ERJ open research
THE BEST IN OPEN ACCESS BASIC, TRANSLATIONAL & CLINICAL RESPIRATORY RESEARCH

Early View

Original research article

Distribution of Type 2 biomarkers and association with severity, clinical characteristics and co-morbidities in the BREATHE real-life asthma population

Laurits Frøssing, Ditte K Klein, Morten Hvidtfeldt, Nicolai Obling, Gunilla Telg, Jonas S Erjefält, Uffe Bodtger, Celeste Porsbjerg

Please cite this article as: Frøssing L, Klein DK, Hvidtfeldt M, *et al*. Distribution of Type 2 biomarkers and association with severity, clinical characteristics and co-morbidities in the BREATHE real-life asthma population. *ERJ Open Res* 2022; in press (https://doi.org/10.1183/23120541.00483-2022).

Title page:

Title:

Distribution of Type 2 biomarkers and association with severity, clinical characteristics and co-morbidities in the BREATHE real-life asthma population

Authors:

Laurits Frøssing* [1], Ditte K Klein* [1], Morten Hvidtfeldt[1], Nicolai Obling[2], Gunilla Telg[3],

Jonas S Erjefält[4], Uffe Bodtger[2,5], Celeste Porsbjerg[1].

1: Respiratory Research Unit, Department of Respiratory Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg. Denmark.
2: Respiratory Research Unit PLUZ, Department of Respiratory Medicine; Zealand University Hospital Naestved. Denmark.
3: Astrazeneca Nordic, Södertälje, Sweden
4: Unit of Airway Inflammation, Lund University. Sweden.
5: Institute of Regional Health Research, University of Southern Denmark. Denmark

*: Contributed equally

Corresponding author: Laurits Frøssing, froessing@dadlnet.dk.

Funding: The BREATHE study was supported by the Interreg ÖKS [NYPS20201002]; AstraZeneca [unrestricted grant] and TEVA [unrestricted grant].

Conflicts of interest: LF, DKK, NO, JE and UB report no conflicts of interest in relation to the current manuscript. MH and CP report unrestricted grants from TEVA and Astrazeneca. GT is employed by Astrazeneca Nordic.

Wordcount:

Manuscript: 3000 (3000)

Abstract: 250 (250)

Tables and figure: 8 (5 and 3 respectively).

Online supplement: 3 tables.

Introduction

Asthma is increasingly recognized as a heterogenous disease consisting of several endotypes (1); overall T2 inflammation, driven by Th2 and ILC2 cells and mediated through interleukin(IL)-5 and IL4/IL13 signaling pathways, is perceived as the main pathogenetic mechanism in the majority of asthma cases.

The introduction of treatments targeting the key T2 cytokines has provided important insights into their relation to the 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, the IL-5 receptor (IL-5r) and thymic stromal lymphopoietin (TSLP) (5–9), whereas treatments targeting IgE (10) and the IL-4 receptor-α (IL-4Ra) (11) does not. FeNO is induced by IL-13 at the bronchial epithelium, reflect airway IL-13 activity (12–14) and is reduced by treatments targeting IgE, IL-4/13Ra 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 utilized as a surrogate marker of eosinophilic airway inflammation(15) albeit recent evidence has highlighted 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 the T2 biomarkers in the general asthma population and across asthma severity is 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 hypothesized that single-pathway activation was a sign of more benign disease and consequently that co-activation of inflammatory pathways as well as global eosinophilic inflammation across compartments was more prevalent in patients with severe disease.

Methods

Design

BREATHE was a multicentre, cross-sectional study recruiting patients with asthma and/or COPD 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). See previous publication for elaboration (18).

Study population

Patients with an asthma diagnosis recruited at a specialist care unit was included in this study; as patients from primary care (n=290) did not have sputum collected nor IgE measured(18).
Patients without a complete biomarker panel; i.e. measurement of FeNO; IgE; and an evaluation of eosinophilia (blood- and/or sputum eosinophil count); 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 ERS/ATS criteria for possible SA (19).

Assessments

Sputum was collected following mannitol provocation test, or induction with 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 IgEs 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 sensitization was defined as elevated (>0.35 kU/L) specific IgE for a minimum 1 of the 10 tested aeroallergens.

Statistical analyses

Binary cut-offs for elevated biomarker expression was utilised: blood eosinophilia was defined as B-EOS ≥0.3 $*10^9$/L; elevated FeNO: FeNO ≥25 ppb; elevated IgE: total IgE ≥150 U/mL and sputum eosinophilia: sputum eosinophils (S-EOS) ≥3% (7,11,23–28).
A conservative cut-off for IgE was utilised in an attempt to ensure well defined groups as median IgE levels in previous severe asthma cohorts have been markedly above the normal range (109-126 U/mL) (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-square- or fishers exact test when needed. To correct for multiple testing a P value of .0025 was considered significant in exploratory analyses.

A multiple linear regression analyses including FeNO, IgE and B-EOS and controlled for age and sex was performed to assess independent association of the individual T2 biomarkers with key clinical characteristics

(ACQ-score, FEV1 and exacerbation rate). Analyses were performed using SAS Studio (SAS Institute, Cary, NC, USA).

Results

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

Patients with MMA were younger (41 vs 49 years, p<0.001), had lower BMI (25.9 vs 28.0, p=0.002) and less allergic sensitization (47% vs 58%, p=0.05), higher FEV1 percent predicted (95% vs 85%, p<0.001) and FVC percent predicted (104%L vs 98%, p=0.01) compared to patients with SA, who had higher levels of blood- (0.22 vs 0.17 $*10^9$/L, p=0.01) and sputum (3.0% vs 1.5%, p=0.01) eosinophils as well as total IgE (143 vs 57 IU/ml, p<0.001) (Table 1).

Based on the quality-of-life(QoL) questionnaires SF12 and miniAQLQ, patients with MMA reported a significantly better physical health (SF12) and asthma related quality of life (miniAQLQ) compared to patients with SA (Table 2).

Patients with SA had significantly more exacerbations (during the past year) compared to patients with MMA (p<0.001); and uncontrolled asthma defined by either ACQ5 > 1.5 or ACT ≤ 19, was significantly more prevalent in SA (76% vs 51%, p<0.001 and 73% vs 56%, p=0.003 for ACQ5 and ACT respectively (Table 2).

No differences in symptom burden, exacerbations or QoL was observed in patients with severe asthma based on the presence of blood eosinophilia, whereas patients with sputum eosinophilia had more exacerbations than those without albeit this did not remain significant when correcting for multiple comparisons (p=0.05) (Table S1 in the online supplement).

