

Comprehensive Characterization of Difficult-to-Treat Asthma Reveals Near Absence of T2-Low Status



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What is already known about this topic? Difficult-to-treat/severe asthma is stratified into T2-high and low endotypes, but the real-world prevalence and clinical characteristics remain poorly understood.

What does this article add to our knowledge? This real-world study shows that multicomponent and longitudinal characterization of T2 status reveals a near absence of T2-low status among biologic-naïve, difficult-to-treat/severe asthma patients.

How does this study impact current management guidelines? This study emphasizes the need to undertake a comprehensive assessment of T2 status in difficult-to-treat and severe asthma to guide treatment options accurately.

BACKGROUND: Asthma is conventionally stratified as type 2 inflammation (T2)-high or T2-low disease. Identifying T2 status has therapeutic implications for patient management, but a real-world understanding of this T2 paradigm in difficult-to-treat and severe asthma remains limited.

OBJECTIVES: To identify the prevalence of T2-high status in difficult-to-treat asthma patients using a multicomponent definition and compare clinical and pathophysiologic characteristics between patients classified as T2-high and T2-low.

METHODS: We evaluated 388 biologic-naïve patients from the Wessex Asthma Cohort of difficult asthma (WATCH) study in the United Kingdom. Type 2–high asthma was defined as 20

parts per billion or greater FeNO, 150 cells/μL or greater peripheral blood eosinophils, the need for maintenance oral corticosteroids, and/or clinically allergy-driven asthma.

RESULTS: This multicomponent assessment identified T2-high asthma in 93% of patients (360 of 388). Body mass index, inhaled corticosteroid dose, asthma exacerbations, and common comorbidities did not differ by T2 status. Significantly worse airflow limitation was found in T2-high compared with T2-low patients (FEV₁/FVC 65.9% vs 74.6%). Moreover, 75% of patients defined as having T2-low asthma had raised peripheral blood eosinophils within the preceding 10 years, which left only seven patients (1.8%) who had never had T2 signals.

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The Wessex Asthma Cohort of difficult asthma (WATCH) study has been supported by the National Institute for Health and Care Research Southampton Biomedical Research Centre and Clinical Research Facility at University Hospital Southampton National Health Service Foundation Trust, United Kingdom. The WATCH study itself is not externally funded. Funding assistance for database support for the WATCH study was initially obtained from a nonpromotional grant from Novartis (£35,000). Funding assistance for sputum assessment was provided

within a nonpromotional investigator-led grant from Boehringer-Ingelheim. Funding assistance for patient costs (eg, parking) was initially provided by a charitable grant (£3,500) from the Asthma, Allergy & Inflammation Research Charity.

Conflicts of interest: H. Rupani reports speaker and consultancy fees from AstraZeneca, GlaxoSmithKline, Teva, Novartis, and Chiesi. P. Howarth reports employment by GSK outside the submitted work. A. Azim reports employment from AstraZeneca outside the submitted work. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 15, 2023; revised April 19, 2023; accepted for publication May 17, 2023.

Available online May 26, 2023.

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2213-2198

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<https://doi.org/10.1016/j.jaip.2023.05.028>

Abbreviations used

BMI- Body mass index

GINA- Global Initiative for Asthma

ICS- Inhaled corticosteroid

mOCS- Maintenance oral corticosteroid

PBE- Peripheral blood eosinophil

T2- Type 2 inflammation

WATCH- Wessex Asthma Cohort of difficult asthma

Incorporation of sputum eosinophilia 2% or greater into the multicomponent definition in a subset of 117 patients with induced sputum data similarly found that 96% (112 of 117) met criteria for T2-high asthma, 50% of whom (56 of 112) had sputum eosinophils 2% or greater.

CONCLUSIONS: Almost all patients with difficult-to-treat asthma have T2-high disease; less than 2% of patients never display T2-defining criteria. This highlights a need to assess T2 status comprehensively in clinical practice before labeling a patient with difficult-to-treat asthma as T2-low. © 2023 The Authors. Published by Elsevier Inc. on behalf of the American Academy of Allergy, Asthma & Immunology. This is an open access article under the CC BY license (<http://creativecommons.org/licenses/by/4.0/>). (J Allergy Clin Immunol Pract 2023;11:2812-21)

Key words: Difficult-to-treat asthma; Eosinophils; T2 inflammation; T2-low asthma; Phenotypes

INTRODUCTION

Airway inflammation is the hallmark of asthma and underscores typical features of the disease: airway remodeling and hyperresponsiveness and variable airflow limitation. In recent years, endotypic classification of asthma has focused on the paradigm of type 2 (T2) inflammation (T2)-high or T2-low status. Although there is no agreed-upon consensus regarding the definition of T2-high status, it is widely acknowledged to be orchestrated by key interleukins including IL-4, IL-5, and IL-13 and characterized by eosinophilic inflammation. Type 2-low asthma is less well-understood and frequently is loosely defined as the absence of prominent T2-pathway signatures.¹ Patients with T2-low asthma have been characterized as being poorly responsive to corticosteroids and present with significant symptomatology, high medication requirements, and numerous comorbidities.^{1,2}

The identification of T2-high disease is clinically relevant because it has prognostic and therapeutic implications in an evolving landscape of effective T2-focused biologic therapies.^{3,4} This is particularly important in difficult-to-treat and severe asthma, which is characterized by a high symptom burden and poor disease control despite high-dose inhaled corticosteroid therapy.⁵ Measuring sputum eosinophils is often considered the reference standard test for airway inflammation, and sputum eosinophilia reliably predict T2 gene expression in induced sputum.⁶ However, sputum induction is impractical in routine clinical practice. Therefore, several noninvasive T2 markers have been explored, including serum IgE, peripheral blood eosinophils (PBE), and FeNO. The UK Severe Asthma Registry used raised

PBE and FeNO to define T2-high asthma and reported that 45% of 2,225 patients could be considered T2-high.⁷ In our own real-world, difficult-to-treat asthma cohort,⁸ we recently found that 40.3% of 500 enrolled participants had raised PBE at cross-sectional assessment, but this rose to 83.4% with a longitudinal perspective of PBE.⁹ The International Severe Asthma Registry used a multicomponent eosinophil gradient algorithm similarly to show that 83.8% of severe asthma patients were most likely to have eosinophilic inflammation¹⁰ (which is predominantly T2-driven). Subsequently, we applied this algorithm to the difficult-to-treat asthma cohort and incorporated longitudinal PBE measurements to show that a comparable proportion of patients (77.4%) could be classified as most likely to have an eosinophilic phenotype.¹¹

However, debate is ongoing regarding the proportionate distribution and nature of T2-high and T2-low disease in biologic-naïve patients with difficult-to-treat and severe asthma. We therefore sought to (1) assess the multicomponent definition of T2 status proposed by Global Initiative for Asthma (GINA) 2021¹² using the four clinical elements of that definition (150 cells/ μ L or greater PBE, 20 parts per billion [ppb] or greater FeNO, the need for daily maintenance corticosteroids [mOCS], and/or clinically allergy-driven asthma) in this group of patients; (2) understand the clinical and pathophysiologic characteristics of patients who fall into T2-high and T2-low asthma groups, using longitudinal data including PBE measurements to verify T2 status; and (3) investigate the further value of adding sputum eosinophilia to the clinical multicomponent assessment of T2-high status.

