch's ANOVA; [+]: Chi-squared test; [§]: Kruskal–Wallis test.

Table 3 depicts baseline characteristics in patients with MMA by T2 biomarker expression subgroups and shows significant differences in the prevalence of allergic sensitisation (least pronounced in patients with elevated B-EOS expression, p<0.001) between the eight subgroups.

In patients with SA (table 4), we found significant differences in age (Welch's ANOVA, p=0.002) across the eight subgroups after correcting for multiple comparisons, but no major differences related to BMI, smoking, allergic sensitisation or other lung function parameters.

In a multiple regression analysis including F_{eNO}, IgE and B-EOS and controlled for sex and age, we found B-EOS was significantly inversely associated with FEV_1 % predicted (β= −15.8, p=0.0003) and positively associated with ACQ5 score (β=0.82, p=0.005) and the number of exacerbations (β=0.67, p=0.01) in MMA. F_{eNO} was significantly positively associated with ACQ5 score (β=0.004, p=0.04) in MMA and with the number of exacerbations (β=0.01, p=0.02) in SA.

Eosinophilia defined by blood and sputum eosinophil count

A total of 511 out of 542 patients (94%) had a complete biomarker panel when eosinophilia was defined as blood and/or sputum eosinophilia: 421 with MMA and 90 with SA. Among patients with an elevated T2 biomarker, eosinophilia was the most pronounced T2 trait in patients both with SA (75%) and MMA (66%) (figure 1b). Again, elevated expression of all three T2 biomarkers (figure 2b) was more pronounced in patients with SA than in patients with MMA (28.4% *versus* 13.6%, p=0.0007).

TABLE 2 Symptoms, quality of life, comorbidities and medication in patients with mild-to-moderate *versus* severe asthma

Variable	Mild-to-moderate asthma	Severe asthma	p-value
Patients total (n)	421	90	
Symptoms			
ACQ5 score	1.6 (0.8–2.4)	2.2 (1.6–3.0)	<0.0001¶
ACQ5 score >1.5	210/415 (51%)	68/90 (76%)	<0.0001+
ACT score	18 (15–21)	15 (11–20)	<0.0001¶
ACT score ⩽19	228/410 (56%)	63/86 (73%)	0.003+
mMRC score	1.7±0.8	2.0±0.9	0.005§
Exacerbations previous year#			
Prednisolone-requiring	0.4±0.9	1.7±1.8	<0.0001
	0 (0–0)	1 (0–3)	<0.0001¶
ED visits	0.3±0.7	0.6±1.0	0.008
	0 (0–0)	0 (0–1)	0.002¶
Hospitalisations	0.2±0.6	0.5±1.0	0.004§
	0 (0–0)	0 (0–1)	0.0004¶
Quality of life			
SF-12			
PCS	47.7 (39.0–53.8)	41.5 (34.2–50.0)	0.0002¶
MCS	52.3 (44.3–57.8)	49.7 (41.2–57.6)	0.2¶
miniAQLQ overall	5.4±1.1	4.9±1.3	0.001§
Symptoms	5.2±1.3	4.7±1.4	0.003§
Activity	5.7±1.1	5.1±1.5	0.0009§
Emotional	5.4±1.4	4.7±1.7	0.0007§
Environmental	5.4±1.4	5.1±1.6	0.2§
miniRQLQ overall	1.5±1.0	1.5±1.0	0.8§
Activity	1.4±1.3	1.6±1.4	0.3§
Practical	1.6±1.4	1.7±1.4	0.4§
Nose	1.7±1.4	1.6±1.3	0.3§
Eyes	1.0±1.2	0.8±0.9	0.2§
Non-nose and eyes	1.7±1.3	1.8±1.4	0.8§
Comorbidities			
Nijmegen	16.9±9.7	17.9±9.4	0.4§
SNOT22	20.4±13.7	23.0±15.9	0.2§
Epworth sleepiness scale	6.3±4.0	5.8±3.9	0.3§
Medication			
ICS, budesonide equivalents (µg)	753±318	1797±435	NA
OCS for asthma	-	11 (12%)	NA
OCS for asthma (mg)	-	10 (5–15)	NA
LABA (µg)	25.0±34.5	47.7±36.0	<0.0001§

Data are presented as n/N (%), mean±SD or median interquartile range (IQR), unless otherwise stated. ACQ5: Asthma Control Questionnaire; ACT: Asthma Control Test; mMRC: modified Medical Research Council dyspnoea scale; ED: emergency department; SF-12: 12-item Short Form Health Survey; PCS: physical component score; MCS: mental component score; miniAQLQ: Mini Asthma Quality of Life Questionnaire; miniRQLQ: Mini Rhinoconjunctivitis Quality of Life Questionnaire; Nijmegen: hyperventilation; SNOT22: Sino-Nasal Outcome Test; ICS: inhaled corticosteroid; OCS: oral corticosteroid; LABA: long-acting β_2-agonist; NA: not applicable. #: reported both as mean±SD and median (IQR); ¶: Kruskal–Wallis test; +: Chi-squared test; §: Welch's ANOVA.

