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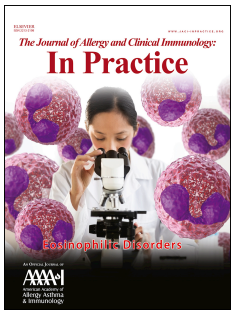

Comprehensive characterisation of difficult-to-treat asthma reveals near absence of T2-low status

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Title:

Comprehensive characterisation of difficult-to-treat asthma reveals near absence of T2-low status

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Conflict of Interest Statement

Dr. Hitasha Rupani reports speaker and consultancy fees from AstraZeneca, GlaxoSmithKline, Teva, Novartis and Chiesi. Professor Peter Howarth reports employment by GSK outside of the submitted work. Dr. Adnan Azim reports employment from Astra Zeneca outside of the submitted work. Mohammed Aref Kyyaly, Rana Abadalkareen, Anna Freeman, Paddy Dennison, Ratko Djukanovic, Pandurangan Vijayanand, Gregory Seumois, S Hasan Arshad, Hans Michael Haitchi, Ramesh J Kurukulaaratchy declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Abbreviations:

BMI	Body mass indx
FeNO	Fractional exhaled nitroen oxde
FEV1	Forced expiratory volume in 1 second
FVC	Forced vtal capacity
GINA	Global Initiative for Asthma
ICS	Inhaled corticosteroid
IL	Interleukin
mOCS	Maintenance oral corticosteroid
NHS	National Health Service
PBE	Peripheral blood eosinophil
T2	Type-2 inflammation
WATCH	Wessex Asthma Cohort of difficult asthma

Abstract:

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 real-world understanding of this T2 paradigm in difficult-to-treat/ severe asthma remains limited.

Objectives

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

Methods

388 biologic naïve patients from the Wessex Asthma Cohort of difficult asthma (WATCH) study, United Kingdom, were evaluated. T2-high asthma was defined as fractional exhaled nitric oxide (FeNO)≥20ppb and/or peripheral blood eosinophils (PBE) ≥150 cells/ul and/or need for maintenance oral corticosteroids and/or clinically allergy-driven asthma.

Results

This multicomponent assessment identified T2-high asthma in 93% (360/388) of patients. 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 to T2-low patients (FEV_1/FVC 65.9% vs 74.6%). 75% patients defined as T2-low asthma had raised PBE within the preceding 10-years, leaving only 7 patients (1.8%) who never had T2-signals. Incorporation of sputum eosinophilia ≥2% into the multicomponent definition in a subset of 117 patients with induced sputum data similarly found that 96% (112/117) met criteria for T2-high asthma of which 50% (56/112), had sputum eosinophils ≥2%.

Conclusion

Almost all patients with difficult-to-treat asthma have T2-high disease with <2% of patients never displaying T2-defining criteria. This highlights a need to comprehensively assess T2 status in clinical practice before labelling a patient with difficult-to-treat asthma as T2-low.

Abstract: 250 words

ClinicalTrials.gov Registration Number

NCT03996590

Highlights Box

- **What is already known about this topic?**

Difficult-to-treat/ severe asthma is stratified into T2-high and low endotypes but their 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 characterisation 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 emphasises the need to undertake comprehensive assessment of T2-status in difficult-to-treat and severe asthma in order to accurately guide treatment options.

Introduction

Airway inflammation is the hallmark of asthma and underscores the typical features of the disease: airway remodelling and hyperresponsiveness and variable airflow limitation. In recent years, endotypic classification of asthma has focused on the paradigm of type 2-inflammation high (T2-high) or type 2-inflammation low (T2-low) status. While there is no agreed consensus on the definition of T2-high status, it is widely acknowledged as being orchestrated by key interleukins (IL) including IL-4, IL-5 and IL-13 and characterized by eosinophilic inflammation. T2-low asthma is less well understood and frequently loosely defined as the absence of prominent T2-pathway signatures.[1] Patients with T2-low asthma have been characterised 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 as it has prognostic and therapeutic implications in an evolving landscape of effective T2-focused biological therapies.[3, 4] This is

particularly important in difficult-to-treat and severe asthma which is characterized by high symptom burden and poor disease control despite high dose inhaled steroid therapy.[5] Measuring sputum eosinophils is often considered the gold standard test for airway inflammation and sputum eosinophilia reliably predicts T2 gene expression in induced sputum.[6] However, sputum induction is impractical in routine clinical practice. Therefore several non-invasive T2-markers have been explored including serum IgE, peripheral blood eosinophils (PBE) and fractional exhaled nitric oxide (FeNO). The UK Severe Asthma Registry used raised PBE and FeNO to define T2-high asthma and reported that 45% of their 2225 patients can 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 (ISAR) used a multi-component eosinophil gradient algorithm to similarly show that 83.8% of severe asthma patients are 'most likely' to have eosinophilic inflammation[10] (which is predominantly T2-driven). Subsequently, we applied this algorithm to our difficult-to-treat asthma cohort and incorporated longitudinal PBE measurements to show that a comparable proportion of patients, 77.4%, can be classified as 'most likely' to have an eosinophilic phenotype.[11]

However, there remains ongoing debate on the proportionate distribution and nature of T2-high and T2-low disease in biologic naïve patients with difficult-to-treat and severe asthma. In this paper, we therefore sought to: (i) assess the multi-component definition of T2-status proposed by GINA 2021 using the 4 clinical elements of that definition (PBE ≥150 cells/ul and/or FeNO≥20ppb and/or need for daily maintenance corticosteroids [mOCS] and/or clinically allergy driven asthma) in this group of patients, (ii) understand the clinical and

pathophysiological characteristics of patients that fall into T2-high and T2-low asthma groups, utilizing longitudinal data including PBE measures to verify T2-status and (iii) 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) is a prospective observational study of well-characterised 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 (BTS) Adult Asthma Management Guidelines 2016.[12] All patients provided written informed consent (REC reference 14/WM/1226). Detailed study methodology has been previously published[8] and we have summarised the inclusion and exclusion criteria along with a study flow chart in the supplement (**Supplementary Table E1**, **Supplementary Figure E1**).