METHODS

The Wessex Asthma Cohort of difficult asthma (WATCH) study ($n = 500$) was a prospective observational study of well-characterized patients with difficult-to-treat and severe asthma managed in the tertiary difficult asthma clinic at University Hospital Southampton.⁸

Difficult asthma was defined as asthma with ongoing symptoms despite high-dose therapies and/or continuous or frequent use of oral steroids, according to the British Thoracic Society Adult Asthma Management Guidelines 2016.¹³ All patients provided written informed consent (REC Reference 14/WM/1226). The detailed study methodology was previously published elsewhere.⁸ Table E1 and Figure E1 (in this article's Online Repository at www.jaci-inpractice.org) present inclusion and exclusion criteria along with a study flowchart.

Because this was a real-world cohort, the presence of comorbidities or other medical conditions (eg, diabetes or ischemic heart disease) did not preclude patients from being enrolled. We previously showed that the WATCH cohort is broadly representative of the wider clinic population from which it is drawn, and there were no major differences between the WATCH cohort and other patients in the difficult asthma clinic apart from a higher prevalence of mOCS dependence.¹⁴ Clinical data including detailed clinical, health, and disease-related questionnaires, anthropometry, allergy skin prick testing, blood tests, and lung function testing were captured at enrollment to the WATCH study. Only biologic-naïve patients were included in the current analysis ($n = 388$). Sputum induction was performed in a subset of patients ($n = 117$), as previously described.⁸ Sputum inflammatory phenotypes were determined using a cutoff of greater than 2% for sputum eosinophils and greater than 61% for sputum neutrophils. Clinically requested

TABLE 1. Clinical characteristics for patients with type 2 (T2)-high and T2-low status using clinical and biomarker variables

Clinical characteristics	T2-high (n = 360)	T2-low (n = 28)	P
Age at diagnosis, y	20* (36)	27.6† (17.7)	.22
Adult-onset asthma (aged ≥18y) (%/n)	54.7 (197/360)	67.9 (19/28)	.18
Male patients (%/n)	33 (119/360)	35.7 (10/28)	.8
Ethnicity (% White/n)	91.7 (330/360)	96.4 (27/28)	.93
Body mass index	29.6 (9.8)	29.3 (16.8)	.81
Never-smokers (%/n)	51.4 (185/360)	51.9‡ (14/27)	1
Ever intubated for acute asthma (%/n)	11.1 (40)	28.6 (8)	.01
One or more asthma-related hospitalizations in past 12 mo (%/n)	27.9 (100/358)	44.4 (12/27)	.07
Two or more exacerbations treated with corticosteroids in preceding 12 mo (%/n)	65.3 (209/320)	61.5 (16/26)	.70
Started biologics after enrollment (%/n)	32.8 (118/360)	10.7 (3/28)	.015
Dose of ICS (BDP equivalent, µg)	1,500‡ (2,000)	1,500‡ (2,000)	.74
ICS dose ≥1,000 µg (BDP equivalent) (%/n)	88.5 (292/330)	88.9 (24/27)	1
ICS dose ≥2,000 µg (BDP equivalent) (%/n)	46.7‡ (154/330)	40.7‡ (11/27)	.55
Receiving inhaled long-acting β ₂ -agonist therapy (%/n)	86.7 (312/360)	85.7 (24/28)	.89
Receiving inhaled long-acting antimuscarinic therapy (%/n)	67.5 (243/260)	64.2 (18/28)	.73
Receiving leukotriene receptor antagonists (%/n)	69.7 (251/260)	67.8 (19/28)	.84
Peripheral blood eosinophil count at study enrollment (median), cells/µL	200 (300)	100 (100)	<.01
Peak blood eosinophil count in past 10 y, cells/µL	500§ (600)	200 (300)	<.01
Aeroallergen-positive skin prick tests (%/n)	69.9 (197/282)	22.7 (5/22)	<.01
Total IgE (IU/L)	84.7 (229.2)	17.3 (25.4)	<.01
FeNO at enrollment (parts per billion)	20.3 (25.9)	10.0 (10.0)	<.01

BDP, beclomethasone dipropionate; ICS, inhaled corticosteroid.

T2 status was classified based on the presence of blood eosinophils of 150cells/µL or greater, FeNO of 20 parts per billion or greater, the need for maintenance oral corticosteroids, or allergen-driven asthma. Values are medians and interquartile ranges unless otherwise specified. *P* reflects χ^2 comparisons for categorical variables, Mann-Whitney test for continuous variables with nonnormal distribution, and Fisher exact test when observed cell counts were <5.

*Data were unavailable for 16 patients.

†Data were unavailable for one patient.

‡Data were unavailable for 30 patients.

§Data were unavailable for six patients.

||Data were unavailable for 67 patients.

blood tests were processed by the fully accredited hospital pathology laboratory, in compliance with ISO142819 standards.

Blood cytokine levels (in picograms per milliliter) were measured in plasma from 360 WATCH study participants using a human magnetic Luminex multiplex assay (Bio-Techne Ltd, Abington, UK) for C-C motif chemokine ligand (CCL)2/monocyte chemoattractant protein-1, CCL3/macrophage inflammatory protein-1 α (MIP-1 α), IL-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, IFN- γ , periostin, and TNF- α , according to the manufacturer's instructions (R&D Systems, Bio-Techne Ltd, Abington, UK). To control for a batch effect, we prepared five 96-well plates, pipetted all 360 available plasma samples at the same session, and sequentially ran the five 96-well plates. Each plate contained the eight standard curve dilutions in duplicate wells and two blank wells.

Using the four clinical measures of the multicomponent algorithm proposed by GINA 2021, we defined T2-high status as the presence of any of these clinically relevant parameters: FeNO 20 parts per billion (ppb) or greater; PBE 150 cells/µL or greater; the need for mOCS; and/or clinically allergy-driven asthma (defined as a positive skin test to any aeroallergen, plus an answer of yes to the question posed to patients, "Any allergic triggers for asthma?")

By interrogating available electronic health records, historical values for PBE were reviewed within the preceding 10 years with a median of 10 measurements reviewed per patient.¹⁵ Only baseline FeNO measurements were available.

Statistical analysis

We performed statistical analysis using SPSS software (IBM SPSS statistics, version 25.0, Armonk, NY: IBM Corp), GraphPad Prism 9 (GraphPad Software, LLC, La Jolla, Calif), Venny's online tool,¹⁶ and R software (Vienna, Austria). Continuous clinical variables are presented as medians and interquartile ranges, and categorical variables as frequencies (percentages). Between-group differences were assessed by the Mann-Whitney, Kruskal Wallis, χ^2 , or Fisher exact test, as appropriate.

