Exacerbation Profile and Risk Factors in a T2-Low Severe Asthma Population

McDowell, P. J., Busby, J., Hanratty, C. E., Djukanovic, R., Woodcock, A., Walker, S., Hardman, T. C., Arron, J. R., Choy, D. F., Bradding, P., Brightling, C. E., Chaudhuri, R., Cowan, D., Mansur, A. H., Fowler, S. J., Diver, S. E., Howarth, P., Lordan, J., Menzies-Gow, A., ... Heaney, L. G. (2022). Exacerbation Profile and Risk Factors in a T2-Low Severe Asthma Population. *American Journal of Respiratory and Critical Care Medicine*. Advance online publication. https://doi.org/10.1164/rccm.202201-0129OC

Published in:
American Journal of Respiratory and Critical Care Medicine

Document Version:
Peer reviewed version

Exacerbation Profile and Risk Factors in a T2-Low Severe Asthma Population

Sub-title: Exacerbation profile in T2-low severe asthma

PJ McDowell PhD, Busby J PhD, Hanratty CE PhD, Djukanovic R MD, Woodcock A MD, Walker S PhD, Hardman TC PhD, Arron JR PhD, Choy DF PhD, Bradding P MD, Brightling CE FMedSci, Chaudhuri R MD, Cowan D MD, Mansur AH MD, Fowler SJ MD, SE Diver PhD, Howarth P MD, Lordan J MD, Menzies-Gow A MD, Harrison T MD, Robinson DS MD, Holweg CTJ PhD, Matthews JG MD, Pavord ID FMedSci, Heaney LG MD, on behalf of the investigators for the MRC Refractory Asthma Stratification Program*

Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences, Queen's University, Belfast, UK (Prof L Heaney MD, J Busby PhD, CE Hanratty PhD, PJ McDowell);

23andMe, Sunnyvale, California, USA (J Matthews MD);

Gartnavel General Hospital, Glasgow and University of Glasgow, Glasgow, UK (Prof R Chaudhuri MD);

Oxford Respiratory NIHR BRC, Nuffield Department of Medicine, The University of Oxford, Oxford, UK (Prof ID Pavord FMedSci);

Niche Science & Technology Ltd., Unit 26, Falstaff House, Bardolph Road, Richmond TW9 2LH, UK (TC Hardman PhD);

Genentech Inc., South San Francisco, California, USA (JR Arron MD, CTJ Holweg PhD, DF Choy BS);

Department of Respiratory Sciences, Institute for Lung Health and Leicester NIHR Biomedical Research Centre, University of Leicester, Leicester, UK (Dr SE Diver, Prof P Bradding DM, Prof C Brightling PhD);

NHS Greater Glasgow and Clyde, Stobhill Hospital, Glasgow, UK (D Cowan MD);

School of Clinical and Experimental Sciences, University of Southampton, NIHR Southampton Biomedical Research Centre, Southampton, UK (Prof R Djukanovic MD, Prof PH Howarth MD);

Nottingham Respiratory NIHR Biomedical Research Centre, University of Nottingham, Nottingham, UK (Prof TW Harrison MD);

Division of Infection, Immunity and Respiratory Medicine, School of Biological Sciences, The University of Manchester; Manchester Academic Health Science Centre and NIHR Manchester Biomedical Research Centre, Manchester University Hospitals NHS Foundation Trust, Manchester, UK (Prof A Woodcock MD, SJ Fowler MD);

The Newcastle upon Tyne NHS Foundation Trust, Newcastle upon Tyne, UK (JL Lordan MD);

University of Birmingham and Heartlands Hospital, University Hospitals Birmingham NHS Foundation Trust, Birmingham, UK (Prof AH Mansur PhD);

Royal Brompton & Harefield Hospitals, London, UK (Prof A Menzies-Gow MD);

University College Hospitals NHS Foundation Trust, London, UK (Prof D Robinson MD);

Asthma UK & British Lung Foundation Partnership, 18 Mansell Street, London, UK (SM Walker PhD).