After correction for multiple comparisons, no significant within-group (ie. severe- and mild-moderate asthma respectively) differences in biomarker expression were identified (Table S2 in the online supplement).

Overlapping biomarker expression

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

<u>Eosinophilia defined by blood eosinophil count alone</u>

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

Elevated FeNO was the most prevalent T2 biomarker (33%) in patients with MMA, while elevated IgE (30%) and B-EOS (27%) were almost as frequent (Figure 1A). In patients with SA, elevated IgE (49%) was more prevalent T2 biomarker than FeNO (39%) and B-EOS (38%).

Among patients with an elevated T2 biomarker, expression of all three biomarkers was markedly more pronounced in patients with SA (18.8% vs. 6.3%, p=0.00001) (Figure 2A), while expression of one or two T2 biomarker was not (66% vs 56%, p= 0.12 and 21% vs 21%, p=0.93, respectively)

Table 3 depicts baseline characteristics in patients with MMA by T2 biomarker expression sub-groups and shows significant differences in the prevalence of allergic sensitization (least pronounced in patients with elevated B-EOS expression, p<0.001) between the 8 sub-groups.

In patients with SA (Table 4), we found significant differences in age (Welch's ANOVA, p=0.002) across the 8 sub-groups after correcting for multiple comparisons, but no major differences related to BMI, smoking, allergic sensitization or other lung function parameters.

In a multiple regression analysis including FeNO, IgE and B-EOS and controlled for sex and age we found B-EOS significantly inversely associated with FEV1-percent 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. FeNO was significantly positively associated with ACQ-5 score (β=0.004, p=0.04) in MMA; and to the number of exacerbations (β=0.01, p=0.02) in SA.

Eosinophilia defined by blood- and sputum eosinophil count

A total of 511 (94%, 511/542) patients had a complete biomarker panel when eosinophilia was defined as blood- and/or sputum eosinophil eosinophilia**:** 421 with MMA and 90 with SA. Among patients with an elevated T2 biomarker, eosinophilia was now the most pronounced T2 trait in both patients with SA (75%) and MMA (66%) (Figure 1B). Again, co-expression of all T2 biomarkers (Figure 2B) was more pronounced in patients with SA compared to patients with MMA (28.4% vs. 13.6%, p= 0.0007).

Expression of more than one T2 biomarker was significantly more prevalent in patients with SA (46% vs. 32%, p= 0.01) and three-fourths of patients with SA showed expression of at least one T2 marker compared to two-thirds of patients with MMA (74% vs. 65%, p=0.07) (Figure 2B).

Airway versus systemic eosinophilia

Paired sputum- and blood eosinophil count was available in 364 (364/511, 71%) patients: 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 sputum- (≥3%) and blood eosinophil count (≥0.3*10^9/L), Table S3 in the online supplement contains the contingency tables while Table 4 lists the clinical characteristics of these groups. Discordant eosinophilia (33% vs 34%) was equally prevalent in MMA and SA with isolated sputum eosinophilia twice as prevalent as isolated blood eosinophilia (11% vs 22% and 12% vs 22% for MMA and SA respectively). The proportion with isolated sputum eosinophilia relative to all with eosinophilia $\frac{isolated\ S{-}EOS}{\mathrm{S{-}EOS} \cup \mathrm{B{-}EOS}}$) was equal across 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 FEV1 (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). AHR towards mannitol was significantly different across the groups (p=0.03), with a markedly higher prevalence in the subgroups with airway eosinophilia (‡blood eosinophilia).

FeNO was significantly higher in the group with concomitant eosinophilia in both patients with MMA and SA (p<0.0001 and p=0.0002 respectively).

In all patients, we found a significant good-to-excellent agreement between S-EOS and B-EOS (0.97, p>0.001), and FeNO (0.93, p>0.001) using intraclass correlation (Table S4 in the online supplement). Similar levels of relationship were found in patients with SA (B-EOS 0.87, <0.001; FeNO 0.74, p<0.001) and MMA (B-EOS 0.96, <0.001; FeNO 0.91, p<0.001) respectively. 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 *10^9/L; kappa 0.34, p=0.003) and elevated FENO (≥25 ppb; kappa 0.34, p=0.003) whereas agreement in patients with MMA was only modest (kappa 0.21, p=0.0002 and kappa 0.20, p=0.0005 respectively) (Table S4 in the online supplement). Again, agreement using elevated IgE was not significant (≥150 IE; p=0.08 and 0.25 respectively).

Discussion

In this real-life study of a large population of patients with asthma, co-expression of more than one T2 biomarker was significantly more prevalent in patients with SA compared to MMA and co-expression of all three types of T2 biomarkers – eosinophils, FeNO 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. Hence, the results offer a real-life 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 FeNO associated with increased exacerbation risk in mild-moderate and severe asthma; 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).

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

In continuation, we recognize the cross-sectional design of this study as a potential limitation in the comparison of T2 biomarker expression across asthma severity; as severity is defined by dosage of ICS and all the while all biomarkers (except IgE) are considered responsive to ICS. Further, we are - given our study design - 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 as 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 a marked heterogeneity within the T2 high entity supporting the notion that the assessed T2 biomarkers reflect activation of the distinct immune pathways that predominantly drive their induction and their expression may therefore inform us about the types of T2 inflammatory mechanisms involved.

Co-activation of IL5 and IL4/IL13 pathways (measured by B-EOS and FeNO 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 signaling pathway are 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; and while they have all provided a significant reduction (50-60%) of severe exacerbations and a small improvement in airflow obstruction (FEV1) a large proportion of patients are still left with a significant disease burden which in some has led to treatment with more than one biologic(28).

The airway epithelium, and in particular the upstream alarmins TSLP and IL-33, are increasingly recognized as key players in initiating and driving type 2 inflammation in asthma(45) and anti-alarmin treatment provide 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 patients with concomitant elevated biomarkers (46,47) – a patient group which we found highly prevalent in this this generalizable real-life population of asthma patients.

