

The triple type 2 signature confers more frequent exacerbations and worse midexpiratory flow in moderate to severe asthma

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Clinical Implications

The triple T2 inflammatory signature was associated with worse FEF₂₅₋₇₅% and greater severe exacerbation frequency. This may explain why biologics that suppress all three biomarkers have the greatest impact on exacerbations in T2 high patients with severe asthma.

Type 2 (T2) inflammation plays a key role in persistent asthma and is primarily driven by upstream epithelial alarmins such as thymic stromal lymphopoietin and IL-33 in response to external stimuli.¹ These in turn perpetuate the inflammatory cascade by increasing the expression of downstream cytokines including IL-4, IL-5, and IL-13.² In real-life clinical practice, the presence of T2 inflammation can be assessed using surrogates such as peripheral blood eosinophil (PBE) count, fractional exhaled nitric oxide (FENO), and allergy as IgE. This process is especially important because pivotal decisions surrounding biologics for severe asthma usually depend on the underlying T2 disease phenotypic signature.

Most clinical trials studying biologics have specific inclusion criteria necessitating the recruitment of volunteers with particular T2 signatures. In this regard, the LIBERTY ASTHMA QUEST study,³ assessing the efficacy and safety of the anti-IL-4R α biologic dupilumab in moderate to severe asthma, allowed accurate determination of the relative prevalence of T2 inflammation because initial enrollment was independent of biomarkers. In QUEST, the proportion of patients with combined PBE count greater than or equal to 150 cells/ μ L and FENO greater than or equal to 25 parts per billion (ppb) was 42%.

Therefore, we aimed to investigate the effect of the triple T2 inflammatory signature on spirometry and exacerbation frequency in our cohort of patients with moderate to severe asthma.

Between January 2016 and March 2023, 215 consecutive respiratory physician—diagnosed Global Initiative for Asthma—defined moderate-to-severe asthma patients taking a daily beclomethasone dipropionate equivalent inhaled corticosteroid dose of greater than or equal to 800 μ g and at least 1 second-line controller were identified from our National Health Service specialist respiratory clinics. Fifty-three were excluded because they did not have the full data set regarding PBE, FENO, and total IgE, and therefore 162 patients were included in the analyses. Patients with other clinically relevant respiratory conditions such as chronic obstructive pulmonary disease or bronchiectasis were excluded. Data on PBE count, FENO, total IgE, spirometry, and the number of severe exacerbations over the preceding year, defined as that requiring prednisolone 40 mg daily for at least 5 days, were collected. Values for all T2

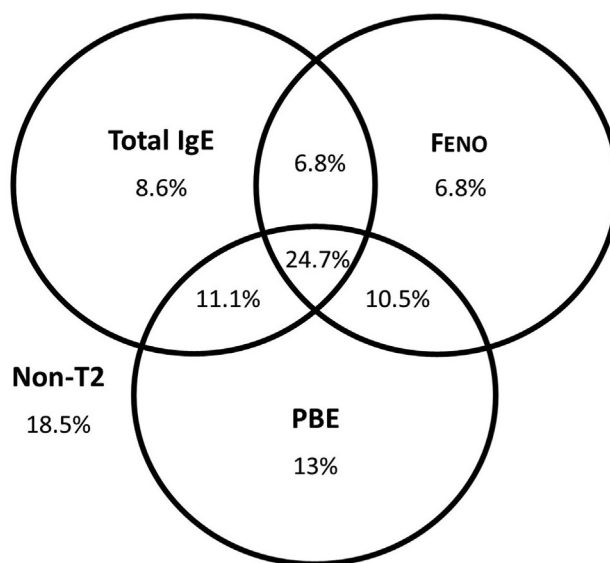


FIGURE 1. Venn diagram depicting the relative prevalence of T2 phenotypes. Cutoff points used: PBE count greater than or equal to 300 cells/ μ L; FENO greater than or equal to 25 ppb; and total IgE greater than or equal to 100 kU/L.

biomarkers and spirometry were taken before initiation of any biologics or oral corticosteroids. Notably, values for PBE count were taken as the mean of values over the previous year to obviate variability over time.