Elevated expression of more than one T2 biomarker was significantly more prevalent in patients with SA (46% *versus* 32%, p=0.01) and three quarters of patients with SA showed elevated expression of at least one T2 marker compared to two thirds of patients with MMA (74% *versus* 65%, p=0.07) (figure 2b).

Airway versus *systemic eosinophilia*

A paired S-EOS and B-EOS was available in 364 of 511 patients (71%): 73 with SA and 291 with MMA.

A significantly larger proportion of patients with SA had concomitant sputum and blood eosinophilia while complete absence of eosinophilia was significantly more prevalent in patients with MMA.

Figure 3 illustrates the agreement in classification based on S-EOS (⩾3%) and B-EOS ($\geqslant 0.3\times10^9\cdot L^{-1}$). Supplementary table S3 contains the contingency tables while table 4 lists the clinical characteristics of

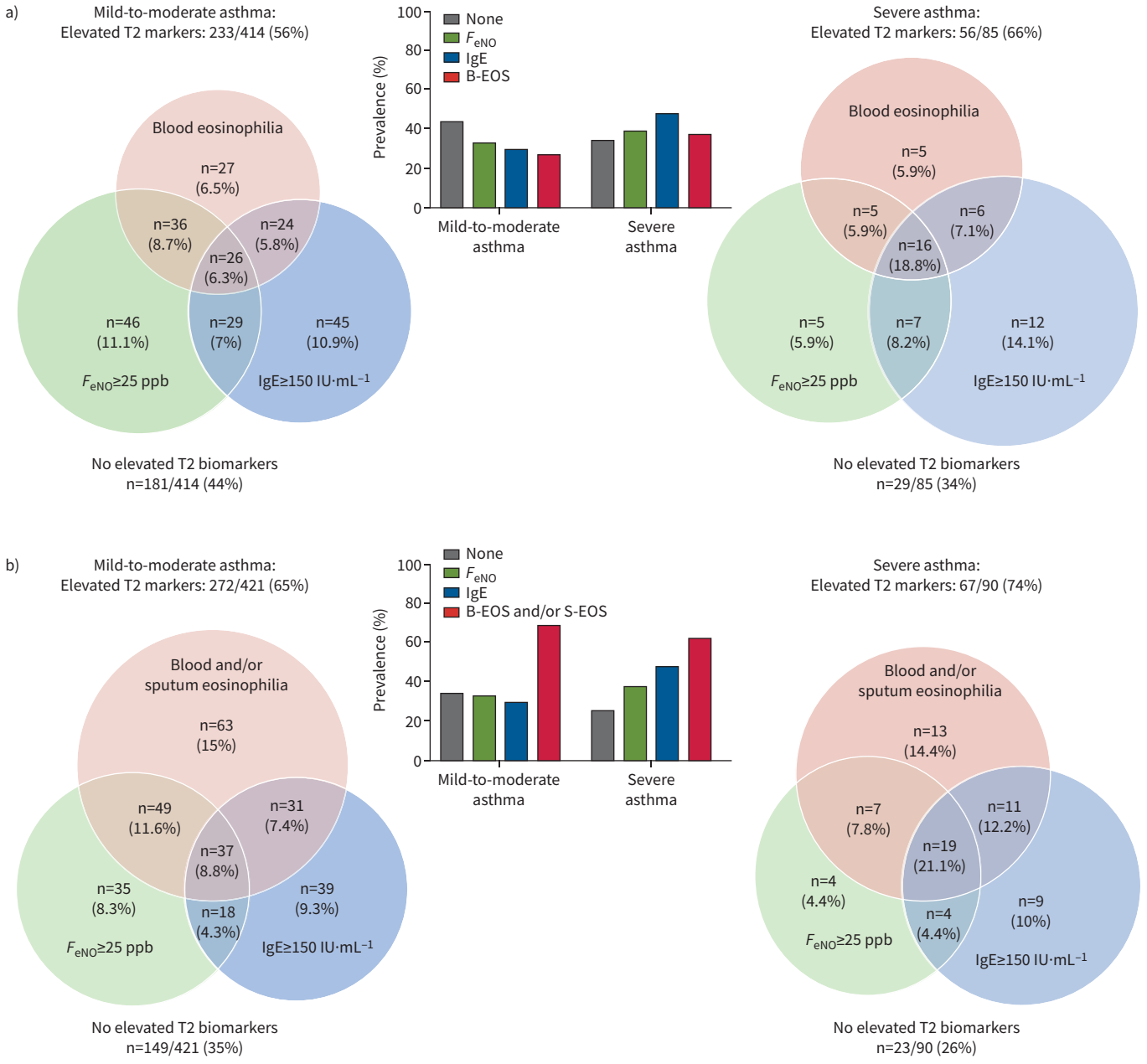

FIGURE 1 Prevalence and co-expression of type 2 (T2) biomarkers in patients with mild-to-moderate *versus* severe asthma. a) Eosinophilia defined as elevated blood eosinophil count (B-EOS). b) Eosinophilia defined as elevated B-EOS and/or sputum eosinophil count (S-EOS). F_{eNO}: exhaled nitric oxide fraction; IgE: immunoglobulin E.