For the cytokine measurement and analysis, using GraphPad Prism 9, we used the Kruskal-Wallis test on mean ranks and corrected for multiple comparisons using Dunn's test. Multiple comparisons were accounted for by controlling the false discovery rate with the two-stage step-up method of Benjamini, Krieger, and Yekutieli for comparing each cytokine between the T2-high and T2-low groups. Because these tests showed no significant differences, we used the nonparametric Mann-Whitney test on the sum of and median of ranks to compare the T2-high versus T2-low group for each cytokine.

RESULTS

Of the 500 patients in the WATCH cohort, 388 biologic-naïve patients (either with at least one positive GINA 2021-defined T2-clinical parameter or negative to all four of these parameters) were included in the present analysis

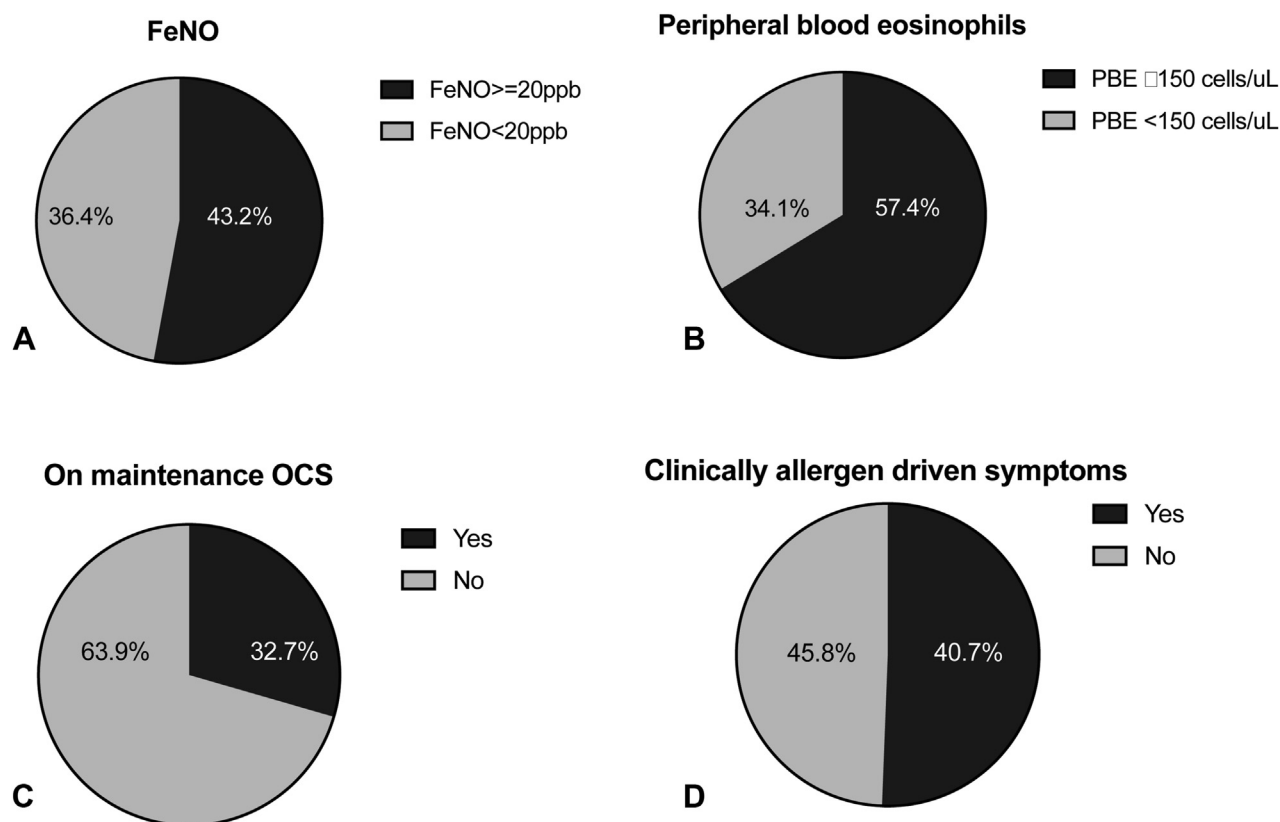


FIGURE 1. Relative distribution of type 2 (T2)-defining parameters in 360 patients classified as T2-high, defined by (A) FeNO, (B) peripheral blood eosinophils (PBE), (C) receiving maintenance oral corticosteroids (OCS), and (D) having clinically allergen-driven symptoms. Data are missing for (A) 67 patients, (B) 28 patients, (C) 11 patients, and (D) 48 patients.

(Figure E1). Biologic-naïve patients were comparable in core clinical characteristics to the rest of the WATCH cohort (see Table E2 in this article's Online Repository at www.jaci-inpractice.org).

Type 2—high status overwhelmingly predominates in patients with difficult-to-treat and severe asthma

We identified T2-high status in 93% (360 of 388) of biologic-naïve WATCH participants (Table I). Of these T2-high patients, 52.9% had raised FeNO, 66.3% had raised PBE, 29.5% were receiving mOCS, and 50.6% had clinically allergen driven symptoms (Figure 1, A–D). Of the 314 of 388 patients who had all four criteria measured, 4.8% ($n = 15$) fulfilled all four criteria, 36.9% ($n = 116$) had raised PBE and allergy-associated symptoms, 30.8% ($n = 97$) had raised PBE and FeNO, 30.3% ($n = 95$) had raised FeNO and allergy-associated symptoms, and 25.9% ($n = 81$) were receiving mOCS and still had raised FeNO or PBE (Figure 2). Peripheral blood eosinophils and FeNO are commonly used T2 biomarkers; 80% of patients ($n = 251$) had either biomarker raised at baseline, and PBE was historically raised in a further 30 patients.

Generally in clinical practice, higher thresholds for PBE and FeNO are used to identify patients with T2-high disease, especially when considering eligibility criteria for biologic therapy. We therefore reviewed the proportion of T2-high and T2-low asthma in the current cohort using PBE of 300 cells/ μ L or greater and FeNO of 25 ppb or greater (along with mOCS use

and allergic tendencies). This analysis revealed that 81% of patients (316 of 388) could be classified as T2-high (vs 93% classified as T2-high using the lower thresholds for PBE and FeNO). Omission of OCS use as a criterion for T2-high disease revealed 75% (291 of 388) who would still be classified as T2-high.

Using the previously defined GINA 2021 T2 classification, eight patients were classified as T2-high solely based on the need for mOCS. Deeper scrutiny of these patients showed that two had historical evidence of PBE ≥ 150 cells/ μ L, whereas one patient developed raised PBE of 150 cells/ μ L or greater during the follow-up after study enrollment. A further patient had confirmed sensitization to a perennial aeroallergen and was subsequently given anti-IgE therapy. This indicated the presence of other markers of T2-high disease in most of these patients. The remaining four patients did not show other or historical evidence of T2-high disease.