As this was a real-world cohort, the presence of comorbidites or other medical conditions e.g. diabetes or ischaemic heart disease, did not preclude patients from being enrolled. We have previously shown that the WATCH cohort is broadly representative of the wider clinic population that it is drawn from and there are no major differences between the WATCH-cohort and other patients in our difficult asthma clinic apart from a higher prevalence of mOCS dependence.[13] Clinical data including detailed clinical, health and disease-related questionnaires, anthropometry, allergy skin prick testing, blood tests and lung function

testing were captured at enrolment to the WATCH study. Only biologic naïve patients were included in the present 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 >2% cut off for sputum eosinophils and >61% cut off for sputum neutrophils. Clinically requested blood tests were processed by the fully accredited hospital pathology laboratory, compliant to ISO142819 standards.

Blood cytokine levels (pg/ml) were measured in plasma from 360 WATCH study participants using a human magnetic Luminex® multiplex assay for C-C Motif Chemokine Ligand 2/Monocyte chemoattractant protein-1 (CCL2/MCP-1), C-C Motif Chemokine Ligand 3/Macrophage Inflammotry Protein-1 Alpha (CCL3/MIP-1-Alpha), Interleukin (IL)-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, interferon (IFN)-gamma, Periostin (POSTN) and Tumor Necrosis Factor-Alpha (TNF-Alpha) 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 and pipetted all the 360 available plasma samples at the same session and sequentially ran the five 96-well plates. Each plate contained the 8 standard curve dilutions in duplicate wells and 2 blank wells.

T2-high status was defined using the 4 clinical measures of the multicomponent algorithm proposed by GINA 2021, as presence of any of the following clinically relevant parameters:

i. FeNO≥20ppb and/or
ii. PBE ≥150cells/uL and/or
iii. Need for mOCS and/or

iv. Clinically allergy driven asthma (defined as positive skin test to any aeroallergen plus '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.[14] Only baseline FeNO measurements were available.

Statistical analysis

Statistical analysis was performed using SPSS 25 (IBM, SPSS Inc., NY, USA), GraphPad Prism 9 (Graphpad Software, LLC, La Jolla, California, USA), Venny's on-line tool[15] and R (Vienna, Austria). Continuous clinical variables are presented as median plus interquartile range (IQR) and categorical variables as frequencies (percentages). Between group differences were assessed by Mann Whitney, Kruskal Wallis, Chi Squared or Fisher's 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 the 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. As these tests showed no significant differences, we used the non-parametric Mann-Whitney test on sum of and median ranks to compare the T2-high versus the T2-low group for each cytokine.

Results

Of the 500 patients in the WATCH cohort, 388 biologic naïve patients (either with at least 1 positive GINA 2021 defined T2-clinical parameter or negative to all 4 of these parameters)

were included in the present analysis (**Supplementary Figure E1**). Biologic naïve patients were comparable in core clinical characteristics to the rest of the WATCH cohort (**Supplementary Table E2**).

T2-high status overwhelmingly predominates in patients with difficult-to-treat and severe asthma:

T2-high status was identified in 93% (360/388) of the biologic-naïve WATCH participants (**Table 1**). Of these T2-high patients, 52.9% had a raised FeNO, 66.3% had raised PBE, 29.5% were on mOCS and 50.6% had clinically allergen driven symptoms (**Figure 1,A-D**). Of the 314/388 patients that had all 4 criteria measured, 4.8% (n=15) fulfilled all 4 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 on mOCS and still had a raised FeNO or PBE (**Figure 2**). PBE and FeNO are commonly used T2-biomarkers and 80% (n=251) of patients had either one biomarker raised at baseline with PBE 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 our cohort using PBE ≥300 cells/l and FeNO≥25ppb (along with mOCS use and allergic tendencies). This analysis revealed that 81% of patients (316/388) could be classified as T2-high (versus 93% classified as T2-high using the lower thresholds for PBE and FeNO). Omission of OCS use as a criteria for T2-high disease revealed 75% (291/388) 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 2 had historical evidence of PBE ≥150 cells/μl, while 1 patient developed raised PBE ≥150 cells/μL during the follow-up period after study enrolment. A further 1 patient was found to have confirmed sensitization to a perennial aeroallergen and was subsequently commenced on anti-IgE therapy indicating the presence of other markers of T2-high disease in most of these patients. The remaining 4 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 T2-high status had similar age of asthma onset as those who were classified as T2-low. While 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 BMI, or proportion of never smokers between T2-high and T2-low asthma patients. Prevalence of other comorbidities commonly associated with asthma, including eczema, gastro-oesophageal reflux disease (GORD), rhinitis, breathing pattern disorder, anxiety, bronchiectasis and obesity was similar in patients classified as T2-high or T2-low (**Table 2**).

T2-high asthma patients had significantly worse airflow limitation (FEV_1/FVC 65.9% vs 74.6%) (**Figure 3, A-C**) and MEF_{25-75} (48.3% predicted vs 75.6% predicted) compared to patients with T2-low status at clinic (post-bronchodilator) spirometry (**Figure 3,D**). Measurements of gas exchange were similar in both groups (**Figure 3, E and F**) (**Supplementary Table E3**).

Blood levels of cytokines associated with allergic airway inflammation, IL-4, CCL2/MCP-1 and CCL3/MIP-1 alpha, and IFN-gamma were significantly higher in the presence of T2-high status (**Figure 4,A-D**). However, the levels for IL-4, CCL3/MIP1-alpha and IFN-gamma were very low or at the limit of detection. Conversely, levels of other 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 (**Supplementary Table E4**).

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

A significantly higher proportion of patients with T2-low asthma had ever been intubated for acute asthma (**Table 1**, 28.6%, 8/28 vs 11.1%, 40/360). Of the 8 T2-low patients who had been acutely intubated, 6/8 (75%) had raised PBE within the preceding 10-years, suggesting a masked background T2-high status. Furthermore 5/8 (62.5%) had clinically diagnosed breathing pattern disorder, 3/8 (37.5%) had diagnosed depression, 2/8 (25%) had diagnosed anxiety and 4/8 (50%) were clinically obese (BMI 34.5$\pm$9.8).

Other acute healthcare needs did not differ significantly by T2-status including asthma exacerbations needing oral corticosteroids or acute asthma admissions in the preceding 12 months (**Table 1**); nor did ICS dose.