these groups. Discordant eosinophilia was equally prevalent in MMA and SA (33% *versus* 34%) with isolated sputum eosinophilia twice as prevalent as isolated blood eosinophilia (11% *versus* 22% and 12% *versus* 22% for MMA and SA, respectively). The proportion of patients with isolated sputum eosinophilia relative to all with eosinophilia, calculated as $\frac{\text{isolated S}-\text{EOS}}{\text{S}-\text{EOS} \cup \text{B}-\text{EOS}}$, was equal across disease severity (MMA 45% and SA 35%, p=0.1).

In MMA, patients with concomitant eosinophilic inflammation were significantly older (p=0.004) and had a significantly lower absolute and predicted FEV_1 (p=0.004 and p=0.03, respectively) than those without. In patients with SA, those with airway eosinophilia were significantly older than those without, and a significantly larger proportion of the patients with concomitant airway and systemic eosinophil inflammation were men (71%, p=0.05). Airway hyperresponsiveness towards mannitol was significantly

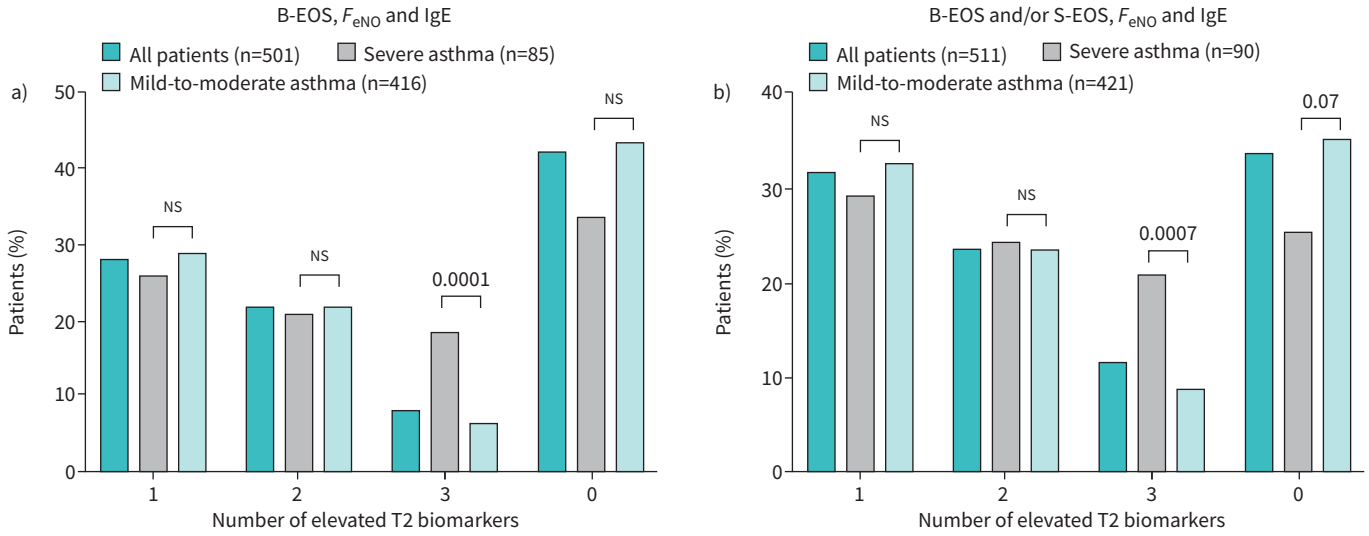

FIGURE 2 Concomitant biomarker elevation in patients with mild-to-moderate *versus* severe asthma with elevated type 2 (T2) biomarkers. a) Eosinophilia defined as elevated blood eosinophil count (B-EOS). b) Eosinophilia defined as elevated B-EOS and/or sputum eosinophil count (S-EOS). F_{eNO}: exhaled nitric oxide fraction; IgE: immunoglobulin E.

different across the groups (p=0.03), with a markedly higher prevalence in the subgroups with airway eosinophilia (±blood eosinophilia).

F_{eNO} was significantly higher in the group with concomitant eosinophilia in patients with both MMA and SA (p<0.0001 and p=0.0002, respectively).