Corresponding Author:
Liam G Heaney
Centre for Experimental Medicine, School of Medicine, Dentistry and Biomedical Sciences,
Queen's University,
Belfast, UK.
Email: l.heaney@qub.ac.uk
Tel: +44 (0) 28 9097 6376

PJ McDowell, PhD, ORCID 0000-0003-2063-7992
Hanratty CE, PhD, ORCID 0000-0003-2453-6021
Djukanovic R, MD, ORCID 0000-0001-6039-5612
Woodcock A, MD, ORCID 0000-0002-5428-8578
Hardman TC, PhD , ORCID 0000-0002-1417-9019
Arron JR, PhD, ORCID 0000-0001-7677-9979
Bradding P, MD, ORCID 0000-0001-8403-0319
Brightling CE, FMedSci, ORCID 0000-0002-9345-4903
Chaudhuri R, MD, ORCID 0000-0001-8007-949X
Mansur AH, MD, ORCID 0000-0002-8615-8778
SE Diver, PhD, ORCID 0000-0002-5803-5121
Menzies-Gow A, MD, ORCID 0000-0001-9707-4986
Pavord ID, FMedSci, ORCID 0000-0002-4288-5973
Heaney LG, MD, ORCID 0000-0002-9176-5564

ABSTRACT

BACKGROUND: The last 25 years have seen huge progress in understanding of the pathobiology of T2 asthma, identification of measurable biomarkers and the emergence of novel monoclonal antibody treatments. Although present in a minority of severe asthmatics, very little is known about the mechanisms underlying T2-low asthma, making it a significant unmet need in asthma research.

METHODS: Exacerbation assessment was a pre-specified secondary analysis of data from a 48-week, multicentre, randomised controlled clinical study comparing the use of biomarkers and symptoms to adjust steroid treatment in a T2-low severe asthma-enriched cohort.

Participants were phenotyped as $T2^{LOW}$(fractional exhaled nitric oxide [FeNO] ≤20 ppb and blood eosinophil count [PBE] ≤150 cells/µL) or $T2^{HIGH}$ (FeNO>20 or PBE>150) at study enrolment and at each exacerbation. Here, we report the findings of the exacerbation analyses, including comparison of exacerbators and non-exacerbators, the physiological changes at exacerbation in those who had evidence of T2 biology at exacerbation versus those that did not, and the stability of inflammatory phenotypes when stable and at exacerbation.

RESULTS: Of the 301 participants, 60.8% (183/301) had one or more self-reported exacerbations (total of 390). Exacerbators were more likely to be female, have a higher BMI and more exacerbations requiring oral corticosteroid (OCS) and unscheduled primary care attendances for exacerbations.

At enrolment, 23.6% (71/301) were $T2^{LOW}$, and 76.4% (230/301) $T2^{HIGH}$. The $T2^{LOW}$ group had more asthma primary care attendances, were more likely to have a previous admission to HDU/ICU and to be receiving maintenance OCS.

At exacerbation the $T2^{LOW}$ events were indistinguishable from $T2^{HIGH}$ exacerbations in terms of lung function (mean fall in $T2^{LOW}$ FEV_1 - 200 [400] mL v $T2^{HIGH}$ 200 [300] mL, p=0.93) and symptom increase (ACQ5: $T2^{LOW}$ 1.4 [0.8] v $T2^{HIGH}$1.3 [0.8], p=0.72), with no increase in T2 biomarkers from stable to exacerbation state in the $T2^{LOW}$ exacerbations.

The inflammatory phenotype within individual patients was dynamic; inflammatory phenotype at study entry did not have a significant association with exacerbation phenotype.

CONCLUSION:

Asthma exacerbations demonstrating a $T2^{LOW}$ phenotype were physiologically and symptomatically similar to $T2^{HIGH}$exacerbations. $T2^{LOW}$ asthma was an unstable phenotype, suggesting that exacerbation phenotyping should occur at the time of exacerbation. The clinically significant exacerbations in participants without evidence of T2 biology at the time of exacerbation highlights the unmet and pressing need to further understand the mechanisms at play in non-T2 asthma.