FeNO is produced at the bronchial epithelium and was for both MMA and SA significantly higher in the group with concomitant elevation of all biomarkers relatively to the group with isolated elevation of FeNO. In addition, FeNO was markedly more elevated relatively to B-EOS and S-EOS in patients with concomitant blood- and sputum eosinophilia compared to the groups with isolated increases in both mild-moderate and severe asthma (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 FeNO in both patients with concomitant pathway activity and global eosinophilia suggest 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 fourth (26%) of patients 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 also benefit more 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 biologic treatment, but routine airway sampling using induced sputum remains restricted to highly specialized 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 (51); as sputum eosinophilia without blood eosinophilia was prevalent in 22% of patients with both MMA and SA . This translates to that eosinophilia is missed in half (45%) of patients with MA and one-third (35%) of patients which means that these patients ultimately will miss out on phenotype-guided treatment - including the opportunity for the currently available biologic treatments - without airway sampling (28). A pragmatic solution could be implementation of algorithms utilizing conventional biomarkers (49,50); however, this approach does not address the potential for spatial heterogeneity.

In conclusion, we have in this generalisable real-life 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 as the best treatable trait – eosinophilia - otherwise is overlooked in a large proportion of patients irrespective of disease severity

Collectively, we believe our findings emphasize 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 type 2 inflammatory pathway.

Acknowledgements

We thank all the BREATHE-study group members of the five recruiting centers for their dedication and effort, and laboratory technicians Öznur Turan, Merve Melike Yilmaz and Lene W Pedersen from the Respiratory Research Unit at Bispebjerg University Hospital for counting all of the sputum samples.
BREATHE study group members: Vibeke Backer[a], Kerstin Romberg[b,c], Leif Bjermer [c], Karsten Kristiansen[d], Ruigi Xu[e], Alexander Silberbrandt[f], Linnea Jarenbäck[c], Abir Nasr[c], Ellen Tufvesson[c], Michiko Mori[g], Lisa Karlsson[g], Ulf Nihlén[c] and Thomas Veje Flintegaard[f]

A: Centre for Physical Activity Research, Rigshospitalet, Copenhagen University, Copenhagen, Denmark; B: Health Care Centre, Näsets Läkargrupp, Höllviken, Sweden; C: Respiratory Medicine and Allergology, Clinical Sciences Lund, Lund University, Lund, Sweden; D: Laboratory of Genomics and Molecular Biomedicine, Department of Biology, University of Copenhagen, Copenhagen, Denmark; E: North Europe Regional Department, BGI-Europe, Copenhagen; F: Respiratory Research Unit, Department of Respiratory Medicine, Bispebjerg University Hospital, Copenhagen, Denmark. G: Unit of Airway Inflammation, Lund University, Lund, Sweden.

Table 1: Baseline characteristics and biomarkers in patients with mild-moderate vs severe asthma

Variable	Mild-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 (packyears)	0 (0-7)	0 (0-8)	0.4¥
Allergic sensitization	196 (47%)	52 (58%)	0.05¤
Medication			
ICS, budesonide eqivalents, µg	753 (±318)	1797 (±435)	na
OCS for asthma, n/N (%)	-	11 (12%)	na
OCS for asthma, mg	-	10 (5-15)	na
Lung function			
FEV1 (L)	3.2 (±0.94)	2.8 (±0.81)	<0.0001‡
FEV1 % 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)	0.17 (0.10-0.30)	0.22 (0.12-0.41)	0.01¥
Blood eosinophils ≥0.3 cells*10^9/L	113 (27%)	32 (36%)	0.06¤
Blood eosinophils ≥0.15 cells*10^9/L	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)	57 (20-193)	143 (48-347)	<0.0001¥
IgE total ≥150IU/ml	125 (30%)	43/90 (48%)	0.0009¤
IgE total 75-150 IU/ml	59/421 (14%)	17/90 (18%)	0.2¤
FeNO (ppb)	17 (10-31)	18 (10-39)	0.6¥
FeNO ≥25ppb	139 (33%)	34 (38%)	0.4¤
FeNO ≥50ppb	62 (15%)	16 (18%)	0.5¤

Data are presented as numbers (n), n/N (%), mean±SD, or median (IQR). BMI: body mass index; ICS: inhaled corticosteroids; OCS: oral corticosteroids; FEV1: forced expiratory volume in 1 sec; FVC: forced vital capacity.
#: Proportion of performed mannitol challenge test that were positive (test performed if FEV1 ≥70% predicted).
Statistical tests: ¤: chi-square §: fishers exact test, ‡: Welch's ANOVA, ¥: Kruskal Wallis, na.: not applicable.

Table 2: Symptoms, quality-of-life, comorbidities and medication in patients with mild-moderate vs severe asthma

Variable	Mild-moderate asthma	Severe asthma	p-value
Patients total, n	421	90	
Symptoms			
ACQ5	1.6 (0.8-2.4)	2.2 (1.6-3.0)	<0.0001¥
ACQ5>1.5	210/415 (51%)	68/90 (76%)	<0.0001¤
ACT	18 (15-21)	15 (11-20)	<0.0001¥
ACT≤19	228/410 (56%)	63/86 (73%)	0.003¤
mMRC	1.7 (±0.8)	2.0 (±0.9)	0.005‡
Exacerbations previous year			
Prednisolone-requiring⊕	0.4 (±0.9) / 0 (0-0)	1.7 (±1.8) / 1 (0-3)	<0.0001/<0.0001¥
ER visits⊕	0.3 (±0.7) / 0 (0-0)	0.6 (±1.0) / 0 (0-1)	0.008/0.002¥
Hospitalizations⊕	0.2 (±0.6) / 0 (0-0)	0.5 (±1.0) / 0 (0-1)	0.004/0.0004¥
Quality of life			
SF12			
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‡
Co-morbidities			
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 eqivalents, µg	753 (±318)	1797 (±435)	na.
OCS for asthma, n/N (%)	-	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 numbers (n), n/N (%), mean±SD or median (IQR). ACQ-5: Asthma Control Questionnaire; ACT: Asthma Control Test; MRC: Medical Research Council dyspnea scale; SF-12: Health condition questionnaire; PCS: physical component score; MCS: mental componenet score; miniAQLQ: Mini Asthma Quality of Life Questionnaire; miniRQLQ: Mini Rhinoconjunctivitis Quality of Life Questionnaire; Nijmegen: Hyperventilation; SNOT22: Sino-Nasal Outcome Test. ⊕: Reported both as mean±SD and median (IQR).
Statistical tests: ¤: chi-square §: fishers exact test, ‡: Welch's ANOVA, ¥: Kruskal Wallis, na.: not applicable.