In all patients, we found a significant good-to-excellent agreement for S-EOS with B-EOS (0.97, p<0.001) and with F_{eNO} (0.93, p<0.001) using intraclass correlation (supplementary table S4). Similar levels of relationship were found in patients with SA (B-EOS 0.87, p<0.001; F_{eNO} 0.74, p<0.001) and MMA (B-EOS 0.96, p<0.001; F_{eNO} 0.91, p<0.001). Agreement with total IgE was poor irrespective of severity.

In patients with SA, we found a fair and significant agreement in the presence of airway inflammation (using sputum eosinophilia ⩾3% as reference) using blood eosinophilia (⩾$0.3\times10^9\cdot L^{-1}$; κ 0.34, p=0.003) and elevated F_{eNO} (⩾25 ppb; κ 0.34, p=0.003), whereas agreement in patients with MMA was only modest (κ 0.21, p=0.0002, and κ 0.20, p=0.0005, respectively) (supplementary table S4). Again, agreement using elevated IgE (⩾150 $IU\cdot mL^{-1}$) was nonsignificant (p=0.08 and p=0.25, respectively).

Discussion

In this real-world study of a large population of patients with asthma, co-expression of more than one elevated T2 biomarker was significantly more prevalent in patients with SA than with MMA and co-expression of all three types of T2 biomarkers, *i.e.* eosinophils, F_{eNO} and IgE, was a particular characteristic of SA. These findings support our hypothesis that SA is more commonly associated with activation of several T2 pathways, indicating that treatments targeting SA may need to act more broadly on T2 inflammatory pathways.

The present study is the first to report on the prevalence of co-expression of the conventionally available T2 biomarkers across asthma severity in a broad population. The results offer a real-world estimate of the prevalence of elevated biomarkers and their co-expression across MMA to SA.

Currently, the relative importance of overlapping activity of the T2 inflammatory pathways is largely unclear. Previous studies on co-expression of T2 biomarkers have shown that co-expression is associated with poorer outcomes in asthma [31–36], with concomitant elevation of B-EOS and F_{eNO} associated with increased exacerbation risk in MMA and SA, and a higher prevalence of impaired lung function [32–35]. Similarly, concomitantly elevated B-EOS and IgE has been associated with increased exacerbation risk in moderate-to-severe asthma [36].

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TABLE 3 Clinical characteristics in subgroups by type 2 biomarker expression (B-EOS, F_{eNO} and IgE), mild-to-moderate asthma

	None	B-EOS	F_{eNO}	IgE	B-EOS+F_{eNO}	B-EOS+IgE	F_{eNO}+IgE	B-EOS+IgE+F_{eNO}	p-value
Patients (n)	181	27	46	44	36	24	29	26	
Age (years)	41±17	45±18	41±17	37±16	50±16	42±16	35±16	40±21	0.02[¶]
Sex (female)	120±66	18±67	18±39	24±55	21±58	13±54	15±52	15±58	0.06[+]
BMI (kg·m^{-2})	26.0±5.6	25.8±5.0	26.3±5.1	24.7±4.2	26.2±5.1	28.1±7.5	24.6±4.8	25.4±4.2	0.3[¶]
Smoking (pack-years)	0 (0–8)	1.5 (0–25.8)	0 (0–3.5)	0 (0–10)	0 (0–8)	0 (0–10)	0 (0–0.75)	0 (0–0)	0.03[§]
Allergic sensitisation	44±24	7±26	23±50	32±73	17±47	22±92	23±79	24±92	<0.001[+]
Lung function									
FEV_1 (L)	3.2±0.9	3.3±0.9	3.7±1.1	3.3±0.8	2.9±1.0	2.9±0.8	3.6±0.7	3.1±1.2	0.001[¶]
FEV_1 % predicted	94.4±16.4	91.7±15.5	99.8±14.6	91.2±14.8	89.9±19.4	86.8±14.1	95.3±14.6	88.4±20.3	0.01[¶]
FVC (L)	4.2±1.1	4.1±1.1	4.8±1.4	4.5±1.2	4.0±1.4	4.0±1.0	4.9±1.1	4.1±1.5	0.001[¶]
FVC % predicted	104.2±15.8	104.5±18.6	108.2±14.0	103.5±13.4	104.2±20.0	100.4±15.2	109.5±14.3	98.1±19.7	0.1[¶]
AHR to mannitol[#]	81/156 (52%)	12/24 (50%)	23/44 (52%)	18/38 (47%)	21/27 (78%)	13/22 (59%)	20/27 (74%)	14/19 (74%)	0.05[+]
PD15 to mannitol	268 (106–471)	265 (56–452)	290 (123–415)	233 (83–308)	243 (80–371)	153 (46–226)	212 (45–371)	117 (18–204)	0.08[§]
Biomarkers									
Blood eosinophils (cells×10^9·L^{-1})	0.1 (0.08–0.17)	0.36 (0.31–0.40)	0.17 (0.11–0.20)	0.15 (0.09–0.19)	0.44 (0.36–0.62)	0.39 (0.32–0.46)	0.17 (0.11–0.24)	0.52 (0.39–0.80)	<0.001[§]
Sputum eosinophils (%)	0.75 (0.25–3.2)	2.25 (0.38–3.6)	1.75 (0.29–6.0)	1.0 (0.25–2.0)	20.1 (2.4–50.3)	3.25 (1.0–8.0)	2.5 (1.0–8.7)	6.25 (0.75–17)	<0.001[§]
Sputum neutrophils (%)	39 (14.3–64.1)	32.5 (17.2–58.1)	33.3 (16.4–62.1)	34.7 (19.5–73.8)	26.7 (9.7–51.8)	42.3 (20.0–65.5)	21.9 (10.8–61.8)	47 (30.8–59.5)	0.8[§]
IgE total (IU·mL^{-1})	23 (10–50)	25 (11–52)	48 (31–100)	279 (222–494)	59 (31–96)	348 (216–662)	419 (215–627)	309 (262–490)	<0.001[§]
F_{eNO} (ppb)	11 (8–16)	16 (10–19)	38 (29–528)	13 (7–17)	49 (39–75)	15 (11–22)	40 (30–66)	60 (37–101)	<0.001[§]