Similarities and differences in characteristics of T2-high and T2-low asthma patients

Patients classified as having T2-high status had a similar age of asthma onset as those who were classified as T2-low. Although there was a female predominance overall, the proportion of men was similar for T2-high and T2-low groups. Similarly, there were no differences in body mass index (BMI), or proportion of never-smokers between T2-high and T2-low asthma patients. The prevalence of other comorbidities commonly associated with

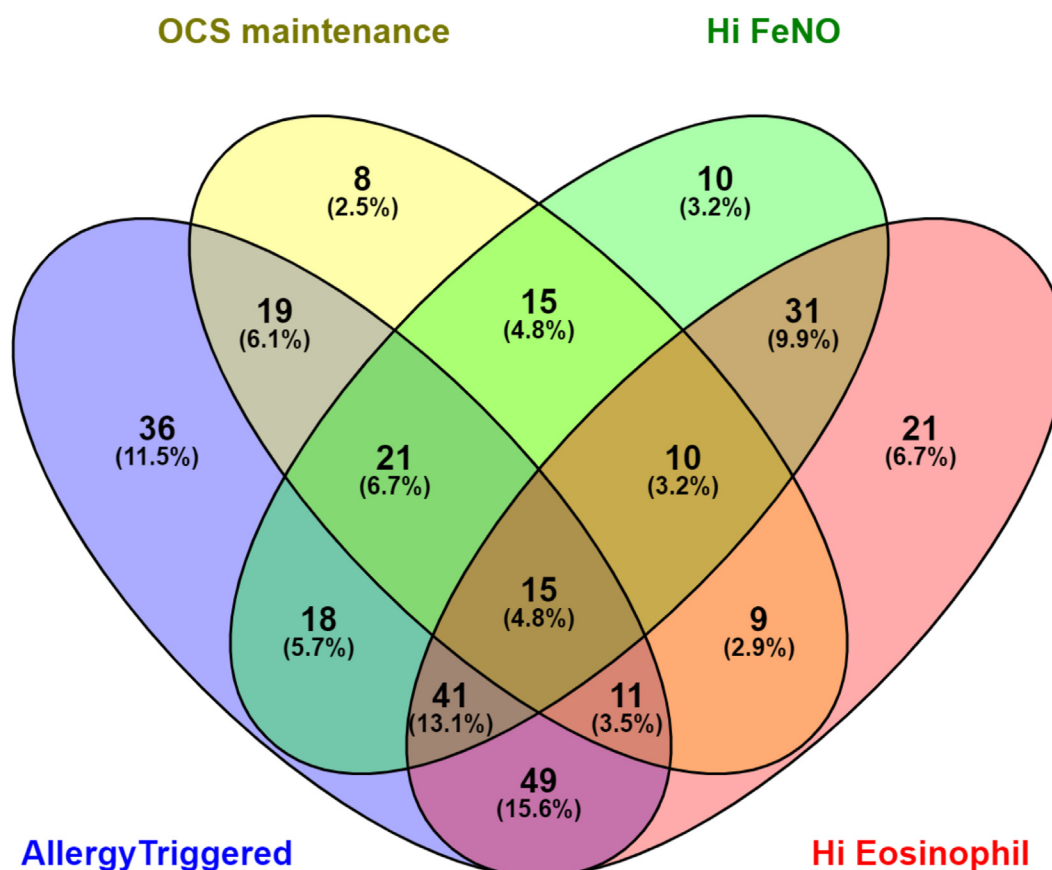


FIGURE 2. Overlap between the presence of four criteria used to define type 2–high disease status in 314 patients with difficult-to-treat and severe asthma: maintenance oral corticosteroids (OCS) use, FeNO of 20 parts per billion or greater (Hi FeNO), peripheral blood eosinophils of 150 cells/ μ L or greater (Hi eosinophil), and allergy-associated symptoms (AllergyTriggered).

TABLE II. Presence of comorbidities in patients classified as type 2 (T2)-high and T2-low status

Comorbidities	T2-high (n = 360)	T2-low (n = 28)	P
Nasal polyps (%/n)	24.3 (82/332)	15.4 (4/26)	.3
Depression (%/n)	38.1 (125/328)	33.3 (9/27)	.62
Anxiety (%/n)	34.8 (114/328)	24 (6/25)	.27
Dysfunctional breathing (%/n)	48.3 (166/344)	59.3 (16/27)	.27
Eczema (%/n)	23.9 (85/356)	14.8 (4/24)	.28
Obstructive sleep apnea (%/n)	7.4 (26/352)	7.4 (2/27)	1
Vocal cord dysfunction (%/n)	13.3 (43/324)	20 (5/25)	.35
Gastroesophageal reflux disease (%/n)	63.5 (221/348)	71.4 (20/28)	.4
Obesity (body mass index ≥ 30) (%/n)	46.9 (169/360)	50 (14/28)	.79

T2 status was classified based on the presence of blood eosinophils of 150 cells/ μ L or greater, FeNO of 20 parts per billion or greater, the need for maintenance oral corticosteroids, or allergen-driven asthma. *P* reflects χ^2 comparisons.

asthma, including eczema, gastroesophageal reflux disease, rhinitis, breathing pattern disorder, anxiety, bronchiectasis, and obesity, was similar in patients classified as T2-high or T2-low (Table II).

The T2-high asthma patients had significantly worse airflow limitation (FEV₁/FVC, 65.9% vs 74.6%) (Figure 3, A-C) and maximal expiratory flow of 25% to 75% (48.3% predicted vs 75.6% predicted) compared with patients with T2-low status at clinic (postbronchodilator) spirometry (Figure 3, D).

Measurements of gas exchange were similar in both groups (Figure 3, E and F; and Table E3 in this article's Online Repository at www.jaci-inpractice.org).

Blood levels of cytokines associated with allergic airway inflammation, IL-4, CCL2/monocyte chemoattractant protein-1 and CCL3/MIP-1 α , and IFN-gamma were significantly higher in the presence of T2-high status (Figure 4, A-D). However, the levels for IL-4, CCL3/MIP-1 α , and IFN-gamma were very low or at the limit of detection. Conversely, levels of other