TABLE 3 Clinical characteristics in sub-groups by T2 biomarker expression (B-EOS, FeNO and IgE**), mild-moderate asthma**

	None	EOS	FeNO	IgE	EOS + FeNO	EOS + IgE	FeNO + IgE	EOS + IgE + FeNO	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 (packyears)	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 sensitization	44 (±24)	7 (±26)	23 (±50)	32 (±73)	17 (±47)	22 (±92)	23 (±79)	24 (±92)	<0.001¤
Lung function									
FEV1 (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‡
FEV1 % 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 ($cells*10^9/L$)	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)	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¥
FeNO (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 numbers (n), n/N (%), mean±SD, or median (IQR). BMI: body mass index; FEV1: forced expiratory volume in 1 sec; FVC: forced vital capacity.
#: Proportion of performed mannitol challenge test that were positive (test performed if FEV1 ≥70% predicted). Statistical tests: ¤: chi-square, ‡: Welch's ANOVA, ¥: Kruskal Wallis.

TABLE 4 Clinical characteristics in sub-groups by T2 biomarker expression (B-EOS, FeNO and IgE), severe asthma

	None	EOS	FeNO	IgE	EOS + FeNO	EOS + IgE	FeNO + IgE	EOS + IgE + FeNO	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/m2)	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 (packyears)	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 sensitization	13 (±44)	2 (±40)	2 (±40)	10 (±83)	1 (±20)	4 (±67)	5 (±71)	11 (±69)	0.1§
Lung function									
FEV1 (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‡
FEV1 % 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)	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 (.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)	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¥
FeNO (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 numbers (n), n/N (%), mean±SD, or median (IQR). #: Proportion of performed mannitol challenge test that were positive (test performed if FEV1 ≥70% predicted). Statistical tests: §: fishers exact test, ‡: Welch's ANOVA, ¥: Kruskal Wallis.

Table 5 Baseline characteristics in subgroups based on the presence of B-EOS and S-EOS

	Severe asthma					Mild-moderate				
Variable	**None**	**S-EOS and B-EOS**	**S-EOS**	**B-EOS**	**p**	**None**	**S-EOS and B-EOS**	**S-EOS**	**B-EOS**	**p**
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¤
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 (packyears)	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 sensitization	17 (61%)	12 (57%)	7 (44%)	4 (50%)	0.8§	63 (42%)	22 (52%)	27 (42%)	24 (67%)	0.05¤
Lung function										
FEV1 (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‡
FEV1 % 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¤
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										
Blood eosinophils (cells*10^9/L)	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.
Sputum eosinophils (%)	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)	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 ≥150IU/ml	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¤
FeNO (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¥
FeNO ≥25ppb	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¤
FeNO ≥50ppb	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¤

Data are presented as numbers (n), n/N (%), mean±SD, or median (IQR). B-EOS: blood eosinophils ≥ 0.3 cells*10^9/L); S-EOS: sputum eosinophils ≥3%. #: Proportion of performed mannitol challenge test that were positive (test performed if FEV1 ≥70% predicted). Statistical tests: ¤: chi-square §: fishers exact test, ‡: Welch's ANOVA, ¥: Kruskal Wallis, na.: not applicable.

References

1. Lambrecht BN, Hammad H. The immunology of asthma. Nat Immunol [Internet]. 2014;16(1):45–56. Available from: http://www.ncbi.nlm.nih.gov/pubmed/25521684

2. Frøssing L, Silberbrandt A, Von Bülow A, Kjærsgaard Klein D, Ross Christensen M, Backer V, et al. Airway gene expression identifies subtypes of type 2 inflammation in severe asthma. Clin Exp Allergy [Internet]. 2021 Jun 17 [cited 2021 Jun 21];cea.13966. Available from: https://onlinelibrary.wiley.com/doi/10.1111/cea.13966

3. Frøssing L, Silberbrandt A, Von Bülow A, Backer V, Porsbjerg C. The Prevalence of Subtypes of Type 2 Inflammation in an Unselected Population of Patients with Severe Asthma. J Allergy Clin Immunol Pract [Internet]. 2020 [cited 2021 Jan 22]; Available from: https://doi.org/10.1016/j.jaip.2020.09.051

4. Matsusaka M, Fukunaga K, Kabata H, Izuhara K, Asano K, Betsuyaku T. Subphenotypes of type 2 severe asthma in adults. J Allergy Clin Immunol Pract [Internet]. 2018;6(1):274-276.e2. Available from: https://doi.org/10.1016/j.jaip.2017.06.015

5. Castro M, Mathur S, Hargreave F, Boulet LP, Xie F, Young J, et al. Reslizumab for poorly controlled, eosinophilic asthma: A randomized, placebo-controlled study. Am J Respir Crit Care Med. 2011;184(10):1125–32.

6. Haldar P, Brightling CE, Hargadon B, Gupta S, Monteiro W, Sousa A, et al. Mepolizumab and exacerbations of refractory eosinophilic asthma. N Engl J Med [Internet]. 2009 Mar 5 [cited 2016 Nov 18];360(10):973–84. Available from: http://www.nejm.org/doi/abs/10.1056/NEJMoa0808991

7. Ortega HG, Liu MC, Pavord ID, Brusselle GG, FitzGerald JM, Chetta A, et al. Mepolizumab Treatment in Patients with Severe Eosinophilic Asthma. N Engl J Med [Internet]. 2014;371(13):1198–207. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1403290

8. Hanania NA, Wenzel S, Roseń K, Hsieh HJ, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: An analysis of biomarkers in the EXTRA study. Am J Respir Crit Care Med. 2013;187(8):804–11.

9. Castro M, Corren J, Pavord ID, Maspero J, Wenzel S, Rabe KF, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma.

N Engl J Med [Internet]. 2018 Jun 28 [cited 2019 Jun 18];378(26):2486–96. Available from: http://www.nejm.org/doi/10.1056/NEJMoa1804092

10. Lane C, Knight D, Burgess S, Franklin P, Horak F, Legg J, et al. Epithelial inducible nitric oxide synthase activity is the major determinant of nitric oxide concentration in exhaled breath. Thorax. 2004 Sep;59(9):757–60.

11. Korhonen R, Lahti A, Kankaanranta H, Moilanen E. Nitric oxide production and signaling in inflammation. Curr Drug Targets Inflamm Allergy. 2005;4(4):471–9.

12. Chibana K, Trudeau JB, Mustovitch AT, Hu H, Zhao J, Balzar S, et al. IL-13 induced increases in nitrite levels are primarily driven by increases in inducible nitric oxide synthase as compared with effects on arginases in human primary bronchial epithelial cells. Clin Exp Allergy. 2008;38(6):936–46.