Following WATCH enrolment, subsequent initiation of asthma biologic therapy was significantly greater in patients defined as T2-high compared to T2-low status (**Table 1**, 32.8% vs 10.7%). Biologics would not typically be started in patients with T2-low asthma. However, review of the 3/28 T2-low defined patients subsequently commenced on a biologic revealed that 1 patient developed raised PBE following enrolment and was therefore suitable for treatment with an anti-IL5/IL-5 receptor biologic. The other 2 were commenced on anti-IgE therapy. They were atopic on skin prick testing but had not reported allergy driven asthma or met any of the other T2 classifying criteria at WATCH enrolment. Furthermore, all 3 patients had historical evidence of raised PBE (≥300 cells/μL) in the prior decade.

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

T2-low status was defined in 7% (28/388) patients 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 (21/28) demonstrated raised PBE (≥150 cells/μL) within the preceding 10-years. Therefore, in our cohort of 388 patients only 7 patients (1.8%) did not have a current or historical T2-signal. Furthermore, of these 7 patients, 3 patients were on treatment with very high dose ICS (≥2000mcg BDP equivalent)-treatment that could have suppressed T2-signal at WATCH enrolment.

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

Induced sputum was obtained from 117 of these 388 WATCH participants (**Table 3**). 48% (56/117) had eosinophils ≥2%, 26% (31/117) had neutrophils ≥61%. Of the 87 patients with raised sputum eosinophils or neutrophils, 13 patients (11% of 117) had mixed granulocytic

sputum (eosinophils≥2% and neutrophils≥61%). 37% (43/117) had paucigranulocytic sputum.

96% (111/117) met our applied definition of T2-high asthma. Using sputum eosinophilia as an additional defining criterion alongside the other 4 components used to define T2-status identified just 1 more patient as having T2-high asthma. Of note, historically, this patient had PBE ≥150 cells/μL in the preceding decade.

Of the 112 patients classified as T2-high, 50% (56/112) had sputum eosinophils ≥2%, 25% (28/112) had ≥61% neutrophils. Of the 84/112 patients with raised spuum eosinophils or neutrophils, 13 had mixed granulocytic sputum. Furthermore, T2-high patients accounted for 93% of those with sputum neutrophilia (**Table 3**).

Discussion

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

The criteria we used to define T2-status are closely aligned to those previously proposed by GINA[16] and most are readily available clinical variables. However, the GINA 2021 criteria also 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 and 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 does not increase the number of patients identified as T2-high, over and above those identified by readily available clinical and biomarker variables. In fact, in our cohort it only added one additional patient to the 'T2-high' group. Furthermore, this patient had historically raised PBE, suggesting an underlying T2-high phenotype that was probably suppressed at the time of enrolment and further emphasises the importance of longitudinal evaluation of biomarkers. Therefore, while sputum induction is considered by many to be the gold-standard investigation for airway inflammation, our findings, coupled with the finding that PBE are an accurate surrogate marker for sputum eosinophils with a reported ROC AUC of 89%[17] supports a move away from placing heavy emphasis on sputum analysis to define T2-status in daily clinical practice.

There remains ongoing debate on 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, presence of nasal polyps and adult onset disease, Heaney et al showed that 83.8% of patients with severe asthma are most likely to have an eosinophilic phenotype[10] while the UK Severe Asthma Registry identified 44.6% patients as T2-high (defined based on FeNO≥25ppb and PBE ≥150 cells/μL) and 9.4% as T2-low.[7] However, the historical highest median PBE count in the T2-low group was 350 cells/μL indicating that many from this group were probably T2-high but had suppressed biomarkers at the time of sampling. The prevalence of T2-high status was higher in our cohort and this is likely to be attributed to utilisation of a multi-component definition, use of

lower defining cut-offs for PBE as recommended by GINA, and incorporation of historical PBE values.

Over-reliance on cross-sectional evaluation of T2-biomarkers is hampered by inherent disease variability and suppression of biomarkers by inhaled and oral corticosteroids. Our analysis adds to the growing body of literature supporting longitudinal consideration of these biomarkers when evaluating disease phenotype.[9, 11] When using baseline clinical variables, 7% (28/388) of patients in our cohort did not show a T2-high signal with this proportion dropping to only 1.8% (7/388) when we included longitudinal PBE values. We have previously demonstrated that persistent historical PBE is associated with worse lung function[9] further highlighting the relevance of longitudinal review in providing insight on disease progression.

Frossing *et al* recently reported that 70% of the 166 patients in their severe asthma cohort had elevation of at least one T2-biomarker (FeNO, PBE, total IgE) with 31% having two or more elevated biomarkers.[18] In our cohort, a similar proportion (up to 37% of patients) had 2 or more elevated T2-biomarkers. Interestingly, most patients on mOCS (75%) had raised biomarkers (PBE and FeNO) despite being on mOCS. This may reflect underlying disease severity and potential steroid resistance but could also be due to treatment non-adherence to ICS and OCS. In our study we did not collect medicines-possession ratio data and so are unable to corroborate medication use.

While there is no agreed consensus on the definition of T2-high asthma, the criteria recommended by GINA 2021 are pragmatic and incorporate clinical and biomarker

variables. Raised PBE and FeNO are uncontested T2-biomarkers though both are time and treatment dependent. The detailed clinical characterisation possible within our analysis enabled careful scrutiny of patients that were classified as T2-low. Three quarters of T2-low patients had evidence of historically raised PBE indicating the presence of a T2 signal. Therefore, less than 2% of patients (7/388) with difficult-to-treat and severe asthma could be classified as T2-low. GINA 2022 has updated the classification of T2-inflammation in severe asthma and mOCS use is no longer included as a defining criterion. Within our cohort only 7/388 patients (1.8%) were classified as T2-high solely based on mOCS use with only 3 of these patients having no evidence of historically raised PBE or atopic sensitisation. This would suggest that the contribution of this criteria in identifying T2-high status is low and rightly removed from the GINA 2022 classification.