Data are presented as n/N (%), mean±SD or median (interquartile range), unless otherwise stated. B-EOS: peripheral blood eosinophil count; F_{eNO}: exhaled nitric oxide fraction; IgE: immunoglobulin E; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; AHR: airway hyperresponsiveness; PD15: provocative dose causing a 15% drop in FEV_1. [#]: proportion of performed mannitol challenge tests that were positive (test performed if FEV_1 ⩾70% predicted); [¶]: Welch's ANOVA; [+]: Chi-squared test; [§]: Kruskal–Wallis test.

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TABLE 4 Clinical characteristics in subgroups by type 2 biomarker expression (B-EOS, F_{eNO} and IgE), severe asthma

	None	B-EOS	F_{eNO}	IgE	B-EOS+F_{eNO}	B-EOS+IgE	F_{eNO}+IgE	B-EOS+IgE+F_{eNO}	p-value
Patients (n)	29	5	5	12	5	6	7	16	
Age (years)	42±12	49±16	54±18	49±18	58±11	45±10	51±13	57±9	0.02[¶]
Sex (female)	22±76	3±60	3±60	7±58	2±40	1±17	3±43	4±25	0.03[+]
BMI (kg·m^{-2})	28.3±5.7	25.1±1.2	28.8±7.6	28.1±5.9	31.1±4.6	27.6±3.1	25.3±5.5	27.2±5.2	0.6[¶]
Smoking (pack-years)	0.5 (0–5)	0 (0–30)	4 (0.5–9.5)	8 (0–14.3)	12 (5–16)	0 (0–9.5)	0 (0–0)	1.5 (0–12)	0.1[§]
Allergic sensitisation	13±44	2±40	2±40	10±83	1±20	4±67	5±71	11±69	0.1[+]
Lung function									
FEV_1 (L)	2.9±0.9	2.8±0.8	2.8±0.6	2.6±0.7	2.5±0.9	3.0±1.0	2.8±0.7	2.4±0.7	0.6[¶]
FEV_1 % predicted	95.3±21.7	81.2±14.9	83.2±7.3	84.0±16.4	77.0±26.2	77.3±21.9	86.3±18.4	71.7±22.8	0.04[¶]
FVC (L)	3.8±0.9	3.9±1.1	3.8±0.8	3.6±1.0	3.4±0.7	4.3±1.2	4.1±0.7	3.9±1.1	0.8[¶]
FVC % predicted	106.2±19.6	94.6±17.6	93.4±9.9	96.5±20.1	88.0±19.0	90.3±14.0	104.1±19.6	91.1±25.7	0.2[¶]
AHR to mannitol[#]	9/17 (53%)	2/2 (100%)	3/4 (75%)	3/7 (43%)	1/3 (33%)	1/3 (33%)	4/5 (80%)	4/7 (57%)	0.6[+]
PD15 to mannitol	222 (75–418)	107 (32–181)	485 (439–531)	196 (114–293)	343	356	83 (21–145)	314 (155–473)	0.2[§]
Biomarkers									
Blood eosinophils (cells×10^9·L^{-1})	0.14 (0.1–0.21)	0.45 (0.38–0.53)	1.0 (0.1–0.26)	0.13 (0.1–0.16)	0.47 (0.42–1.0)	0.39 (0.34–0.56)	0.16 (0.02–0.23)	0.58 (0.42–0.69)	<0.001[§]
Sputum eosinophils (%)	1.1 (0.3–5.3)	3.3 (0.88–69.2)	3.0 (1.6–34.2)	1.5 (0–7.3)	12.2 (3.6–17.3)	2.6 (1.0–4.4)	4 (0.44–12.9)	14.3 (4.8–38.5)	0.01[§]
Sputum neutrophils (%)	64 (27.0–81.3)	38 (7.1–82.6)	62.8 (26.6–83.1)	33 (14.6–47.5)	51.8 (31.9–62)	55.4 (40.8–85.4)	38 (20.6–83.6)	35 (21.8–59.5)	0.4[§]
IgE total (IU·mL^{-1})	40 (18.5–88.5)	92 (44.5–138.5)	65 (40–122.5)	310 (180–905)	54 (23–71)	389 (258–1873)	653 (267–829)	320 (197–651)	<0.001[§]
F_{eNO} (ppb)	10 (8.0–18.5)	7 (4–12)	28 (27–59.5)	11 (9–16.8)	44 (39.5–56.5)	13.5 (10–20.3)	37 (26–55)	66.5 (39.5–91)	<0.001[§]