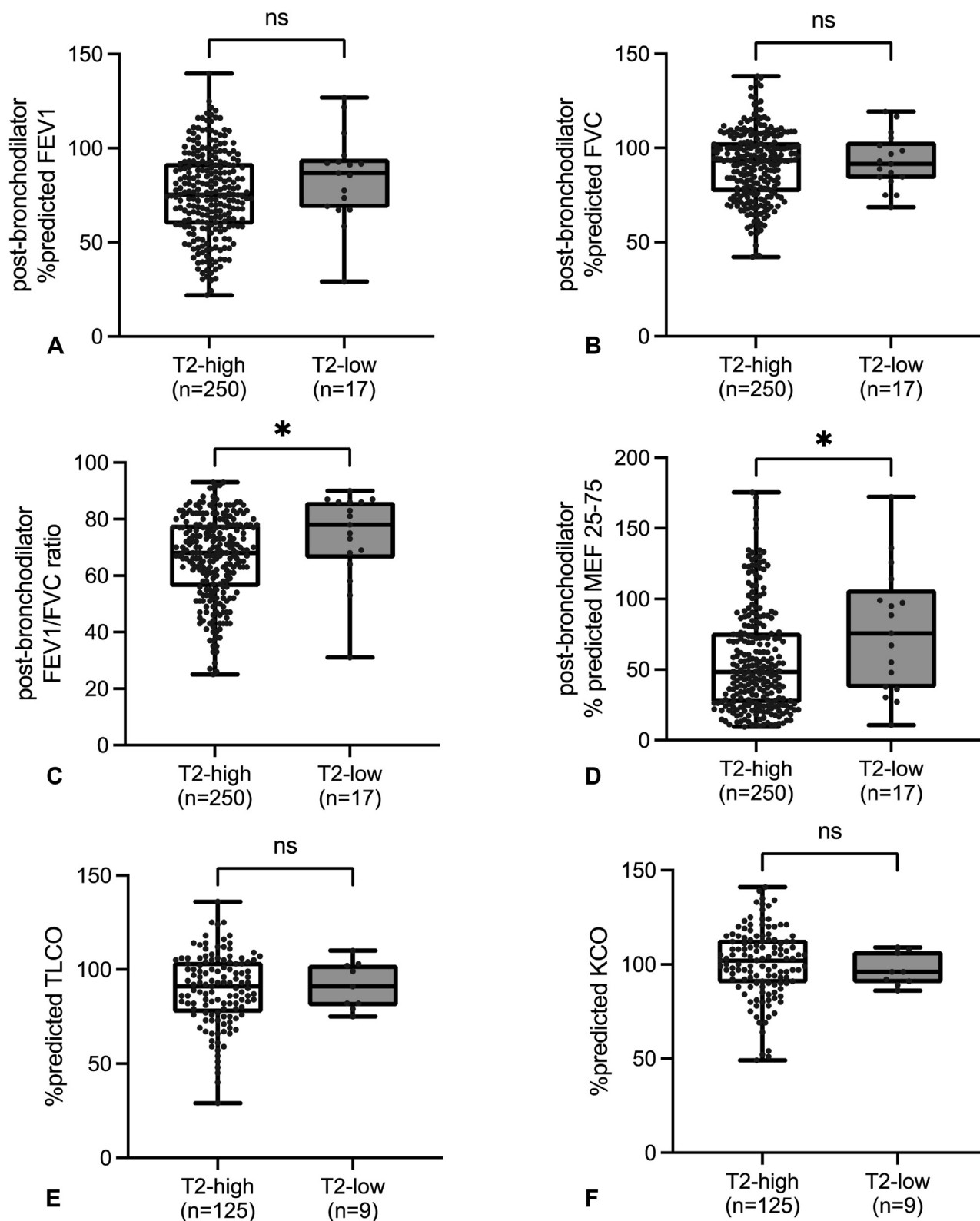


FIGURE 3. Lung function of patients characterized as type 2 (T2)-high and T2-low. (A) Postbronchodilator percent predicted FEV₁, (B) postbronchodilator percent predicted FVC, (C) FEV₁/FVC ratio, (D) postbronchodilator percent predicted maximal expiratory flow of 25% to 75%, (E) transfer factor for carbon monoxide (TLCO), and (F) transfer factor divided by the alveolar volume (KCO). **P* < .05. ns = not significant.

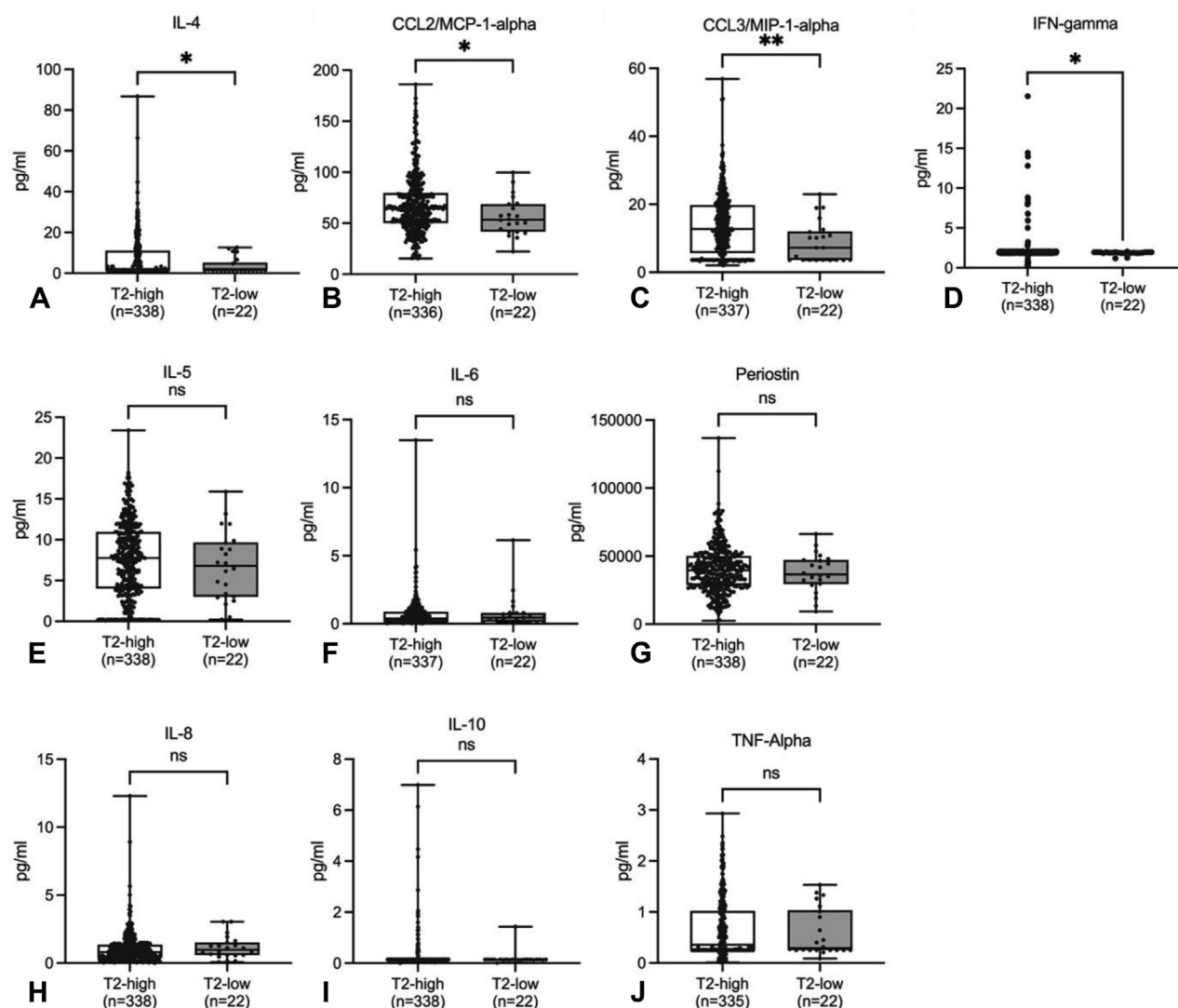


FIGURE 4. Expression of inflammatory cytokines in blood (plasma, pg/mL) in patients with asthma defined as type 2 (T2)-high or T2-low. (A) IL-4, (B) chemokine ligand (CCL)2/monocyte chemoattractant protein-1 (CCL2/MCP-1), (C) CCL3/macrophage inflammatory protein-1 α (MIP-1 α), (D) IL-5, (E) IL-6, (F) periostin, (G) IL-8, (H) IL-10, and (I) TNF- α (TNF-alpha).