T2-low asthma has previously been associated with higher medication requirements, obesity, significant symptomatology, multimorbidity and increased health care burden.[20-22] Registry studies suggest that patients with T2-high or eosinophilic asthma have a higher age of asthma onset.[7, 10] However, in our cohort, clinical characterisation of patients identified as T2-high and T2-low showed broad comparability with no difference in ICS dose, BMI or age of 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, hospitalisation for asthma and exacerbations treated with steroids were similar in the T2-high and T2-low patients. While the overall small number of T2-low patients may partly explain why we did not detect a difference, we also show that most of the patients classified as T2-low using baseline PBE have evidence of historically raised PBE and so may in fact have T2-high disease that is simply masked at the

time of sampling. Therefore, the absence of significant clinical differences between the two cohorts may reflect that T2-high signal is 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 two patient groups.

T2-low asthma is frequently described as 'neutrophilic' asthma.[22] In our cohort a quarter of patients classified as T2-high had elevated sputum neutrophils while most (93%) participants with sputum neutrophilia 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 investigating reasons for the presence of this inflammatory cell in the sputum e.g. bacterial colonisation. We have demonstrated that colonisation 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. [23] Furthermore, we recently added further mechanistic insight into how bacterial pathogens like Haemophilus Influenzae drive neutrophilic inflammation in severe asthma, demonstrating associations with persistent infection of alveolar macrophages.[24] 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 a T2-low status and clinicians should target treatments at the airway microbiome over attempts at reducing airway neutrophilia. Recently, the association between airway neutrophilia and dysbiosis has been extended to blood neutrophils with increased blood neutrophil levels being linked to more prescription for antibiotic courses over a 12 year period in people with asthma.[25]

A strength of our study is that it reflects a real-world UK cohort of biologic naïve patients thereby excluding any confounding effects of biologics on baseline clinical and biomarker characteristics. Detailed clinical characterisation enabled us to scrutinise the presence of clinical variables in sub-groups of patients. One potential limitation of our T2 classification was that it used largely cross-sectional criteria, apart from longitudinal PBE levels. Use of broader longitudinal data might have given greater insights, though the multicomponent nature of our scrutiny will have compensated to a degree. Another limitation of our study is that it focuses on a relatively homogeneous Caucasian population. Replication in other ethnic populations is required.

In summary, our results demonstrate that in a UK population of difficult-to-treat and severe asthma, the majority of patients have T2-high status and there is a near absence of T2-low asthma. T2-high signals typically represent steroid responsive disease and our results support the use of inhaled steroids in all patients with asthma, regardless of underlying severity. Our results also allow us to propose that rather than dichotomising asthma as T2-high or T2-low, asthma should be considered a T2-inflammatory disease with periods when patients are biomarker-low or biomarker high. Designation of T2-low status should only be made in clinical practice after stringent multidimensional and longitudinal characterisation. This infers that with careful characterisation, most difficult-to-treat and severe asthma patients would suit higher level biologic therapies if needed. Finally, to better understand the clinical diversity of difficult-to-treat and severe asthma there is a need to look beyond a simple T2-high/-low paradigm and identify the role of additional pathophysiological pathways alongside a holistic view of the patient.

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Figure legends:

Fig.1: Relative Distribution of T2 Defining Parameters in the 360 patients classified as T2-high defined by A: FeNO, B: Peripheral blood eosinophils, C: On maintenance OCS, and D: clinically allergen driven symptoms. (data missing A: 67 patients, B: 28 patients, C: 11 patients, D: 48 patients). Abbreviations: FeNO: fractional exhaled nitric oxide, PBE: peripheral blood eosinophil count, OCS: oral corticosteroids.

Figure 2: Overlap between the presence of the four criteria used to define T2-high disease status in 314 patients with difficult-to-treat and severe asthma. The four criteria: (i) maintenance OCS use, (ii) FeNO ≥20ppb (Hi FeNO), (iii) peripheral blood eosinophils ≥150cells/μL (Hi eosinophil) and (iv) allergy associated symptoms (allergy triggered).

Figure 3: Lung function of patients characterised as T2-high and T2-low. A: Post-bronchodilator %predicted FEV1, B: Post-bronchodilator %predicted FVC, C: FEV1/FVC ratio, D: Post-bronchodilator %predicted MEF 25-75, E: Transfer Factor TLco, F: Transfer Factor Kco. *$p<0.05$, ns = not significant

Figure 4: Expression of inflammatory cytokines in blood (plasma: pg/ml) in patients with asthma defined at T2-high or T2-low. A: IL-4, B: CCL2/MCP-1, C: CCL3/MIP-1alpha, D: IL-5, E: IL-6, F: Periostin, G: IL-8, H: IL-10, I: TNF-alpha

584 **Table 1:** Clinical characteristics for patients with T2-high and T2-low status using clinical and
585 biomarker variables
586

	T2-high n= 360	T2-low n=28	P value
Age at diagnosis (years)	20[¶] (36)	27.6[¶¶] (17.7)	0.22
Adult onset asthma (≥18-years) (%/n)	54.7 (197/360)	67.9 (19/28)	0.18
Male patients (%/n)	33 (119/360)	35.7 (10/28)	0.8
Ethnicity- % Caucasian (n)	91.7 (330/360)	96.4 (27/28)	0.93
BMI	29.6[f] (9.8)	29.3 (16.8)	0.81
Never smokers (%/n)	51.4 (185/360)	51.9[¶¶] (14/27)	1
Ever intubated for acute asthma (%/n)	11.1 (40)	28.6 (8)	0.01
≥1 asthma-related hospitalization in the last 12 months (%/n)	27.9 (100/358)	44.4 (12/27)	0.07
≥2 exacerbations treated with steroids in preceding 12 months (%/n)	65.3 (209/320)	61.5 (16/26)	0.70
Started biologics after enrolment (%/n)	32.8 (118/360)	10.7 (3/28)	0.015
Dose of ICS (BDP equivalent; mcg)	1500[§§] (2000)	1500[¶¶] (2000)	0.74
ICS dose ≥1000 mcg (BDP equivalent) (%/n)	88.5 (292/330)	88.9 (24/27)	1
ICS dose ≥2000 mcg(BDP equivalent) (%/n)	46.7[§§] (154/330)	40.7[¶¶] (11/27)	0.55
On inhaled long-acting beta-2 agonist therapy (%/n)	86.7 (312/360)	85.7 (24/28)	0.89
On inhaled long-acting anti-muscarinic therapy (%/n)	67.5 (243/260)	64.2 (18/28)	0.73
On leukotriene receptor antagonists (%/n)	69.7 (251/260)	67.8 (19/28)	0.84
Peripheral blood eosinophil count at study enrolment (median), cells/uL	200 (300)	100 (100)	<0.01
Peak blood eosinophil count in past 10-years, cells/uL	500[‡] (600)	200 (300)	<0.01
Aeroallergen positive skin prick tests (%/n)	69.9 (197/282)	22.7 (5/22)	<0.01
Total IgE (IU/L)	84.7 (229.2)	17.3 (25.4)	<0.01
FeNO at enrolment (ppb)	20.3^ (25.9)	10.0 (10.0)	<0.01