Data are presented as n/N (%), mean±SD or median (interquartile range), unless otherwise stated. B-EOS: peripheral blood eosinophil count; F_{eNO}: exhaled nitric oxide fraction; IgE: immunoglobulin E; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; AHR: airway hyperresponsiveness; PD15: provocative dose causing a 15% drop in FEV_1. [#]: proportion of performed mannitol challenge tests that were positive (test performed if FEV_1 ⩾70% predicted); [¶]: Welch's ANOVA; [+]: Fisher's exact test; [§]: Kruskal–Wallis.

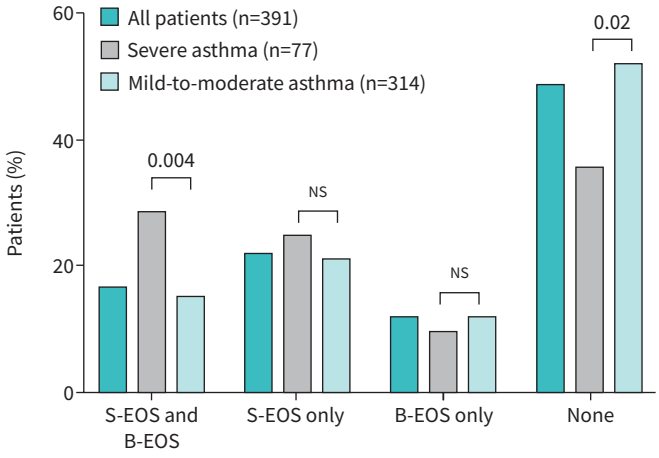

FIGURE 3 Concordance in identification of eosinophilia using blood eosinophil count (B-EOS) and sputum eosinophil count (S-EOS) in patients with mild-to-moderate *versus* severe asthma.

So far, reports on the prevalence of patients without T2 inflammation have been varied [37, 38]. We found no evidence of a predominant neutrophilic subgroup in either MMA or SA, but, given the cross-sectional nature of our study, we were unable to assess whether the difference in prevalence of T2 inflammation across asthma severity was due to the higher levels of maintenance inhaled corticosteroids (ICS) [39], which has been suggested to promote neutrophilic inflammation, or perhaps due to a higher prevalence of the late-onset obese non-eosinophilic phenotype in SA (significant differences in age, BMI and lung function across severity).

We recognise that the cross-sectional design of this study is a potential limitation in the comparison of T2 biomarker expression across asthma severity because severity is defined by dosage of ICS, and all biomarkers (except IgE) are considered responsive to ICS. Further, given our study design, we were unable to address the impact of the intra-individual variability in B-EOS reported by Corren *et al*. [40].

Mannitol, rather than methacholine, was used for bronchial provocation testing, which we speculate may have put us at risk of under-diagnosing asthma because mannitol has a higher specificity but lower sensitivity compared to methacholine, especially when patients are already treated with ICS [41].

In line with others [3, 4, 11, 42, 43], we have in this study reported a large incomplete overlap of patients identified using T2 biomarkers including a marked discrepancy between airway and systemic eosinophilia. Rather than poor diagnostic accuracy [44], we believe this is reflective of marked heterogeneity within the T2-high population, supporting the notion that the assessed T2 biomarkers reflect the activation of the distinct immune pathways that predominantly drive their induction, and expression of these T2 biomarkers may therefore inform us about the types of T2 inflammatory mechanisms involved.

Co-activation of IL-5 and IL-4/IL-13 pathways (measured by B-EOS and F_{eNO} and/or IgE), which has been associated with increased exacerbation risk in both MMA and SA [33, 36], was more prevalent in patients with SA, who were also found to have significantly higher exacerbation rates, suggesting that more than one signalling pathway is concomitantly activated in SA and may be a hallmark of the exacerbation-prone phenotype.