13. Buhl R, Humbert M, Bjermer L, Chanez P, Heaney LG, Pavord I, et al. Severe eosinophilic asthma: A roadmap to consensus [Internet]. European Respiratory Journal European Respiratory Society; May 1, 2017. Available from: https://doi.org/10.1183/13993003.00634-2017

14. Erjefält JS. Unravelling the complexity of tissue inflammation in uncontrolled and severe asthma. Curr Opin Pulm Med [Internet]. 2019;25(1):79–86. Available from: http://insights.ovid.com/crossref?an=00063198-900000000-99172

15. Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Seidel L, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. Eur Respir J [Internet]. 2014 [cited 2020 May 12];44(1):97–108. Available from: http://ow.ly/sLtYr

16. Backer V, Klein DK, Bodtger U, Romberg K, Porsbjerg C, Erjefält JS, et al. Clinical characteristics of the BREATHE cohort – a real-life study on patients with asthma and COPD. Eur Clin Respir J [Internet]. 2020 Jan 1 [cited 2020 Mar 17];7(1):1736934. Available from: https://www.tandfonline.com/doi/full/10.1080/20018525.2020.1736934

17. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, et al. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J [Internet]. 2014 [cited 2016 Oct 5];43(2):343–73. Available from: http://ow.ly/roufI

18. Bafadhel M, McCormick M, Saha S, McKenna S, Shelley M, Hargadon B, et al. Profiling of sputum inflammatory mediators in asthma and chronic

obstructive pulmonary disease. Respiration [Internet]. 2012 [cited 2019 Apr 3];83(1):36–44. Available from: www.karger.com

19. Alvarez-Puebla MJ, Olaguibel JM, Almudevar E, Echegoyen AA, Vela C, De Esteban B. Mannitol versus hypertonic saline: Safety and efficacy of mannitol and hypertonic saline in sputum induction and bronchial hyperreactivity assessment. Chron Respir Dis [Internet]. 2015 Aug 11 [cited 2019 Dec 2];12(3):197–203. Available from: http://journals.sagepub.com/doi/10.1177/1479972315576144

20. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: Assessment and identification using induced sputum. Respirology [Internet]. 2006 Jan [cited 2016 Oct 13];11(1):54–61. Available from: http://www.ncbi.nlm.nih.gov/pubmed/16423202

21. Amaral R, Fonseca JA, Jacinto T, Pereira AM, Malinovschi A, Janson C, et al. Having concomitant asthma phenotypes is common and independently relates to poor lung function in NHANES 2007-2012. Clin Transl Allergy [Internet]. 2018 [cited 2022 Jan 21];8:13. Available from: https://doi.org/10.1186/s13601-018-0201-3

22. Mogensen I, Alving K, Jacinto T, Fonseca J, Janson C, Malinovschi A. Simultaneously elevated FeNO and blood eosinophils relate to asthma morbidity in asthmatics from NHANES 2007-12. Clin Exp Allergy. 2018;48(8):935–43.

23. Price DBB, Bosnic-Anticevich S, Pavord IDD, Roche N, Halpin DMGMG, Bjermer L, et al. Association of elevated fractional exhaled nitric oxide concentration and blood eosinophil count with severe asthma exacerbations. Clin Transl Allergy. 2019 Dec;9(1).

24. Malinovschi A, Fonseca ao A, Jacinto T, Alving K, Janson C. Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects. J Allergy Clin Immunol [Internet]. 2013 [cited 2019 Oct 22];132:821-827.e5. Available from: http://dx.doi.org/10.1016/j.jaci.2013.06.007

25. Malinovschi A, Janson C, Borres M, Alving K. Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity. J Allergy Clin Immunol [Internet]. 2016 [cited 2019 Oct 22];138(5):1301-1308.e2. Available from: http://dx.doi.org/10.1016/j.jaci.2016.01.044

26. Haughney J, Morice A, Blyth KG, Lee AJ, Coutts A, McKnight E, et al. A retrospective cohort study in severe asthma describing commonly measured biomarkers: Eosinophil count and IgE levels. Respir Med [Internet]. 2018 [cited 2019 May 7];134:117–23. Available from:

https://doi.org/10.1016/j.rmed.2017.12.001

27. McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, et al. A large subgroup of mild-to-moderate asthma is persistently noneosinophilic. Am J Respir Crit Care Med. 2012 Mar 15;185(6):612–9.

28. Lazarus SC, Krishnan JA, King TS, Lang JE, Blake K V., Covar R, et al. Mometasone or Tiotropium in Mild Asthma with a Low Sputum Eosinophil Level. N Engl J Med. 2019;380(21):2009–19.

29. Cowan DC, Cowan JO, Palmay R, Williamson A, Taylor DR. Effects of steroid therapy on inflammatory cell subtypes in asthma. Thorax [Internet]. 2010 May [cited 2016 Nov 21];65(5):384–90. Available from: http://www.ncbi.nlm.nih.gov/pubmed/19996343

30. Sverrild A, Porsbjerg C, Francis Thomsen S, Backer V, Copenhagen Dms. Airway hyperresponsiveness to mannitol and methacholine and exhaled nitric oxide: A random-sample population study. 2010;

31. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): A multicentre, double-blind, placebo-controlled trial. Lancet. 2012 Aug;380(9842):651–9.

32. Shrimanker R, Keene O, Hynes G, Wenzel S, Yancey S, Pavord ID. Prognostic and predictive value of blood eosinophil count, fractional exhaled nitric oxide and their combination in severe asthma: a post-hoc analysis. Am J Respir Crit Care Med. 2019;

33. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: A systematic review and meta-analysis. Lancet Respir Med [Internet]. 2015 [cited 2020 Jan 22];3(4):290–300. Available from: http://dx.doi.org/10.1016/

34. Coverstone AM, Seibold MA, Peters MC. Diagnosis and Management of T2-High Asthma. J Allergy Clin Immunol Pract [Internet]. 2020;8(2):442–50. Available from: https://doi.org/10.1016/j.jaip.2019.11.020

35. Porsbjerg CM, Sverrild A, Lloyd CM, Menzies-Gow AN, Bel EH. Anti-alarmins in asthma: Targeting the airway epithelium with next-generation biologics [Internet]. Vol. 56, European Respiratory Journal. 2020 [cited 2021 Dec 29]. Available from: https://doi.org/10.1183/13993003.00260-