FENO was measured using NIOX VERO (Circassia, Oxford, UK) according to manufacturer's instructions and American Thoracic Society/European Respiratory Society guidelines. Spirometry (Micromedical, Chatham, UK) was performed according to American Thoracic Society and European Respiratory Society guidelines.

Statistical analysis using SPSS v27 involved logistic regression to obtain odds ratios (ORs) for the triple T2 signature (PBE count ≥ 300 cells/ μ L, FENO ≥ 25 ppb, and total IgE ≥ 100 kU/L) associated with spirometry and 2 or more exacerbations in the past year compared with all other biomarker groups. ORs were subsequently adjusted for important characteristics such as age, sex, body mass index, smoking history, presence of nasal polyps, and those taking long-acting β -agonists, long-acting muscarinic antagonists, and leukotriene receptor antagonists.⁴ Values are presented as adjusted OR (95% CI) where the lower CI exceeding unity denotes significance. We considered forced midexpiratory flow rate between 25% and 75% of forced vital capacity (FEF₂₅₋₇₅) to be the prespecified primary outcome of interest. To detect a 10% difference in FEF₂₅₋₇₅% predicted, and assuming an estimated between-group SD of 15%, 36 patients were required in each group to achieve 80% power using a 2-tailed alpha of 0.05. Caldicott approval (IGTCAL8564) was obtained from the NHS Tayside Information Governance Team, waiving the need for ethical approval because this was a retrospective cohort study.

TABLE I. Adjusted ORs (95% CIs) for the association between triple biomarker positive patients vs all other type 2 combinations

T2 profile	FEV ₁	FEF ₂₅₋₇₅	FEV ₁ /FVC	≥2 exacerbations/y
T2 high (n = 40 of 162)	1.68 (0.71-3.94)	2.75 (1.01-7.46) *	1.78 (0.74-4.27)	2.44 (1.03-5.80) *
T2 low (n = 30 of 162)	0.88 (0.35-2.22)	0.33 (0.13-0.85) *	0.30 (0.11-0.81) *	0.75 (0.31-1.85)

FVC, Forced vital capacity.

T2 high denoted by PBE count ≥300 cells/μL, FENO ≥25 ppb, and total IgE ≥100 kU/L, whereas T2 low as PBE count <300 cells/μL, FENO <25 ppb, and total IgE <100 kU/L. Cutoff points as follows: FEV₁ <80%, FEF₂₅₋₇₅ <60%, and FEV₁/FVC <0.70.**P* < .05.

Demographic data are presented in Table E1 in this article's Online Repository at www.jaci-inpractice.org. The prevalence of patients with zero, solitary, dual, and triple biomarker positivity according to cutoff points of 300 cells/μL for PBE count, 25 ppb for FENO, and 100 kU/L for total IgE is shown in Figure 1. Twelve percent of patients exhibited PBE count less than 150 cells/μL, FENO less than 25 ppb, and total IgE less than 100 kU/L.

There was a significantly increased likelihood for patients with the triple T2 inflammatory signature to be associated with the prespecified primary end point of impaired FEF₂₅₋₇₅% and severe exacerbation frequency (Table I) compared with all other phenotypes. Those with T2-low disease were less likely to exhibit impaired FEF₂₅₋₇₅% and FEV₁/forced vital capacity compared with all other T2-high phenotypes although there were no differences in exacerbation frequency.

We believe that this is the first study to describe a significant relationship between the triple T2 inflammatory signature with spirometry as FEF₂₅₋₇₅% but not FEV₁%. We found a 64% increased likelihood of impaired FEF₂₅₋₇₅ compared with those with all other biomarker combinations. This amounted to a clinically relevant mean difference of 0.365 L/s or 10% predicted. FEF₂₅₋₇₅ is a measurement of volume-dependent small airways closure and has been demonstrated to be a more sensitive measure of airway geometry alteration compared with FEV₁.⁵ Similar to our study, 2 previous studies in severe asthma^{6,7} did not detect any differences in FEV₁% when comparing the triple T2 signature with other phenotypes. Intriguingly, patients with the T2-low phenotype (PBE count <300 cells/μL; FENO <25 ppb, and total IgE <100 kU/L) were less likely to have impaired FEF₂₅₋₇₅% and FEV₁/forced vital capacity compared with all other phenotypes combined, presumably because sustained T2 inflammation results in a degree of airway remodeling.