Key words: severe asthma; T2-Low; Exacerbation

INTRODUCTION

Patients with inadequately controlled severe asthma despite optimised controller therapies shoulder much of the disability, economic burden and health care consumption attributed to asthma [1–5]. Exacerbation events that result from type-2 (T2) cytokine-driven eosinophilic inflammation are an important aspect of poor asthma control and T2 biomarkers (peripheral blood eosinophils [PBE], and fractional exhaled nitric oxide [FeNO]) serve well as predictors of exacerbation risk [6–9]. Despite the involvement of cytokine-driven eosinophilic inflammation in exacerbations, patients with well-suppressed T2 biology, including those receiving T2-suppressing biological agents, continue to experience exacerbations [9–14].

A proportion of patients in severe asthma clinics are characterised as having non-eosinophilic, 'type-2 low' asthma; a phenotype often associated with smoking, obesity and recurrent or persistent bacterial infection. Recent studies suggest that in many cases, patients with apparent 'type-2 low' severe asthma, expressing with low disease biomarkers (FeNO and PBE), also have underlying type-2 biology suppressed by corticosteroids administered in response to their poor symptom control [15–17]. Ongoing corticosteroid suppression serves to confound our understanding of disease in this patient group [18–20].

A recent trial comparing biomarker versus symptom-based treatment titration in severe asthmatics enrolled a population of patients with a FeNO of less than 45ppb to enrich for a cohort that included a substantial T2 low population [21]. A prespecified secondary endpoint was analysis of asthma exacerbations. Here we report the findings of the exacerbation analysis in this T2-low enriched severe asthma cohort, including comparison of study exacerbators and non-exacerbators, the physiological changes at exacerbation in those who had evidence of T2 biology at exacerbation versus those that did not, and the stability of inflammatory phenotypes when stable and at exacerbation.

METHODS

Study design

This was a 48-week, multicentre, randomised controlled clinical study in severe asthma, where the primary objective was to compare a treatment algorithm led by composite type-2 biomarkers (FeNO, blood eosinophils and serum periostin) with a symptom/risk-based algorithm to optimise the maintenance dose of corticosteroids (see **Supplementary Appendix** for details on the patient inclusion and exclusion criteria) [15]. Comparison of exacerbation rates between the treatment algorithms was an important pre-specified secondary outcome.

Participants were recruited from 12 specialist Severe Asthma Centres in the United Kingdom between 8th January 2016 and 12th July 2018 (see **Supplementary Appendix**), with the last study visit on 18th June 2019. Full inclusion and exclusion criteria are presented in the supplementary appendix, but of note, all enrolled participants had severe asthma (Global Initiative for Asthma (GINA) steps 4 and 5) [2], were aged 18–80 years old and were enrolled in a non-selective manner. Inclusion criteria included a documented history of ≥12% change in forced expiratory volume in 1 second (FEV_1) within the last 24 months, or a positive methacholine or mannitol challenge, and no 'rescue' oral corticosteroids (OCS) for an exacerbation in the 4 weeks prior to enrolment. The study aimed to enrich for a T2 biomarker low population within the cohort, so patients only proceeded to study enrolment if they had a fractional exhaled nitric oxide (FeNO) of <45 ppb at the screening visit.

All participants had one scheduled study visit at study entry, and all other study visits were unscheduled exacerbation study visits. All participants were asked to contact their study centre for clinical assessment (unscheduled exacerbation study visit) when their asthma control deteriorated to a level where they would usually seek medical advice or activate their personalised asthma action plan (PAAP). During these unscheduled visits, detailed clinical evaluation, medication review and spirometry were performed, asthma control questionnaire-7 (ACQ-7) completed, and FeNO, peripheral blood eosinophils (PBE), and serum C-reactive protein (CRP) measured. A urine sample and a spontaneous sputum sample for differential cell counting and biobanking of the supernatant were collected where feasible. An exacerbation was defined as 'severe asthma symptoms worsening outside of a patient's normal daily variation' and following assessment, a clinical decision was provided by the clinical team on the need to prescribe oral corticosteroids (OCS) and/or antibiotic

therapy. Where participants were unable to attend the clinical centre for this unscheduled study visit, exacerbation information was collected at the subsequent scheduled study visit, which occurred every 8 weeks from the time of enrolment until study completion at 48 weeks.