T2 status was classified based on the presence of blood eosinophils≥150cells/ul or FeNO≥20ppb or need for maintenance OCS or allergen driven asthma. Values are median and interquartile ranges (IQR) unless otherwise specified. The P value reflects chi-square comparisons for categorical variables, Mann-Whitney test for continuous variables with non-normal distribution, and Fisher's exact test where observed cell counts were less than 5. Abbreviations: BMI: body mass index, FeNO: fractional exhaled nitric oxide, ICS: Inhaled corticosteroid, ICU: intensive care unit.

¶ data unavailable for 16 patients, ¶¶ data unavailable for 1 patient, §§ data unavailable for 30 patients, ‡ data unavailable for 6 patients, ^ data unavailable for 67 patients

Table 2: Presence of comorbidities in patients classified as T2-high and T2-low status

	T2-high n= 360	T2-low n=28	P value
Nasal polyps (%/n)	24.3 (82/332)	15.4 (4/26)	0.3
Depression (%/n)	38.1 (125/328)	33.3 (9/27)	0.62
Anxiety (%/n)	34.8 (114/328)	24 (6/25)	0.27
Dysfunctional breathing (%/n)	48.3 (166/344)	59.3 (16/27)	0.27
Eczema (%/n)	23.9 (85/356)	14.8 (4/24)	0.28
Obstructive sleep apnoea (%/n)	7.4 (26/352)	7.4 (2/27)	1
Vocal cord dysfunction (%/n)	13.3 (43/324)	20 (5/25)	0.35
Gastroesophageal reflux disease (%/n)	63.5 (221/348)	71.4 (20/28)	0.4
Obesity (BMI ≥30) (%/n)	46.9 (169/360)	50 (14/28)	0.79

T2 status was classified based on the presence of blood eosinophils≥150 cells/ul or FeNO ≥20ppb or need for maintenance OCS or allergen driven asthma. The p value reflects chi-square comparisons.

Table 3 Sputum characterisation for T2-high and T2-low patients.

	T2-high n=112	T2-low n=5	p-value
Median sputum eosinophils, %	1.9	0.12	0.005
Median sputum neutrophils, %	41.7	67.62	0.19
Percentage of patients with sputum eosinophils ≥2%	50 (56/112)	0	
Percentage of patients with sputum neutrophils ≥61%	25 (28/112)	60 (3/5)	0.11

Percentage of patients with mixed granulocytic disease (eosinophils >2% and neutrophils ≥61%)	11.6 (13/112)	0	<0.01

T2 status was classified based on the presence of blood eosinophils≥150 cells/ul or FeNO ≥20ppb or need for maintenance OCS or allergen driven asthma or sputum eosinophils ≥2%. The p value was calculated using the Fisher's exact test for categorical variables and the Mann-Whitney test for continuous variables.

Figure 1

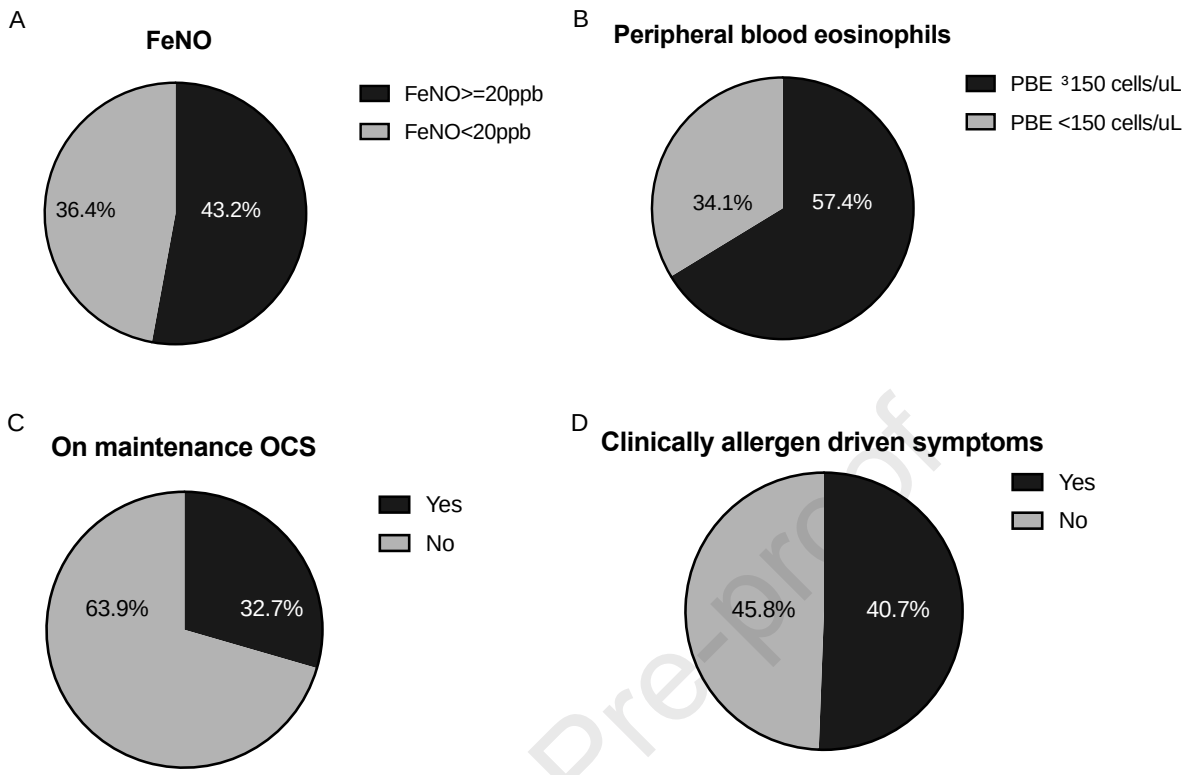


Figure 2 Overlap between the presence of the 4 criteria used to define T2-high disease status in 314 patients with difficult-to-treat and severe asthma.