The currently approved targeted treatments (IL-5, IL-5R, IL-4Ra) all target T2 inflammation downstream. While they have all provided a significant reduction (50–60%) in severe exacerbations and a small improvement in airflow obstruction (FEV_1), a large proportion of patients are still left with a significant disease burden that, in some, has led to treatment with more than one biological [28].

The airway epithelium, and in particular the upstream alarmins TSLP and IL-33, are increasingly recognised as key players in initiating and driving T2 inflammation in asthma [45]. Anti-alarmin treatment provides a more broad anti-inflammatory effect, with phase 2 and 3 studies of tezepelumab showing marked reductions in exacerbations independent of inflammatory phenotype but with increasing efficacy in

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TABLE 5 Baseline characteristics in subgroups based on presence of elevated S-EOS and B-EOS

Variable	Severe asthma					Mild-to-moderate asthma				
	None	S-EOS and B-EOS[#]	S-EOS[#]	B-EOS[#]	p-value	None	S-EOS and B-EOS[#]	S-EOS[#]	B-EOS[#]	p-value
Patients total (n)	28	21	16	8		149	42	64	36	
Age (years)	44±13	54±11	54±14	48±13	0.04[+]	38±15	49±17	41±17	40±17	0.004[+]
Sex (female)	18 (64%)	6 (29%)	10 (63%)	3 (38%)	0.05[§]	87 (58%)	26 (62%)	36 (56%)	20 (56%)	0.9[f]
BMI (kg·m^{-2})	28±6	28±5	28±6	27±4	0.9[+]	25±5	27±5	26±5	26±6	0.3[+]
Smoking (pack-years)	0 (0–8)	5 (0–12)	4 (0–7)	0 (0–4)	0.4[##]	0 (0–4)	0 (0–10)	0 (0–5)	0 (0–9)	0.4[##]
Allergic sensitisation	17 (61%)	12 (57%)	7 (44%)	4 (50%)	0.8[§]	63 (42%)	22 (52%)	27 (42%)	24 (67%)	0.05[f]
Lung function										
FEV_1 (L)	2.9±0.9	2.5±0.8	2.6±0.6	3.0±0.9	0.3[+]	3.4±0.9	2.8±1.0	3.3±1.0	3.3±0.8	0.004[+]
FEV_1 % predicted	90±20	72±23	90±22	85±18	0.06[+]	97±14	88±18	94±18	93±13	0.03[+]
FVC (L)	3.8±1.1	3.8±1.0	3.6±0.5	4.2±1.1	0.5[+]	4.5±1.2	3.8±1.3	4.5±1.2	4.5±1.1	0.01[+]
FVC % predicted	100±18	89±24	104±24	97±15	0.3[+]	107±15	101±19	107±15	107±14	0.3[+]
AHR to mannitol[¶]	7/18 (39%)	6/8 (75%)	6/7 (86%)	1/6 (17%)	0.03[§]	73/139 (52%)	18/31 (58%)	36/54 (67%)	24/36 (67%)	0.2[f]
PD15 to mannitol	165 (23–205)	282 (89–343)	252 (75–418)	181	0.7[##]	281 (104–472)	208 (41–372)	272 (140–400)	252 (73–393)	0.4[##]
Biomarkers										
B-EOS (cells×10^9·L^{-1})	0.14 (0.10–0.20)	0.47 (0.40–0.60)	0.14 (0.10–0.24)	0.41 (0.35–0.69)	NA	0.12 (0.08–0.17)	0.47 (0.34–0.64)	0.16 (0.11–0.22)	0.38 (0.33–0.45)	NA
S-EOS (%)	0.63 (0.25–1.25)	12.2 (5.3–33.0)	7.3 (6.3–11.3)	0.93 (0.5–1.4)	NA	0.5 (0.0–1.3)	13.6 (6.3–35.3)	5.5 (4.0–11.4)	1.0 (0.3–2.3)	NA
Sputum neutrophils (%)	41.1 (13.9–81.1)	49.0 (21.8–60.0)	55.3 (42.5–71.5)	49.2 (36.1–58.8)	0.6[##]	29.5 (11.0–66.0)	36.1 (19.0–47.5)	43.1 (23.4–65.5)	37.7 (11.6–71.5)	0.2[##]
Sputum neutrophils ⩾61%	10/28 (36%)	5/21 (24%)	7/16 (44%)	2/8 (25%)	0.6[§]	42/149 (28%)	5/42 (12%)	22/64 (34%)	11/36 (31%)	0.06[§]
IgE total (IU·mL^{-1})	91 (40–175)	252 (146–440)	130 (27–606)	256 (69–324)	0.05[##]	48 (18–148)	109 (34–270)	41 (20–177)	128 (39–318)	0.007[##]
IgE total ⩾150 IU·mL^{-1}	9/28 (32%)	15/21 (71%)	7/16 (44%)	5/8 (63%)	0.04[§]	36/149 (24%)	18/42 (43%)	18/64 (28%)	15/36 (42%)	0.04[f]
F_{eNO} (ppb)	11 (9–20)	46 (26–68)	17 (11–26)	17 (7–39)	0.0002[##]	14 (9–24)	38 (17–71)	17 (10–40)	20 (13–37)	<0.0001[##]
F_{eNO} ⩾25 ppb	5/28 (18%)	16/21 (76%)	5/16 (31%)	3/8 (38%)	0.0004[§]	34/149 (23%)	25/42 (60%)	23/64 (36%)	13/36 (36%)	<0.0001[f]
F_{eNO} ⩾50 ppb	0/28 (0%)	10/21 (48%)	2/14 (14%)	3/8 (13%)	<0.0001[§]	11/149 (7%)	17/40 (39%)	14/64 (22%)	7/36 (19%)	<0.0001[f]