The inflammatory phenotype was attributed contemporaneously at each study visit, (at baseline and at each exacerbation), using peripheral biomarkers of T2 inflammation, namely FeNO and PBE. Biomarker cut-points similar to those described by the GINA guidelines were used to describe phenotypes; a FeNO ≤20 ppb *and* PBE ≤0.15 x 10^9/L was termed '$T2^{LOW'}$, and FeNO >20 ppb *or* PBE >0.15 x 10^9/L, termed '$T2^{HIGH'}$ to indicate the presence of detectable T2 biology [2].

As this was an observational analysis, all outcome measures were treated as exploratory.

The study protocol and primary study outcome have been previously published [15, 21]. Prior to participant recruitment, the protocol was approved by the Office for Research Ethics Northern Ireland (NI0158), local National Health Service Research and Development approval obtained for each site (NHS R&D) and the study registered on Clinicaltrials.gov (NCT02717689). All participants provided informed, written consent.

Procedures

Spirometry was conducted according to the American Thoracic Society/European Respiratory Society guidelines, with Global Lung Function 2012 (GLI 2012) equations used to calculate FEV_1 and forced vital capacity (FVC) predictive values [22]. NIOX Vero devices (Circassia, USA) were used for FeNO measurement. Spontaneous sputum samples were collected and processed in line with a standard operating procedure applied across all centres; RNA was extracted and underwent analysis following polymerase chain reaction (PCR) using LightCycler® 480 II instrumentation (Roche Molecular Diagnostics) to test for respiratory viruses including influenza A/B, respiratory syncytial virus A/B, rhinovirus, metapneumovirus, adenovirus, parainfluenza 1-4, and coronavirus.

Bacterial load within the DNA extracted from sputum plugs was measured by quantitative PCR (qPCR), using ThermoFisher Quantstudio 5, based on abundance of 16S ribosomal subunit encoding genes and pathogen specific genes including *Moraxella catarrhalis*, *Haemophilus influenzae*, and

Streptococcus pneumonia [23]. We used a specific bacteria threshold of ≥10^6 genome copies/mL on sputum qPCR as significant as this threshold of detection had a 98% concordance with bacteria detection on routine culture and was associated with an increased percentage sputum neutrophils in COPD exacerbations [24].

Statistical analysis

As this was a pre-specified secondary analysis of existing data, no sample size calculation was conducted. Depending on distribution, descriptive statistics are presented as means (SD), medians [IQR] or counts (%). Univariate analyses were conducted using t-tests, chi-square tests and Mann-Whitney U as appropriate. To ensure that bias wasn't introduced as a consequence of repeated exacerbations in an individual participant, each participant's first exacerbation was used when comparing inflammatory phenotypes in the cohort.

The stability of the exacerbation phenotype across multiple exacerbations in an individual patient was assessed using McNemar's test and the Kappa statistic. Data for assessed exacerbations were collected prospectively during exacerbations and there were few missing data. Therefore, all analyses under a complete-case framework. Analyses were conducted using STATA 16 (StataCorp, Texas, USA).

RESULTS

Demographics and baseline clinical descriptors for the study cohort are presented in the **Supplementary Appendix Table E1**. This cohort were generally middle aged (mean (SD) 55.7 (13.1) years), with a female preponderance (64.5%) and high BMIs (31.6 (7.2) Kg/m^2). During the 12 month period prior to enrolment patients reported a median [IQR] of 2 [1,4] exacerbations; one fifth had a history of ICU admission and their symptom-burden and quality of life impairment were high, respectively judged by ACQ-7 and AQLQ patient reported outcomes.

Clinical and biomarker characteristics of exacerbating patients

301 study participants reported a total of 390 exacerbation events, with 60.8% (183/301) experiencing ≥1 exacerbation (**Figure 1 and Table E1 supplement**). Baseline characteristics of those who exacerbated and those who did not are shown in the **Table 1**. When compared to non-exacerbators, patients who exacerbated during the study were more likely to be female [70.5% vs. 55.1%], have a higher BMI [33.1 kgm^{-2} vs. 29.4 kgm^{-2} on enrolment] and had more frequent exacerbations requiring OCS and unscheduled primary care attendances for exacerbations in year prior to the study (**Table 1**). Those who exacerbated were more likely to be receiving maintenance OCS and had a lower sputum eosinophil count at study entry, though other T2 biomarkers (PBE, FeNO or serum periostin) were not different. There was no difference between the exacerbators and non-exacerbators in the proportion of patients who reduced (24.4% vs. 29.3%), maintained (39.6% vs. 38.4%) or increased (36.0% vs. 32.3%) their steroid dose (OCS / ICS) during the study (p=0.66).