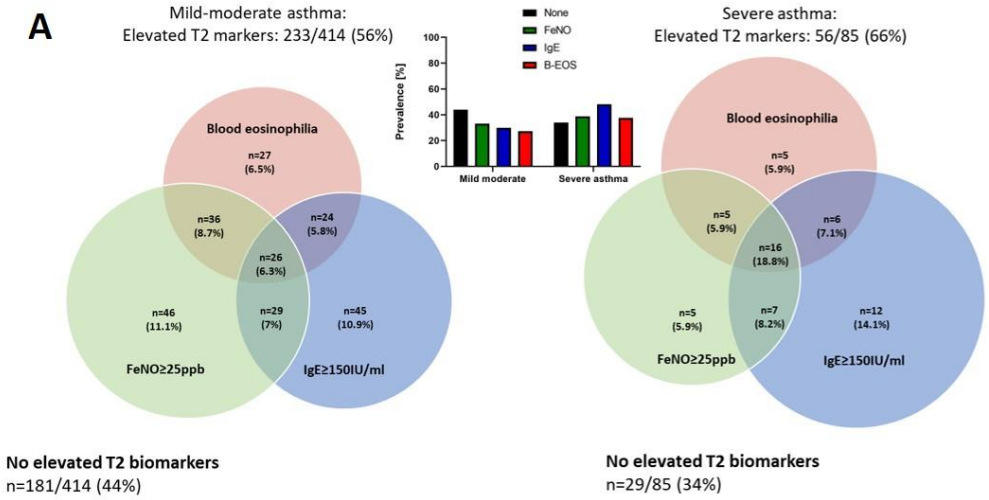
2020

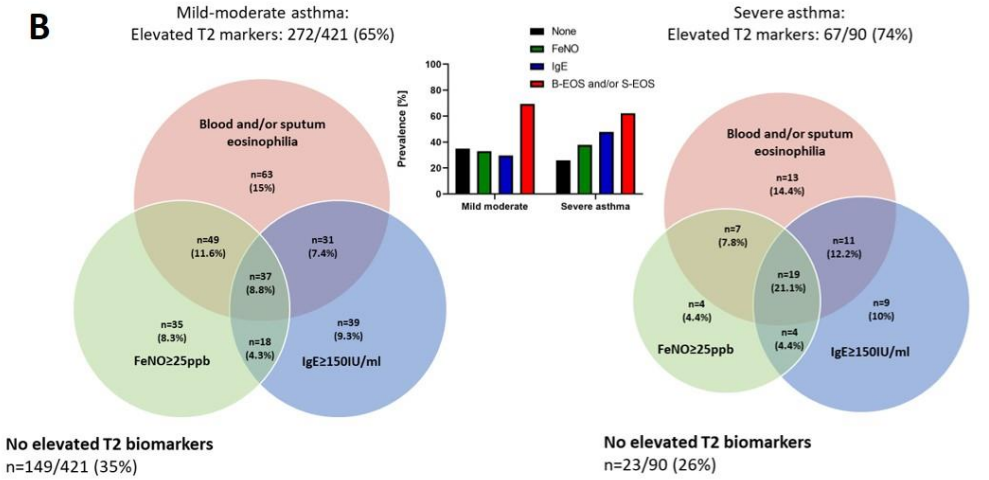
36. Corren J, Parnes JR, Wang L, Mo M, Roseti SL, Griffiths JM, et al. Tezepelumab in adults with uncontrolled asthma. N Engl J Med. 2017;377(10):936–46.

37. Menzies-Gow A, Corren J, Bourdin A, Chupp G, Israel E, Wechsler ME, et al. Tezepelumab in Adults and Adolescents with Severe, Uncontrolled Asthma. N Engl J Med [Internet]. 2021 May 13 [cited 2021 May 21];384(19):1800–9. Available from: http://www.nejm.org/doi/10.1056/NEJMoa2034975

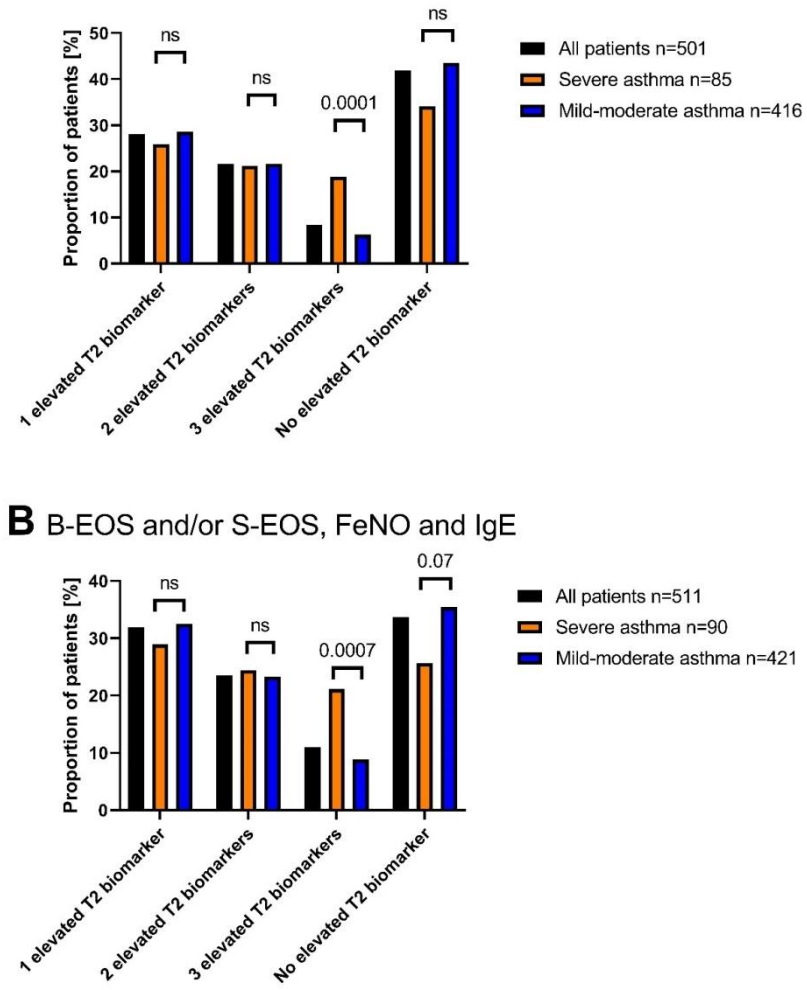
38. Strunk RC, Szefler SJ, Phillips BR, Zeiger RS, Chinchilli VM, Larsen G, et al. Relationship of exhaled nitric oxide to clinical and inflammatory markers of persistent asthma in children. J Allergy Clin Immunol. 2003;112(5):883–92.