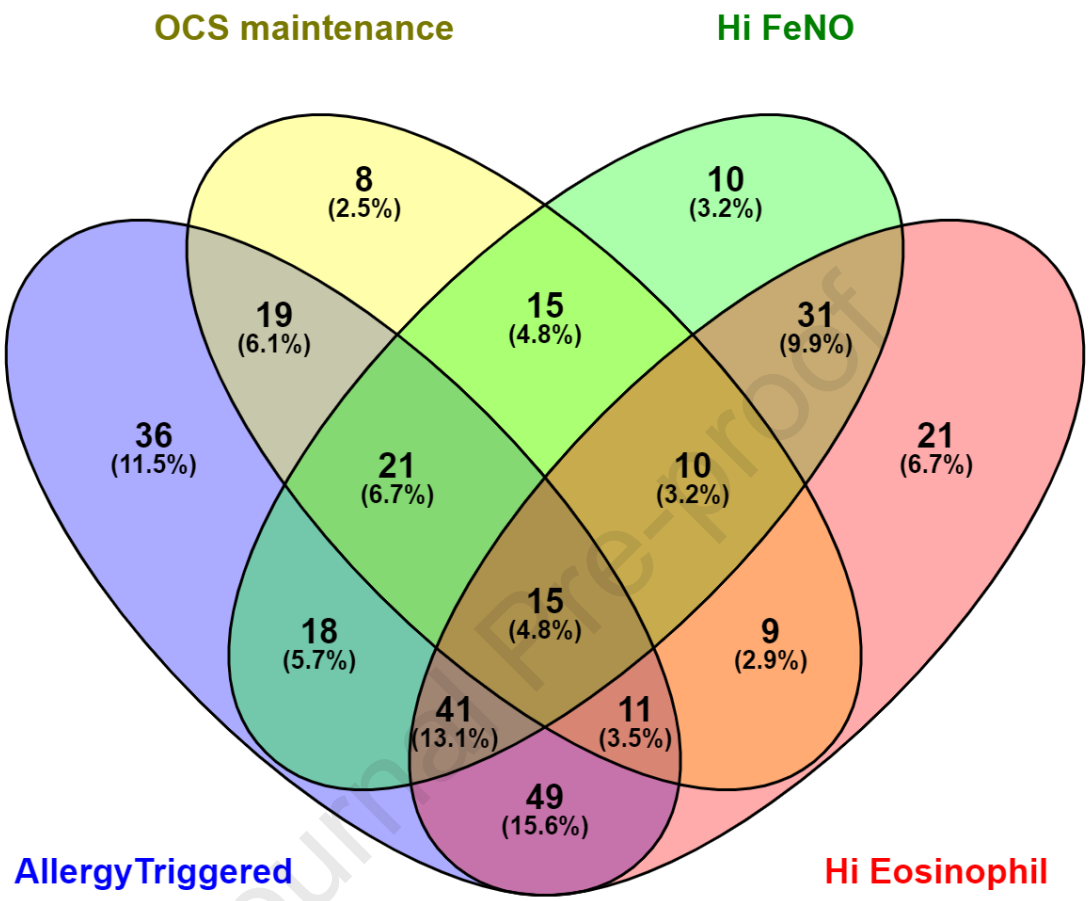


Figure 3:

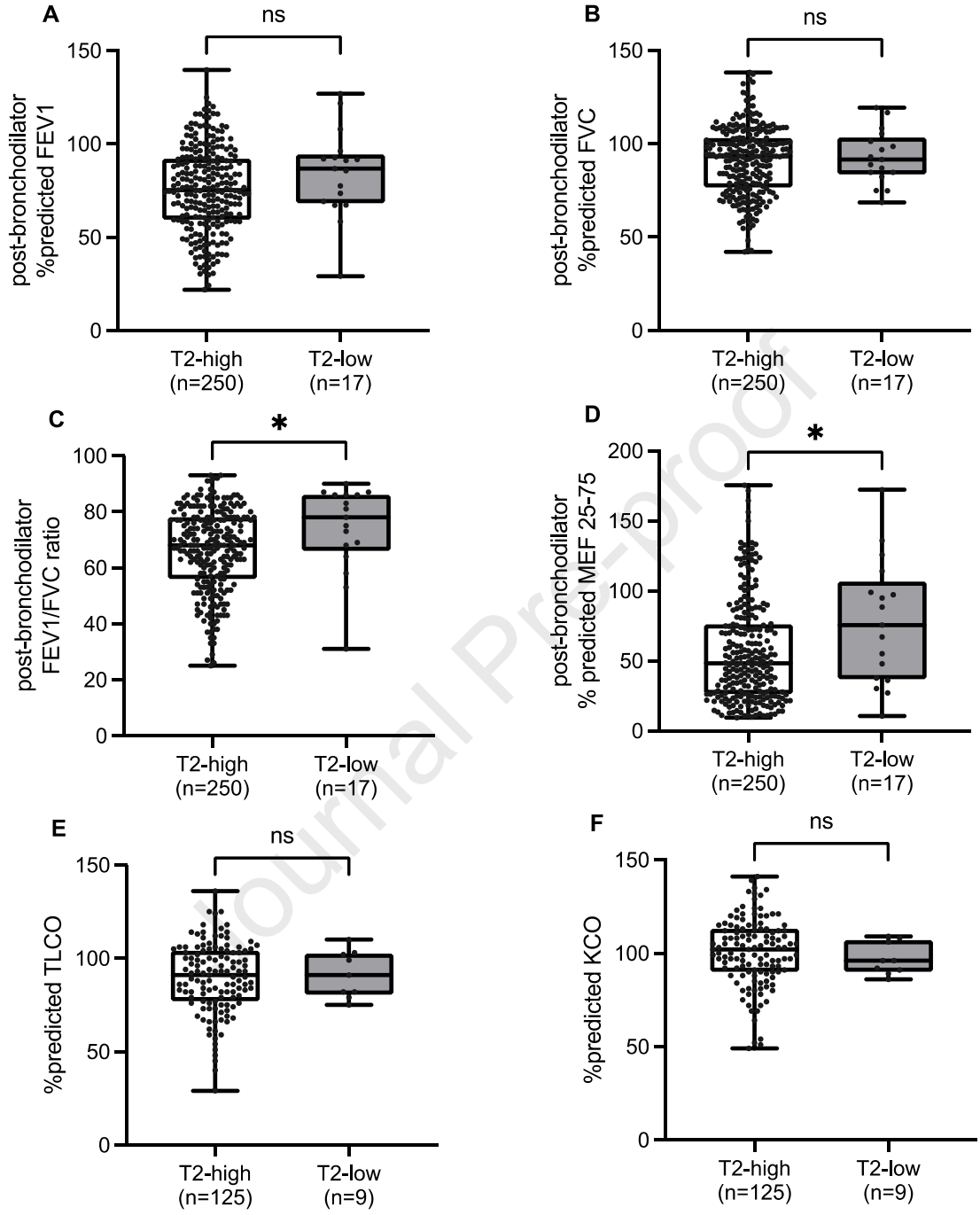
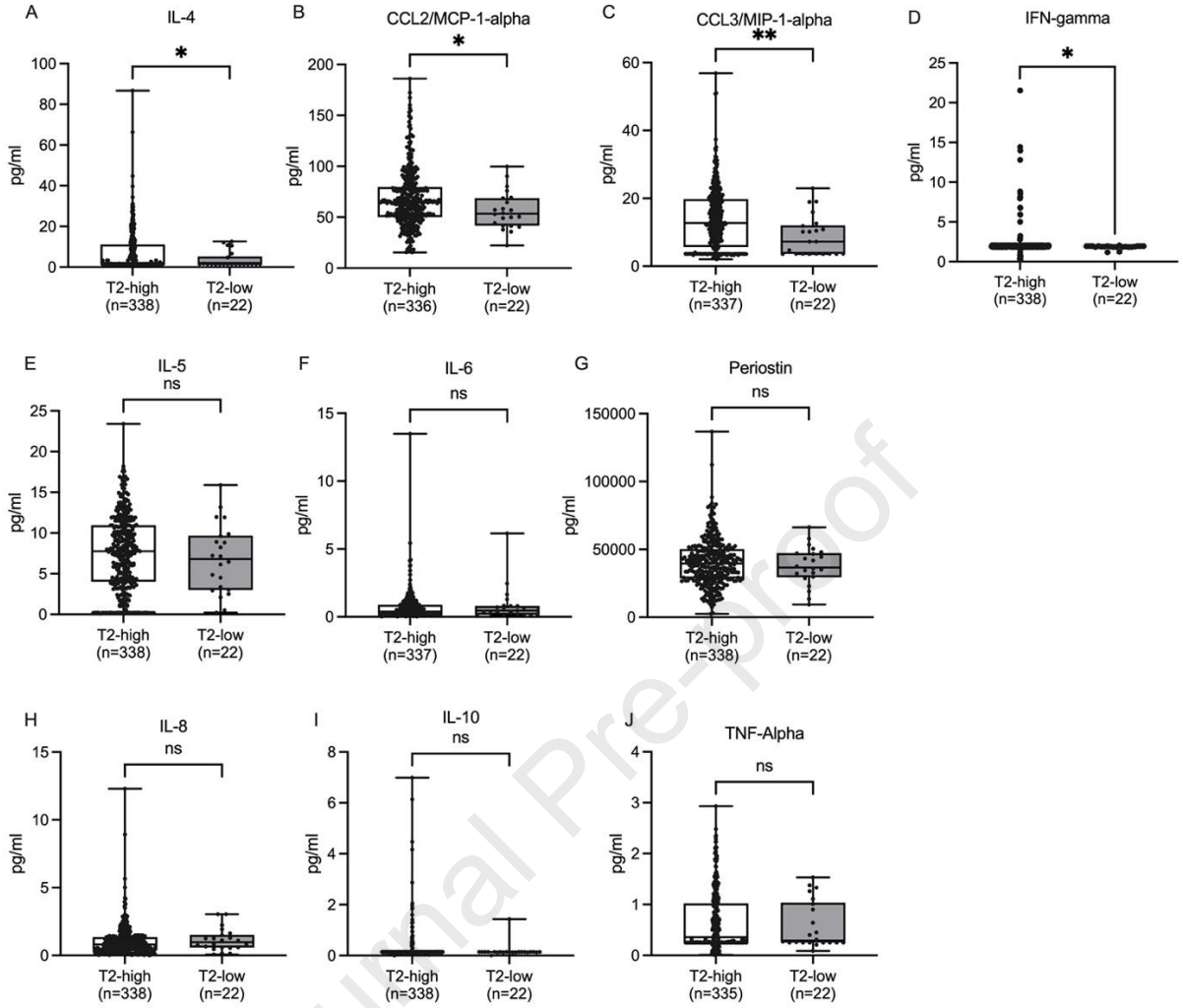


Figure 4



Online Repository Text File

Table E1: WATCH cohort inclusion and exclusion criteria

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

Table E2: Clinical characteristics of patients excluded from T2 analysis due to being on biologic therapy at WATCH enrolment compared to those included (biologic naïve)

Variable	**Biologics naïve N= 388**	**On biologics N= 79**	**P**
Current Age	52 (24)	52 (25)	0.96
Age at diagnosis	21[¶] (36)	14[¶¶] (37)	0.20
Percentage of patients with early onset asthma (≤18-years)	44.1 (171/388)	51.9 (41/79)	0.2
Sex (male:female)	130:258	35:44	0.07
BMI	29.6 (10.4)	29.2 (7.7)	0.90
Percentage of never smokers (n)	51.3[ω] (199/387)	60.8 (47/79)	0.13
Percentage of patients intubated for acute asthma (n)	12.4 (48/388)	17.7 (14/79)	0.2
Percentage of patients with ≥1 asthma related hospitalization in the last 12 months (n)	29.4[¶¶] (113/385)	29.1 (23/79)	0.97
Number of courses of OCS in the last 12 months	3[§] (4)	3[§§] (3)	0.34
Dose of ICS (BDP equivalent; mcg)	1500[‡] (2000)	1000[‡‡] (2000)	0.77
Peak blood eosinophil count (in past 10-years)	0.4[§§] (0.6)	0.6[≠] (0.6)	0.2