When phenotyping at study enrolment, 23.6% (71/301) of participants met the criteria for $T2^{LOW}$ and 76.4% (230/301) had evidence of T2 biology ($T2^{HIGH}$) (**Supplementary Appendix, Table E2**). The $T2^{HIGH}$ group had more obstructive lung function, higher sputum eosinophil count, higher periostin levels and a higher incidence of nasal polyposis. The $T2^{LOW}$ group had more primary care attendances for asthma and were more likely to have had an ICU or HDU admission for asthma. There was no difference in symptom burden (ACQ7: 2.1 vs. 1.9) or impact on quality-of-life (AQL: 4.7 vs. 5.0) between the $T2^{LOW}$ and $T2^{HIGH}$ cohorts. A higher proportion of the $T2^{LOW}$ cohort were receiving

maintenance OCS (34 (47.9%) v 77 (33.5%), p=0.03), at a higher dose (10 mg [10, 12] v 8 mg [5, 10], p=0.04) than the $T2^{HIGH}$ cohort, and were significantly more likely to be advised to down-titrate their CS usage as per the biomarker treatment strategy within the study.

A total of 118 exacerbation events (118/390, 30%) in 80 patients were assessed by study clinicians during unscheduled study visits (**Figure 1**). There was no evidence that the unassessed exacerbations differed in severity to those that were assessed, with a similar proportion of patients admitted to hospital during the event (8 of 118 [6.8%] vs. 23 of 272 [8.5%], p>0.5). As has been described in previous exacerbation studies [20], patients with unassessed exacerbations who followed their PAAP or had non-specialist assessment, were more likely to receive rescue OCS than those treated by asthma specialists during an unscheduled study visit (265 of 272 [97.4%] vs. 84 of 118 [71.2%], respectively; p<0.0001) or antibiotics (145 of 272 [53.3%] vs. 45 of 118 [38.1%], respectively; p=0.0059). In patients assessed by asthma specialists, those who did not receive OCS had a lower FeNO compared to those who received OCS ((15 ppb [IQR: 10, 23)] vs. 28 ppb [IQR: 14, 45], respectively p=0.001). Thirty-four patients did not receive OCS during an unscheduled study visit, and of these, only four (11.8%) went on to have systemic CS from another source (General Practitioners) within the following 7 days to address persistent or worsening symptoms (treatment failure).

Clinical and biomarker characteristics during exacerbations

At first assessed exacerbation for each patient, 27% (19/71) exacerbations were phenotyped as $T2^{LOW}$ and 73% (52/71) of exacerbations as $T2^{HIGH}$ (**Table 2**). The exacerbations in both groups looked clinically similar with comparable increase in symptoms, fall in FEV_1 (mean ~200 mL), decline in FVC, FEV_1/FVC and peak flow from study entry. $T2^{HIGH}$ had a greater increase in FeNO and PBE from study entry to time of exacerbation compared to the $T2^{LOW}$ cohort who had no associated increase in any T2 biomarkers from baseline, despite the similar physiological changes and symptom increase.

Of the first assessed exacerbation episodes for each patient, 26 of 71 (33%) produced spontaneous sputum and had a cell differential: 7/26 (27%) had a sputum eosinophil count <2% and neutrophil count ≥65%; 6/26 (23%) had a sputum eosinophil count <2% and neutrophil count <65%; 4/26 (15%)

a sputum eosinophil count ≥2% and neutrophil count ≥65% and 9/26 (35%) had a sputum eosinophil count ≥2% and neutrophil count <65%. Sputum eosinophils were higher in the $T2^{HIGH}$group, with the $T2^{HIGH}$ group showing a median 2.9% (IQR: -3.8, 10.3) increase in sputum eosinophils from the stable to exacerbation state. The $T2^{LOW}$ group had a greater number of sputum samples positive for virus at exacerbation, and all of the $T2^{LOW}$ cohort had a virus or bacteria detected on qPCR above threshold, however, there was no concomitant elevation of the sputum neutrophil count or serum CRP (described in **Table 2**). The was no significant difference between the $T2^{LOW}$ and $T2^{HIGH}$ cohorts with regards to systemic corticosteroids (CS) or antibiotics prescribed by the asthma team.