The triple signature was also significantly associated with a greater likelihood of 2 or more exacerbations requiring oral corticosteroids occurring in the previous year. Despite the comparison cohort comprising patients with elevated PBE, which itself is predictive of exacerbation risk,⁸ the triple T2 signature was still associated with a 59% increased risk compared with all other phenotypes combined. We postulate the reason for this is that activation of all cytokine pathways including IL-4, IL-5, and IL-13 results in the greatest inflammatory burden. This hypothesis is supported by the phase III NAVIGATOR data demonstrating a 77% mean exacerbation reduction with the anti-thymic stromal lymphopoietin biologic tezepelumab in patients with T2-high disease compared with a 29% mean reduction in those without, with nonoverlapping CIs denoting significance. Pointedly, tezepelumab also suppressed all 3 biomarkers.

A major strength of our study was that analyses were adjusted for inhaled corticosteroid doses and comorbid chronic rhinosinusitis with nasal polyps because these can significantly confound PBE count and FENO values.⁹ In this regard, the prevalence of chronic rhinosinusitis with nasal polyps was 3-fold in the triple T2 group. Moreover, analyses were performed using the mean PBE value over the preceding year because there is significant variability in blood eosinophils in severe asthma.¹⁰

We appreciate our study should be interpreted within the context of limitations including its retrospective nature from a single UK specialist center. However, the prevalence of zero, isolated, dual, and triple biomarker phenotypes in our study was similar to that previously described^{6,7} albeit these studies used total IgE cutoff points of 75 and 150 kU/L to define allergy. The limited sample size might explain why the CIs for the ORs were rather wide for both FEF₂₅₋₇₅% and exacerbations. The prevalence of single and dual biomarker combinations did not allow for sufficient numbers to perform further logistic regression, but we look forward to future studies that may be able to study this area. In conclusion, the triple T2 inflammatory signature was associated with worse FEF₂₅₋₇₅% and greater severe exacerbation frequency. This may explain why biologics that suppress all 3 biomarkers have the greatest impact on exacerbations in T2-high patients with severe asthma.

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Conflicts of interest: R. Chan reports personal fees (talks) and support attending European Respiratory Society (ERS) meetings from AstraZeneca; personal fees (consulting) from Vitalograph; and personal fees (talks) from Thorasys. B. Lipworth reports nonfinancial support (equipment) from GlaxoSmithKline; grants, personal fees (consulting, talks, and advisory board), and other support (attending American Thoracic Society and ERS meetings) from AstraZeneca; personal fees (talks and consulting) from Sanofi; personal fees (consulting, talks, and advisory board) from Circassia in relation to the submitted work; grants, personal fees (consulting, talks, and advisory board), and other support (attending ERS meetings) from Teva; personal fees (talks and consulting) and grants and other support (attending ERS and British Thoracic Society [BTS] meetings) from Chiesi; personal fees (consulting) from Lupin, Glenmark, Dr Reddy, and Sandoz; grants, personal fees (consulting, talks, and advisory board), and other support (attending BTS meetings) from Boehringer Ingelheim; grants and personal fees (advisory board and talks) from Mylan outside of the submitted work; and that his son is presently an employee of AstraZeneca.

Received for publication March 27, 2023; revised April 8, 2023; accepted for publication May 10, 2023.