T2-associated cytokines such as IL-5, periostin, IL-10, and non-T2 associated cytokines such as IL-6, IL-8, and TNF- α did not differ significantly by T2 status (Figure 4, E-J). Other potentially relevant inflammatory cytokines such as IL-13 and IL-17 were not detected or at the limit of detection (see Table E4 in this article's Online Repository at www.jaci-inpractice.org).

However, applying a false discovery rate correction showed no significant difference in any of the measured cytokines, which suggests that baseline levels of these cytokines did not discriminate between groups.

A significantly higher proportion of patients with T2-low asthma had been intubated for acute asthma (Table I) (28.6%, eight of 28 vs 11.1%, 40 of 360). Of the eight T2-low patients who had been acutely intubated, six (75%) had had raised PBE within the preceding 10 years, suggesting a masked background T2-high status. Furthermore, of the eight, five (62.5%) had a clinically diagnosed breathing pattern disorder, three (37.5%)

had diagnosed depression, two (25%) had diagnosed anxiety, and four (50%) were clinically obese (BMI 34.5 ± 9.8).

Other acute health care needs did not differ significantly by T2 status, including asthma exacerbations needing oral corticosteroids or acute asthma admissions in the preceding 12 months (Table I), nor did inhaled corticosteroid (ICS) dose.

After WATCH enrollment, subsequent initiation of asthma biologic therapy was significantly greater in patients defined as T2-high compared with T2-low status (Table I) (32.8% vs 10.7%). Biologics would not typically be given to patients with T2-low asthma. However, review of the three of 28 patients defined as T2-low who were subsequently given a biologic revealed that one patient developed raised PBE after enrollment and was therefore suitable for treatment with an anti-IL5/IL-5 receptor biologic. The other two began anti-IgE therapy. They were atopic upon skin prick testing but had not reported allergy-driven asthma or met other T2-classifying criteria at WATCH

TABLE III. Sputum characterization for type 2 (T2)-high and T2-low patients

Sputum inflammatory characteristics	T2-high (n = 112)	T2-low (n = 5)	P
Median sputum eosinophils (%)	1.9	0.12	.005
Median sputum neutrophils (%)	41.7	67.62	.19
Percentage of patients with $\geq 2\%$ sputum eosinophils	50 (56/112)	0	
Percentage of patients with $\geq 61\%$ sputum neutrophils	25 (28/112)	60 (3/5)	.11
Percentage of patients with mixed granulocytic disease ($>2\%$ eosinophils and $\geq 61\%$ neutrophils)	11.6 (13/112)	0	$<.01$

T2 status was classified based on the presence of blood eosinophils of 150 cells/ μ L or greater, FeNO of 20 parts per billion or greater, the need for maintenance oral corticosteroids, allergen-driven asthma, or sputum eosinophils of 2% or greater. *P* was calculated using Fisher exact test for categorical variables and Mann-Whitney test for continuous variables.

enrollment. Furthermore, all three patients had had historical evidence of raised PBE (≥ 300 cells/ μ L) in the prior decade.

Unmasking T2-high signatures in patients with apparent T2-low status

Type 2–low status was defined in 7% of patients (28 of 388) who did not fit criteria for T2-high asthma. This group was examined in more detail for potential masked features suggestive of T2-high status. Three quarters of non-T2 patients (21 of 28) demonstrated raised PBE (≥ 150 cells/ μ L) within the preceding 10 years. Therefore, in the current cohort of 388 patients, only seven (1.8%) did not have a current or historical T2 signal. Furthermore, of those seven patients, three were receiving treatment with very high-dose ICS ($\geq 2,000$ μ g beclomethasone dipropionate equivalent), treatment that could have suppressed T2 signal at WATCH enrollment.

High prevalence of T2-high asthma status is independent of sputum analysis

We obtained induced sputum from 117 of the 388 WATCH participants (Table III). Forty-eight percent (56 of 117) had eosinophils of 2% or greater and 26% (31 of 117) had neutrophils of 61% or greater. Of the 87 patients with raised sputum eosinophils or neutrophils, 13 (11% of 117) had mixed granulocytic sputum (eosinophils of 2% or greater and neutrophils of 61% or greater). Moreover, 37% (43 of 117) had paucigranulocytic sputum.

In addition, 96% of patients (111 of 117) met our applied definition of T2-high asthma. Using sputum eosinophilia as an additional defining criterion alongside the other four components used to define T2 status identified just one more patient with T2-high asthma. Historically, this patient had PBE of 150 cells/ μ L or greater in the preceding decade.

Of the 112 patients classified as T2-high, 50% (56 of 112) had sputum eosinophils of 2% or greater and 25% (28 of 112) had 61% or greater neutrophils. Of the 84 of 112 patients with raised sputum eosinophils or neutrophils, 13 had mixed granulocytic sputum. Furthermore, T2-high patients accounted for 93% of those with sputum neutrophilia (Table III).

DISCUSSION

Using a multicomponent classification of T2 inflammation in a large cohort of patients with difficult-to-treat and severe asthma, we showed that 93% of patients had T2-high status whereas only 1.8% of patients had no T2 signal after thorough longitudinal characterization.

The criteria we used to define T2 status are closely aligned to those previously proposed by GINA¹⁷; most are readily available

clinical variables. However, the GINA 2021 criteria included sputum eosinophilia, a test that is not readily available for all patients. The WATCH cohort included a group of patients (n = 117) who underwent sputum induction. Therefore, we evaluated the T2-high/T2-low split with and without using sputum eosinophilia as a defining criterion. This analysis revealed that using sputum analysis to identify T2-high status did not increase the number of patients identified as T2-high in addition to those identified by readily available clinical and biomarker variables. In the current cohort, it added only one patient to the T2-high group. Furthermore, this patient had historically raised PBE, which suggests that an underlying T2-high phenotype was probably suppressed at the time of enrollment. Furthermore, it emphasizes the importance of a longitudinal evaluation of biomarkers. Therefore, although sputum induction is considered by many to be the reference standard investigation for airway inflammation, our findings, coupled with the finding that PBEs are an accurate surrogate marker for sputum eosinophils, with a reported area under the curve of the receiver operating characteristic of 89%,¹⁸ supports a move away from placing heavy emphasis on sputum analysis to define T2 status in daily clinical practice.