Data are presented as n/N (%), mean±SD or median (interquartile range), unless otherwise stated. S-EOS: sputum eosinophil count; B-EOS: peripheral blood eosinophil count; BMI: body mass index; FEV_1: forced expiratory volume in 1 s; FVC: forced vital capacity; AHR: airway hyperresponsiveness; PD15: provocative dose causing a 15% drop in FEV_1; IgE: immunoglobulin E; F_{eNO}: exhaled nitric oxide fraction; NA: not applicable. [#]: B-EOS ⩾0.3 cells×10^9·L^{-1} and S-EOS ⩾3%; [¶]: proportion of performed mannitol challenge tests that were positive (test performed if FEV_1 ⩾70% predicted); [+]: Welch's ANOVA; [§]: Fisher's exact test; [f]: Chi-squared test; [##]: Kruskal–Wallis test.

patients with concomitant elevated biomarkers [46, 47], a patient group that we found highly prevalent in this generalisable real-world population of asthma patients.

F_{eNO} is produced at the bronchial epithelium and was for both MMA and SA significantly higher in the group with concomitant elevation of all T2 biomarkers relative to the group with isolated elevation of F_{eNO}. In addition, F_{eNO} was markedly more elevated relative to B-EOS and S-EOS in patients with concomitant blood and sputum eosinophilia than in those with isolated increases, in both MMA and SA (table 5).

These findings point to a synergistic effect of concomitant pathway activity in line with previous reports [3, 43, 48], and we speculate that the consistent and marked elevation of F_{eNO} in patients with both concomitant pathway activity and global eosinophilia suggests a predominantly epithelial-driven disease [12–14]. Whether this reflects more active and treatment-responsive disease as alluded to by Shrimanker *et al.* [43], or a necessity for more upstream targeting as suggested by Porsbjerg *et al.* [45], remains to be uncovered.

At the same time, one quarter of patients (26%) expressed biomarkers indicative of single-pathway activity and, while single-pathway blocking is the most apparent treatment choice in these patients, studies are needed to understand whether these patients should be managed according to T2 biomarker status or if they would also benefit from the more broad anti-inflammatory effect of anti-alarmin treatment.

Eosinophil inflammation is the key treatable trait in asthma and a key criterion for the initiation of biological treatment, but routine airway sampling using induced sputum remains restricted to highly specialised centres despite reports of a marked discrepancy between airway and systemic eosinophilia [3, 17, 28].

Our findings highlight the importance of a continued push towards clinically feasible airway inflammometry, *e.g.* using molecular inflammometry [49], because sputum eosinophilia without blood eosinophilia was prevalent in 22% of patients with both MMA and SA. This translates to eosinophilia being missed in half of patients (45%) with MMA and one third of patients (35%) with SA, which means that these patients ultimately will miss out on phenotype-guided treatment, including the opportunity to receive currently available biological treatments, without airway sampling [28]. A pragmatic solution could be implementation of algorithms using conventional biomarkers [50, 51]; however, this approach does not address the potential for spatial heterogeneity.

In conclusion, we have in this generalisable real-world population of patients found evidence of T2 inflammation in two thirds of patients with SA and approximately half with MMA and identified co-expression of T2 biomarkers, and in particular co-expression of all T2 biomarkers, as a particular characteristic of SA. Our findings highlight the paramount importance of clinically feasible airway inflammometry because the best treatable trait, eosinophilia, is otherwise overlooked in a large proportion of patients, irrespective of disease severity

Collectively, we believe our findings emphasise the complexity of the underlying mechanisms responsible for airway inflammation in asthma, and in particular SA, underlining not only the need for a composite approach to inflammometry but also the relevance of treatments targeting further upstream in the T2 inflammatory pathway.

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