Available online May 26, 2023.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2023.05.021>

REFERENCES

1. Chan R, Stewart K, Misirovs R, Lipworth B. Targeting downstream type 2 cytokines or upstream epithelial alarmins for severe asthma. *J Allergy Clin Immunol Pract* 2022;10:1497-505.
2. Chan R, RuiWen Kuo C, Lipworth B. Pragmatic clinical perspective on biologics for severe refractory type 2 asthma. *J Allergy Clin Immunol Pract* 2020; 8:3363-70.
3. Busse WW, Maspero JF, Rabe KF, Papi A, Wenzel SE, Ford LB, et al. Liberty Asthma QUEST: phase 3 randomized, double-blind, placebo-controlled, parallel-group study to evaluate dupilumab efficacy/safety in patients with uncontrolled, moderate-to-severe asthma. *Adv Ther* 2018;35:737-48.
4. Schatz M, Zeiger RS, Vollmer WM, Mosen D, Cook EF. Determinants of future long-term asthma control. *J Allergy Clin Immunol* 2006;118:1048-53.
5. Chan R, Lipworth BJ. Oscillometry bronchodilator response in adult moderate to severe eosinophilic asthma patients: a prospective cohort study. *Clin Exp Allergy* 2022;52:1118-20.
6. Frøssing L, Klein DK, Hvidtfeldt M, Obling N, Telg G, Erjefält JS, et al. Distribution of type 2 biomarkers and association with severity, clinical characteristics and comorbidities in the BREATHE real-life asthma population. *ERJ Open Res* 2023;9:00483-2022.
7. Denton E, Price DB, Tran TN, Canonica GW, Menzies-Gow A, FitzGerald JM, et al. Cluster analysis of inflammatory biomarker expression in the International Severe Asthma Registry. *J Allergy Clin Immunol Pract* 2021;9:2680-8.e7.
8. Zeiger RS, Schatz M, Li Q, Chen W, Khatry DB, Gossage D, et al. High blood eosinophil count is a risk factor for future asthma exacerbations in adult persistent asthma. *J Allergy Clin Immunol Pract* 2014;2:741-750.e4.
9. Chan R, Lipworth B. Impact of nasal polyps on endotype and phenotype in patients with moderate to severe asthma. *Ann Allergy Asthma Immunol* 2021; 127:548-52.
10. Corren J, Du E, Gubbi A, Vanlandingham R. Variability in blood eosinophil counts in patients with eosinophilic asthma. *J Allergy Clin Immunol Pract* 2021; 9:1224-1231.e9.

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TABLE E1. Patient demographics

Characteristic	All other combinations (n = 122)	Triple T2 (n = 40)	P value
Sex (F/M)	83/39	21/19	.075
Age (y)	51 ± 15	54 ± 13	.342
Ex-smoker (%)	25%	38%	.190
Current smoker (%)	7%	3%	.430
Severe asthma (%)	79%	92%*	.048
BMI (kg/m ²)	31.1 ± 6.7	29.2 ± 6.1	.060
ICS dose (µg)	1807 ± 858	1870 ± 291	.647
LABA (%)	92%	83%	.096
LAMA (%)	57%	48%	.318
LTRA (%)	54%	65%	.227
Theophylline (%)	22%	15%	.331
Oral antihistamine (%)	50%	70%*	.027
FEV ₁ (%)	86.1 ± 21.8	80.6 ± 22.8	.087
FEF ₂₅₋₇₅ (%)	54.7 ± 29.2	44.4 ± 23.3*	.022
FVC (%)	101.5 ± 17.9	99.8 ± 20.6	.617
FEV ₁ /FVC	0.71 ± 0.13	0.66 ± 0.12*	.028
Nasal polyps (%)	18%	53% [†]	<.001
FENO (ppb)	18 (19)	48 (47) [†]	<.001
PBE (cells/µL)	272 (303)	470 (293) [†]	<.001
Total IgE (kU/L)	66 (161)	298 (354) [†]	<.001
OCS exacerbations	1 (4)	3 (4)*	.017

BMI, Body mass index; F, female; ICS, inhaled corticosteroid; IQR, interquartile range; LABA, long-acting β-agonist; LAMA, long-acting muscarinic antagonist; LTRA, leukotriene receptor antagonist; M, male; OCS, oral corticosteroid.

Mean ± SD or median (IQR) analyzed using independent *T* test or Mann-Whitney *U* test, respectively. Categorical variables analyzed using χ^2 test.

**P* < .05.

[†]*P* < .001.