Debate remains regarding the prevalence of T2-high and T2-low status in patients with difficult-to-treat and severe asthma. Using data from an international severe asthma registry and a multicomponent algorithm that included the biomarkers PBE and FeNO, use of medication including mOCS and anti-IL5 treatment, the presence of nasal polyps, and adult onset disease, Heaney et al¹⁰ showed that 83.8% of patients with severe asthma were most likely to have an eosinophilic phenotype. On the other hand, the UK Severe Asthma Registry identified 44.6% patients as T2-high (based on an FeNO of 25 ppb or greater and a PBE of 150 cells/ μ L or greater) and 9.4% as T2-low.⁷ However, the historical highest median PBE count in the T2-low group was 350 cells/ μ L, which indicated that many from that group were probably T2-high but had suppressed biomarkers at the time of sampling. The prevalence of T2-high status was higher in the current cohort. This is likely because of the use of a multicomponent definition, the use of lower defining cutoffs for PBE as recommended by GINA, and the incorporation of historical PBE values.

Overreliance on a cross-sectional evaluation of T2 biomarkers is hampered by inherent disease variability and the suppression of biomarkers by ICS and oral corticosteroids. Our analysis adds to the growing body of literature supporting a longitudinal consideration of these biomarkers when evaluating the disease phenotype.^{9,11} When using baseline clinical variables, 7% of patients in the current cohort (28 of 388) did not show a

T2-high signal. This proportion dropped to only 1.8% (seven of 388) when we included longitudinal PBE values. We previously demonstrated that persistent historical PBE is associated with worse lung function,⁹ which further highlights the relevance of longitudinal review for providing insight into disease progression.

Frossing et al¹⁹ reported that 70% of 166 patients in that severe asthma cohort had elevation of at least one T2 biomarker (FeNO, PBE, and total IgE); 31% had two or more elevated biomarkers. In the current cohort, a similar proportion (up to 37% of patients) had two or more elevated T2 biomarkers. Most patients receiving mOCS (75%) had raised biomarkers (PBE and FeNO) despite using mOCS. This may reflect the underlying disease severity and potential steroid resistance, but it could also result from treatment nonadherence to ICS and oral corticosteroids. In this study, we did not collect data on the medicine–possession ratio, and so we were unable to corroborate the use of medication.

There is no agreed-upon consensus regarding the definition of T2-high asthma, but criteria recommended by GINA 2021 are pragmatic and incorporate clinical and biomarker variables. Raised PBE and FeNO are uncontested T2 biomarkers, although both are time- and treatment-dependent. The detailed clinical characterization possible within the current analysis enabled careful scrutiny of patients who were classified as T2-low. Three-quarters of T2-low patients had evidence of historically raised PBE that indicated the presence of a T2 signal. Therefore, less than 2% of patients with difficult-to-treat and severe asthma (seven of 388) could be classified as T2-low. The Global Initiative for Asthma 2022 has updated the classification of T2 inflammation in severe asthma, and mOCS use is no longer included as a defining criterion. Within the current cohort, only seven of 388 patients (1.8%) were classified as T2-high based solely on mOCS use; only three of those patients had no evidence of historically raised PBE or atopic sensitization. This suggests that the contribution of this criterion in identifying T2-high status is low and may rightly be removed from the GINA 2022 classification.

Type 2–low asthma was previously associated with higher medication requirements, obesity, significant symptomatology, multimorbidity, and increased health care burden.^{20–23} Registry studies suggest that patients with T2-high or eosinophilic asthma have a higher age at asthma onset.^{7,10} However, in the current cohort, a clinical characterization of patients identified as T2-high and T2-low showed broad comparability with no difference in ICS dose, BMI, or age at asthma onset. Similarly, the presence of comorbidities usually associated with high symptomatology, such as breathing pattern disorder, anxiety, and depression, was similar across the two groups. In particular, hospitalization for asthma and exacerbations treated with corticosteroids were similar in T2-high and T2-low patients. Although the overall small number of T2-low patients may partly explain why we did not detect a difference, we also show that most patients classified as T2-low using baseline PBE had evidence of historically raised PBE and so may have had T2-high disease that was simply masked at the time of sampling. Therefore, the absence of significant clinical differences between cohorts may reflect that T2-high signal was present in almost all patients with difficult-to-treat and severe asthma. This concept is supported by our blood cytokine data, which showed that many typical T2-associated cytokines did not differ between the patient groups.

Type 2–low asthma is frequently described as neutrophilic asthma.²³ In the current cohort, a quarter of patients classified as

T2-high had elevated sputum neutrophils, whereas most participants with sputum neutrophilia (93%) were T2-high. Levels of typical neutrophil-associated cytokines and chemokines such as IL-6 and IL-8 were similar in both T2-high and T2-low patients. This suggests that the presence of sputum neutrophilia should not be viewed as an indicator of disease phenotype, but instead should prompt an investigation of reasons for the presence of this inflammatory cell in the sputum (eg, bacterial colonization). We demonstrated that colonization of the airways in patients with severe asthma with potentially pathogenic bacteria (*Moraxella catarrhalis*, *Haemophilus sp*, and *Streptococcus sp*) correlates with bronchoalveolar lavage neutrophilia and IL-13 levels.²⁴ Furthermore, we recently added further mechanistic insight into how bacterial pathogens such as *Haemophilus influenzae* drive neutrophilic inflammation in severe asthma, demonstrating associations with the persistent infection of alveolar macrophages.²⁵ Collectively, these findings suggest that airway neutrophilia is better considered to reflect and be a consequence of airway dysbiosis or infection rather than being definitive of T2-low status, and that clinicians should target treatment at the airway microbiome rather than attempt to reduce airway neutrophilia. Recently, the association between airway neutrophilia and dysbiosis was extended to blood neutrophils, and increased blood neutrophil levels were linked to more prescription for antibiotic courses over a 12-year period in people with asthma.²⁶

A strength of our study is that it reflects a real-world UK cohort of biologic-naïve patients, excluding the confounding effects of biologics on baseline clinical and biomarker characteristics. Detailed clinical characterization enabled us to scrutinize the presence of clinical variables in subgroups of patients. A potential limitation of our T2 classification was that it used largely cross-sectional criteria, apart from longitudinal PBE levels. The use of broader longitudinal data might have provided greater insight, although the multicomponent nature of our scrutiny will have compensated to a degree. Another limitation of our study is that it focused on a relatively homogeneous White population. Replication in other ethnic populations is required.

Our results demonstrate that in a UK population of difficult-to-treat and severe asthma, most patients had T2-high status and there was a near-absence of T2-low asthma. Type 2–high signals typically reveal corticosteroid-responsive disease and our results support the use of inhaled corticosteroids in all patients with asthma, regardless of underlying severity. Our results also allow us to propose that rather than dichotomizing asthma as T2-high or T2-low, it should be considered a T2-inflammatory disease with periods when patients are biomarker-low or biomarker-high. The designation of T2-low status should be made in clinical practice only after stringent multidimensional and longitudinal characterization. This implies that with careful characterization, most difficult-to-treat and severe asthma patients would suit higher-level biologic therapies when needed. Finally, to understand the clinical diversity of difficult-to-treat and severe asthma better, there is a need to look beyond a simple T2-high/T2-low paradigm and identify the role of additional pathophysiologic pathways alongside a holistic view of the patient.