Values are median plus interquartitile range (IQR) unless otherwise specified. [¶] data unavailable for 17 patients, [¶¶] data unavailable for 2 patients, [§]data unavailable for 42 patients, [§§]data unavailable for 6 patients, [‡]data unavailable for 31 patients, [‡‡] data unavailable for 8 patients, [≠]data unavailable for 4 patients, [ω] data unavailable for 1 patient.

Abbreviations: ICS: Inhaled corticosteroid. OCS: oral corticosteroid. ICU, intensive care unit.

Table E3

	T2-high n=250	T2-low n=17	p-value
Post-bronchodilator %predicted FEV1	75.3 (32.7)	86.9 (26.3)	0.14
Post-bronchodilator %predicted FVC	93.2 (26.5)	91.2 (19.8)	0.82
Post-bronchodilator FEV1/FVC ratio	68 (22)	78 (20)	0.02*
Post-bronchodilator % predicted MEF 25-75	48.3 (50)	75.6 (69.7)	0.04*
%predicted TLCO	91 (27)	91 (22)	0.82
%predicted KCO	102 (23)	96 (17)	0.34

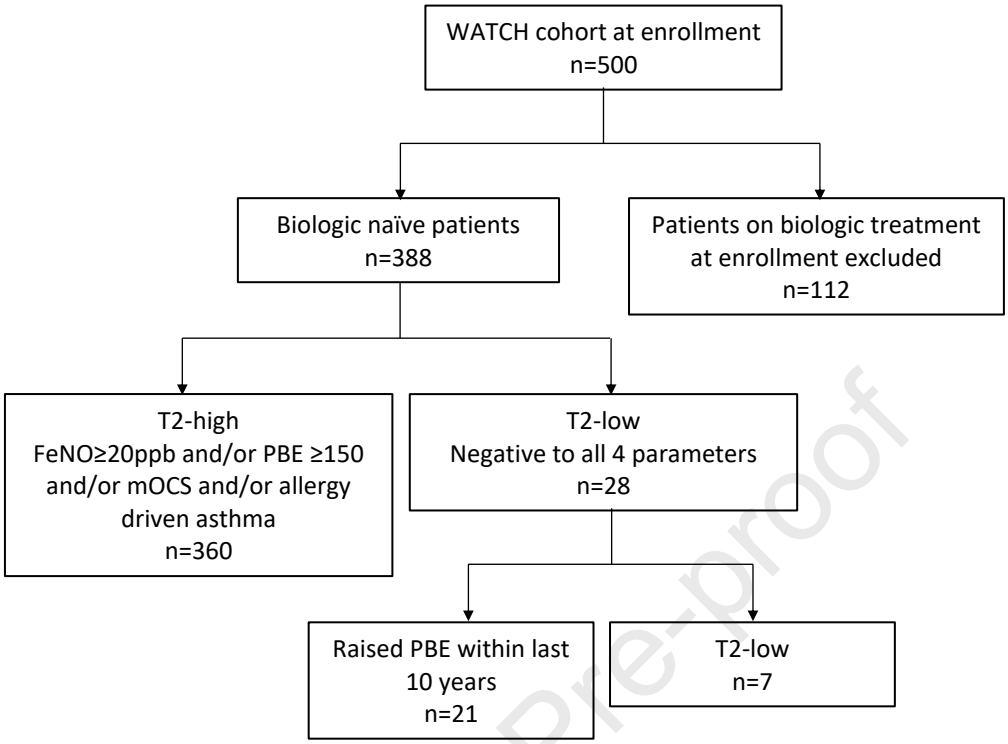
Comparison of lung function parameters between patients classified as T2-high and T2-low. FEV1: forced expiratory value in 1 second, FVC: forced vital capacity, TLCO: Transfer Factor of the lung, KCO: carbom monoxide transfer coefficient; * significant. Values shown are median (IQR).

Table E4

Cytokine levels (pg/ml)	T2-high (n=335-338)	T2-low (n=22)	p-value
CCL2/MCP-1	64.34 (29.53)	53.42 (27.08)	0.048*
CCL3/MIP-1-Alpha	12.72 (14.14)	7.23 (8.43)	0.008**
IL-4#	1.55 (9.6)	1.51 (3.72)	0.047*
IL-5	7.76 (6.94)	6.81 (12.88)	0.44
IL-6#	0.38 (0.74)	0.46 (0.65)	0.65
IL-8#	0.81 (0.97)	0.98 (0.95)	0.3
IL-10#	0.14 (0.01)	0.14 (0.01)	0.85
IL-13	ND	ND	-
IL-17#	0.47 (0.05)	0.47 (0.06)	0.2342
IFN-gamma#	1.94 (0.14)	1.89 (0.1)	0.01*
POSTN	39509 (21599)	36599 (17735)	0.68
TNF-Alpha#	0.36 (0.77)	0.29 (1.28)	0.85

Comparison of lung of cytokine levels in blood (plasma) between patients classified as T2-high and T2-low.
C-C Motif Chemokine Ligand 2/Monocyte chemoattractant protein-1 (CCL2/MCP-1), C-C Motif Chemokine Ligand 3/Macrophage Inflammotry Protein-1 Alpha (CCL3/MIP-1-Alpha), Interleukin (IL)-4, IL-5, IL-6, IL-8, IL-10, IL-13, IL-17, interferon (IFN)-gamma, Periostin (POSTN), Tumor Necrosis Factor-Alpha (TNF-Alpha). *, ** significant; # very low or at limit of detection; not detectable (ND).

Online Repository Figure E1



Online Repository Text: Figure Legends

Online Repository Figure E1: Flowchart of patients from the WATCH study that were included in our analysis