39. Frøssing L, Kjærsgaard Klein D, Backer V, Baines KJ, Porsbjerg C. The six-gene expression signature in whole sampled sputum provides clinically feasible inflammatory phenotyping of asthma. ERJ Open Res [Internet]. 2020 [cited 2020 Jul 7];6(1):00280–2019. Available from: https://doi.org/10.1183/23120541.00280-2019

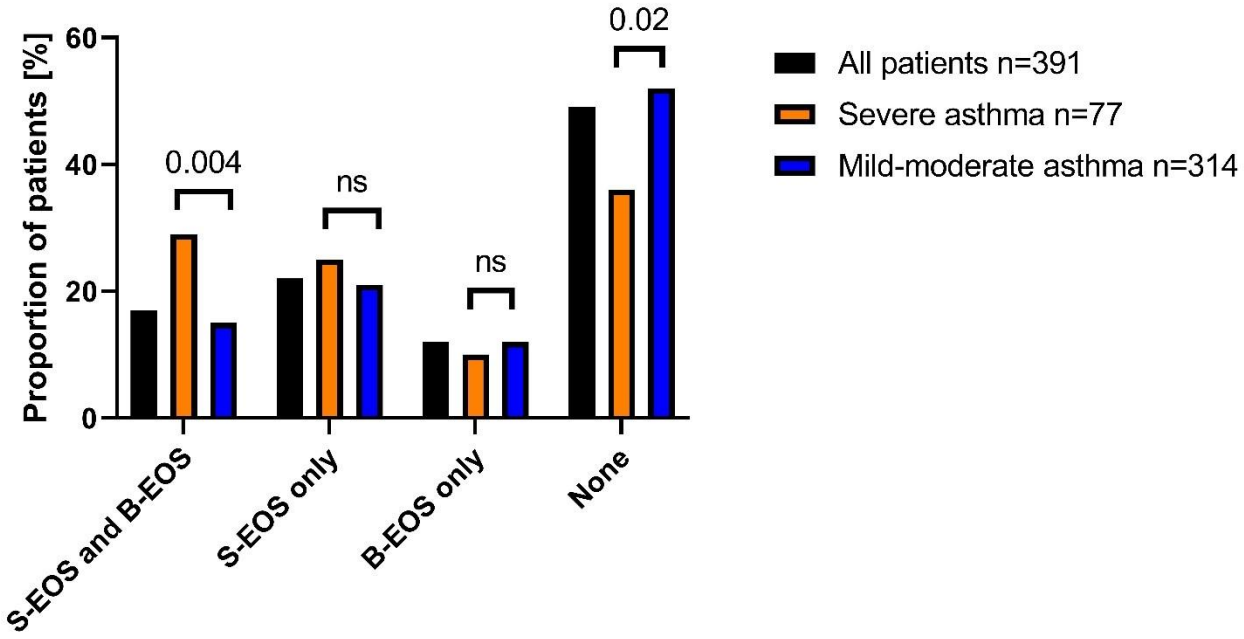




Prevalence and co-expression of T2 biomarkers in patients with mild-moderate vs severe asthma. A) Eosinophilia defined as elevated blood eosinophil count. B) Eosinophilia defined as elevated blood- and/or sputum eosinophil count.



Concomitant biomarker elevation in patients with mild-moderate- vs severe asthma with elevated T2 biomarkers.
A) Eosinophilia defined as elevated blood eosinophil count (B-EOS). B) Eosinophilia defined as elevated blood- and/or sputum eosinophil count (B-EOS and/or S-EOS).



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

Online supplement in relation to:

Distribution of Type 2 biomarkers and association with severity, clinical characteristics and co-morbidities in the BREATHE real-life asthma population

Authors:

Laurits Frøssing* [1], Ditte K Klein* [1], Morten Hvidtfeldt[1], Nicolai Obling[2], Gunilla Telg[3], Jonas S Erjefält[4], Uffe Bodtger[2,5], Celeste Porsbjerg[1].

1: Respiratory Research Unit, Department of Respiratory Medicine, Copenhagen University Hospital, Bispebjerg and Frederiksberg. Denmark.

2: Respiratory Research Unit PLUZ, Department of Respiratory Medicine; Zealand University Hospital Naestved. Denmark.

3: Astrazeneca Nordic, Södertälje, Sweden

4: Unit of Airway Inflammation, Lund University. Sweden.

5: Institute of Regional Health Research, University of Southern Denmark. Denmark

*: Contributed equally

Table S1: Symptoms, quality-of-life, comorbidities and medication in patients with severe eosinophilic and non-eosinophilic asthma

Variable	B-EOS <0.3	B-EOS ≥0.3	p-value	S-EOS <0.3	S-EOS ≥0.3	P
N	53	32		39	39	
ACQ5	2.2 (1.1-3.0)	2.4 (1.7-3.3)	0.4¥	2.2 (1.2-3.0)	2.4 (1.8-3.0)	0.6¥
ACQ5>1.5	37/53 (70%)	26/32 (81%)	0.2¤	28/39	33/39	0.2¤
ACT	17 (12-20)	14 (11-19)	0.1¥	16 (12-20)	14 (11-18)	0.3¥
ACT≤19	35/53 (66%)	23/32 (72%)	0.4¤	27/39	29/39	0.6¤
mMRC	2.0 (0.9)	2.0 (0.9)	0.7¥	2.1 (0.9)	2.0 (0.9)	0.8¥
Exacerbations previous year	1.4 (1.6) / 1 (0-2.0)	1.8 (2.0) / 1 (0-4)	0.4‡/0.5¥	1.2 (1.5) / 1 (0-2)	2.2 (2.0) / 1 (0-5)	0.02‡/0.05¥
ER visits	0.60 (0.97) / 0 (0-1)	0.44 (0.84) / 0 (0-1)	0.4‡/0.5¥	0.64 (1.1) / 0 (0-1)	0.55 (0.93) / 0 (0-1)	0.7‡/0.8¥
Hospitalizations	0.51 (0.93) / 0 (0-1)	0.38 (0.83) / 0 (0-1)	0.5‡/0.4¥	0.38 (0.78) / 0 (0-1)	0.70 (1.2) / 0 (0-1)	0.2‡/5¥
Quality of life						
SF12						
PCS	44 (34-52)	41 (38-44)	0.6¥	44 (36.0-50.3)	40.0 (34.2-46.4)	0.5¥
MCS	46 (40-55)	55 (49-58)	0.007¥	47 (42-53)	54 (48-59)	0.03¥
miniAQLQ overall	4.9 (1.4)	5.0 (1.1)	0.9‡	4.9 (1.4)	4.9 (1.2)	1.0‡
miniRQLQ overall	1.4 (0.9)	1.5 (1.1)	0.8‡	1.4 (1.0)	1.5 (1.1)	0.7‡
Co-morbidities						
Nijmegen	17.0 (9.0)	17.3 (9.5)	0.8‡	18.3 (10.2)	16.8 (9.0)	0.5‡
SNOT22	22.9 (15.3)	21.9 (17.1)	0.9‡	25.1 (16.8)	18.3 (12.9)	0.6‡
Epworth sleepiness scale	5.2 (3.1)	6.8 (4.2)	0.1‡	6.1 (3.9)	5.6 (3.9)	0.6‡
Medication						
LABA, µg	40 (36-40)	36 (24-40)	0.3¥	39 (24-100)	36 (24-40)	0.4¥
ICS, budesonide eqivalents, µg	1788 (433)	1819 (460)	0.7‡	1723 (333)	1894 (594)	0.2‡
OCS for asthma, n	6/53 (11%)	4/32 (13%)	0.9§	3/39 (8%)	8/39 (21%)	0.1§
OCS for asthma, mg	12.5 (5-15)	5 (5-7.5)	0.1¥	12.5 (5.0-17.5)	7.5 (5.0-13.8)	0.6¥