Acknowledgments

The authors wish to thank the patients who are participating in this study. They also wish to acknowledge the contributions of the wider Wessex Asthma Cohort of difficult asthma study team,

including Matthew Harvey, Mae Felongco, Helen Wheeler, June Law, Yvette Thirlwall, Kim Bentley, Laura Presland, Frances Mitchell, Yueqing Cheng, Josune Olza Meneses, Wei Chern Gavin Fong, Kerry Day, and David Hill, and with particular thanks to Clair Barber for sputum processing.

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TABLE E1. Wessex Asthma Cohort of difficult asthma cohort inclusion and exclusion criteria

Inclusion criteria	All patients who attend adult or transitional regional asthma clinic at University Hospital Foundation Trust or satellite outreach clinics on Isle of Wight and are managed with “high-dose therapies” and/or “continuous or frequent use of oral steroids,” according to British Thoracic Society Adult Asthma Management guidelines 2016 Be able to provide informed consent
Exclusion criteria	Patients who attend adult or transitional regional asthma clinic as University Hospital Southampton National Health Service Foundation Trust or satellite outreach clinics on Isle of Wight but are not managed with “high-dose therapies” and/or “continuous or frequent use of oral steroids,” according to British Thoracic Society Adult Asthma Management Guidelines 2016.

TABLE E2. Clinical characteristics of patients excluded from type 2 analysis because they were receiving biologic therapy at Wessex Asthma Cohort of difficult asthma enrollment, compared with included (biologic-naïve) patients

Variable	Biologic-naïve (n = 388)	Receiving biologics (n = 79)	P
Current age, y	52 (24)	52 (25)	.96
Age at diagnosis, y	21* (36)	14† (37)	.20
Patients with early-onset asthma (aged ≤18 y) (%)	44.1 (171/388)	51.9 (41/79)	.2
Sex (male:female)	130:258	35:44	.07
Body mass index	29.6 (10.4)	29.2 (7.7)	.90
Never-smokers (% [n])	51.3‡ (199/387)	60.8 (47/79)	.13
Patients intubated for acute asthma (% [n])	12.4 (48/388)	17.7 (14/79)	.2
Percentage of patients with one or more asthma-related hospitalization in past 12 mo (% [n])	29.4‡ (113/385)	29.1 (23/79)	.97
Courses of oral corticosteroids in past 12 mo (% [n])	3‡ (4)	3§ (3)	.34
Dose of inhaled corticosteroid (beclomethasone dipropionate equivalent, µg)	1,500 (2,000)	1,000¶ (2,000)	.77
Peak blood eosinophil count (in past 10 y)	0.4§ (0.6)	0.6** (0.6)	.2

Values are median plus interquartile range unless otherwise specified.

*Data were unavailable for 17 patients.

†Data were unavailable for two patients.

‡Data were unavailable for 42 patients.

§Data were unavailable for six patients.

||Data were unavailable for 31 patients.

¶Data were unavailable for eight patients.

**Data were unavailable for four patients.

††Data were unavailable for one patient.

TABLE E3. Comparison of lung function parameters between patients classified as type 2 (T2)-high and T2-low

Lung function parameters	T2-high (n = 250)	T2-low (n = 17)	P
Postbronchodilator percent predicted FEV ₁	75.3 (32.7)	86.9 (26.3)	.14
Postbronchodilator percent predicted FVC	93.2 (26.5)	91.2 (19.8)	.82
Postbronchodilator FEV ₁ /FVC ratio	68 (22)	78 (20)	.02*
Postbronchodilator percent predicted maximal expiratory flow of 25% to 75%	48.3 (50)	75.6 (69.7)	.04*
Percent predicted transfer factor of lung	91 (27)	91 (22)	.82
Percent predicted carbon monoxide transfer coefficient	102 (23)	96 (17)	.34

Values are shown as medians (interquartile ranges).

*Significant.

TABLE E4. Comparison of cytokine levels in blood (plasma) between patients classified as type 2 (T2)-high and T2-low

Cytokine level, pg/mL	T2-high (n = 335-338)	T2-low (n = 22)	P
C-C motif chemokine ligand 2/monocyte chemoattractant protein-1	64.34 (29.53)	53.42 (27.08)	.048*
C-C motif chemokine ligand 3/macrophage inflammatory protein-1 α	12.72 (14.14)	7.23 (8.43)	.008*
IL-4 $\ddagger$	1.55 (9.6)	1.51 (3.72)	.047*
IL-5	7.76 (6.94)	6.81 (12.88)	.44
IL-6 $\ddagger$	0.38 (0.74)	0.46 (0.65)	.65
IL-8 $\ddagger$	0.81 (0.97)	0.98 (0.95)	.3
IL-10 $\ddagger$	0.14 (0.01)	0.14 (0.01)	.85
IL-13	ND	ND	—
IL-17 $\ddagger$	0.47 (0.05)	0.47 (0.06)	.2342
IFN-gamma $\ddagger$	1.94 (0.14)	1.89 (0.1)	.01*
Periostin	39,509 (21599)	36,599 (17735)	.68
TNF- α $\ddagger$	0.36 (0.77)	0.29 (1.28)	.85

ND, not detectable.

*Significant.

 $\ddagger$ Very low or at limit of detection.

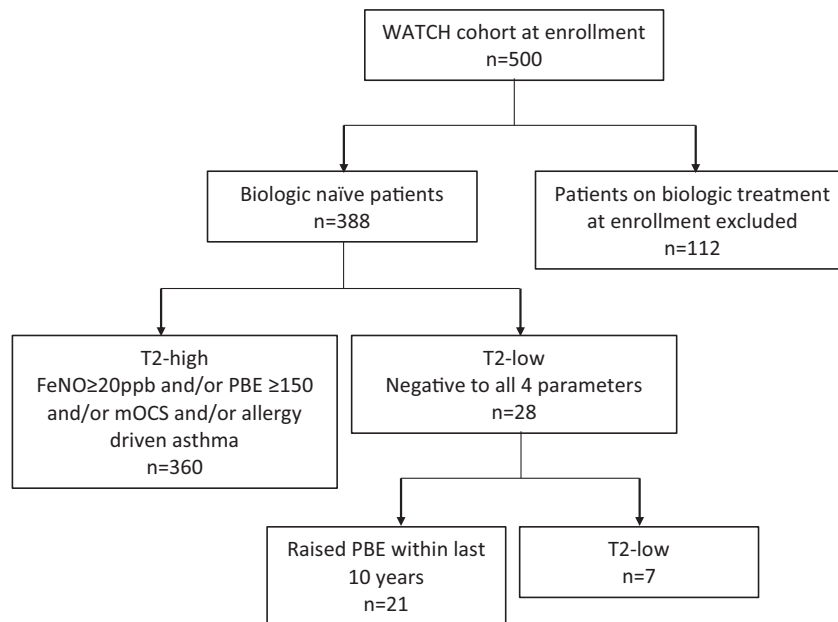


FIGURE E1. Flowchart of patients from Wessex Asthma Cohort of difficult asthma (WATCH) study who were included in the current analysis. *mOCS*, maintenance oral corticosteroid; *PBE*, peripheral blood eosinophil; *ppb*, parts per billion; *T2*, type 2.