Data are presented as numbers (n), n/N (%), mean±SD or median (IQR). ACQ-5: Asthma Control Questionnaire; ACT: Asthma Control Test; MRC: Medical Research Council dyspnea scale; SF-12: Health condition questionnaire; PCS: physical component score; MCS: mental componenet score; miniAQLQ: Mini Asthma Quality of Life Questionnaire; miniRQLQ: Mini Rhinoconjunctivitis Quality of Life Questionnaire; Nijmegen: Hyperventilation; SNOT22: Sino-Nasal Outcome Test; LABA: long-acting β2-agonist; ICS: inhaled corticosteroids; OCS: oral corticosteroids. Statistical tests: ¤: chi-square §: fishers exact test, ‡: Welch's ANOVA, ¥: Kruskal Wallis.

Table S2: Baseline characteristics and biomarkers across asthma severity and -control

Variable	Mild-moderate asthma ¤			Severe asthma		
	Controlled	**Uncontrolled**	**p**	**Controlled**	**Uncontrolled**	**p**
Patients total, n	153	266		16	74	
Age (years)	40 (18)	42 (17)	0.2‡	49 (12)	49 (14)	0.9‡
Sex (female)	77 (50%)	136 (64%)	0.001¤	9 (56%)	38 (51%)	0.5¤
BMI (kg/m^2)	24.9 (4.4)	26.4 (5.7)	0.02‡	26.1 (5.1)	28.4 (5.9)	0.1‡
Smoking (packyears)	0 (0-4)	0 (0-9)	0.1§	2.5 (0-10)	0 (0-8)	0.5§
Atopy	76 (50%)	94 (45%)	0.5¤	11 (69%)	41 (55%)	0.9¤
Lung function						
FEV1 (L)	3.5 (1.0)	3.1 (0.9)	0.0001‡	2.8 (0.7)	2.8 (0.8)	1.0‡
FEV1 % predicted	96 (15)	92 (17)	0.01‡	86 (19)	85 (22)	0.9‡
FVC (L)	4.7 (1.3)	4.1 (1.1)	<0.0001‡	4.1 (0.9)	3.8 (1.0)	0.3‡
FVC % predicted	108 (14)	102 (17)	0.0009‡	104 (18)	97 (21)	0.2‡
Biomarkers						
Blood eosinophils (cells*10^9/L)	0.16 (0.10-0.25)	0.18 (0.10-0.32)	0.1§	0.17 (0.10-0.29)	0.24 (0.13-0.45)	0.2§
Blood eosinophils ≥0.3 cells*10^9/L	34/153 (22%)	78/266 (29%)	0.4	4/16 (25%)	28/69 (41%)	0.2§
Sputum eosinophils (%)	1.1 (0.25-3.8)	1.8 (0.25-5.8)	0.1§	0.6 (0.13-6.8)	3.5 (1.0-7.4)	0.1§
Sputum eosinophils ≥3%	37/106 (35%)	71/195 (36%)	0.07¤	4/12 (33%)	36/67 (54%)	0.2§
Sputum neutrophils (%)	25.8 (10.8-61.3)	42.0 (17.8-66.5)	0.03§	41.2 (19.4-62.6)	49.8 (23.8-72.3)	0.3§
Sputum neutrophils ≥61%	28/106 (26%)	55/195 (28%)	0.4¤	3/12 (25%)	22/67 (33%)	0.8§
IgE total (IU/ml)	57 (24-167)	57 (18-210)	0.8§	163 (97-391)	129 (47-252)	0.1§
IgE total ≥150IU/ml	42/153 (27%)	82/266 (31%)	0.9¤	11/16 (69%)	32/74 (43%)	0.5¤
FeNO (ppb)	17 (10-28)	16 (9-36)	0.6§	19 (13-26)	16 (10-44)	0.8§
FeNO ≥25ppb	16/153 (10%)	45/266 (17%)	0.2¤	5/16 (31%)	29/74 (39%)	0.2§

Data are presented as numbers (n), n/N (%), mean±SD, or median (IQR). BMI: body mass index; FEV1: forced expiratory volume in 1 sec; FVC: forced vital capacity.
Uncontrolled: ACQ5 >1.5 or ACT < 19. ¤: 6 patients with mild-moderate asthma had no ACQ5 or ACT score available and were thus excluded.
Statistical tests: ¤: chi-square §: fishers exact test, ‡: Welch's ANOVA, ¥: Kruskal Wallis.

Table S3:						
		Mild-moderate Asthma, n=291			Severe asthma, n=73	
		B-EOS			B-EOS	
		>= 0.3	<0.3		>= 0.3	< 0.3
S-EOS	>= 3%	42 (14%)	64 (22%)		21 (29%)	16 (22%)
	<3%	36 (12%)	149 (51%)		8 (11%)	28 (38%)

Table S4 identification of airway eosinophilia using conventional T2 biomarkers						
	All		Severe		Moderate	
	Kappa	P	Kappa	P	Kappa	p
B-EOS (≥ 0.3)	0.25	<0.0001	0.34	0.003	0.21	0.0002
FeNO (≥25ppb)	0.24	<0.0001	0.34	0.003	0.20	0.0005
IgE ≥150 IE	0.11	0.03	0.21	0.08	0.07	0.25