Within the cohort who were $T2^{LOW}$ at study entry, those who went on to exacerbate had a higher number of primary care attendances for asthma prior to study entry, more OCS rescue courses in the previous year and higher baseline ACQ-7 score compared to those who did not exacerbate (**Table 3**). Comparing T2 status at baseline (when stable) and exacerbation, 45/75 (60%) of patients had T2 biology detectable at baseline and exacerbation. T2 biology was also evident during exacerbation in 11/17 (65%) who were $T2^{LOW}$ at study entry. The observed change in exacerbation phenotype may be related to changes in corticosteroid exposure, as 5/7 who were $T2^{LOW}$ at baseline and subsequently reduced the dose of CS demonstrated a $T2^{HIGH}$ phenotype at exacerbation (see **Supplementary Appendix Table E3**). However, further analysis showed the inflammatory phenotype at study entry was not associated significantly with the phenotype at exacerbation (p=0.84, kappa=0.12), **Table 4a**). Inflammatory phenotype at first exacerbation was not associated significantly with the phenotype at secondary exacerbation (p=1.00, kappa=0.19), **Table 4b**).

DISCUSSION

The advent of biologic therapies targeting the T2-cytokine axis has reduced the frequency of asthma exacerbations in patients with severe asthma with an underlying T2-driven eosinophilic phenotype [9–12]. Under these conditions, T2 biomarkers (FeNO and PBE) have been shown to perform well both as prognostic biomarkers for exacerbation events (as shown in placebo arms of clinical trials) and as predictors of good therapeutic responses to T2 biologic therapies. The RASP-UK study population for the present analysis was purposefully enriched for patients with severe asthma

expressing a T2 biomarker low phenotype, within a clinical setting where residual exacerbation events represent an unmet medical need as the mechanisms are poorly understood. In this cohort, T2-biomarkers (FeNO, PBE and serum periostin) at randomisation were not prognostic for the frequency of exacerbation events, an observation consistent with mechanisms other than type-2 inflammation being responsible. Baseline sputum eosinophil counts were significantly lower in patients who went on to have an exacerbation, but as this biomarker was only obtained in approximately one third of patients at baseline (which is a common limitation when using sputum as source of a biomarkers), we cannot exclude some selection bias for sputum eosinophil counts in this subgroup. However, the biomarker measurement data appear to confirm recruitment of an exacerbation prone T2-biomarker low non-eosinophilic population as planned for the study.

In the present study, poor asthma symptom control, female sex, obesity, restrictive lung function and multiple unscheduled prior healthcare visits for exacerbation events in the prior year to study emerged as clinical factors associated with patients experiencing exacerbations. Poor asthma control and prior exacerbation history has consistently been associated with exacerbation risk and specifically in heavily treated severe asthma populations [25, 26].

The association between higher symptom burden and higher exacerbation rate was also seen in the MEX study, which examined exacerbation phenotype in patients with severe eosinophilic asthma established on mepolizumab [20], this association between higher symptom burden and higher pre-biologic exacerbation rates was also seen in those patients who went on to exacerbate while being treated with mepolizumab, suggesting that these factors are important for future risk despite significant background treatment. There may be a smaller ‘window’ for symptom deterioration in these highly symptomatic patients with severe asthma, prior to them self-identifying asthma deterioration, as they more easily cross a threshold, whereby they revert to their personal action plan and seek medical intervention.

The interaction between female sex, obesity and severe asthma needs further analysis but importantly the obese female phenotype has been described previously in severe asthma cohorts, and this group is often T2 biomarker low with a high symptom burden despite high dose ICS and

systemic CS [15, 26–28]. These data extend our knowledge for this high symptom burden clinical group, identifying them (both retrospectively and prospectively) as being particularly exacerbation prone. Defining the relationship between these clinical parameters and risk of exacerbation is key to understanding the underlying mechanism.

Those patients who self-treated according to their asthma plan or were seen in primary care almost always received systemic CS (97.4%), whereas those assessed by treating clinicians with broader access to clinical assessments, specifically FeNO measurement (as blood eosinophil data would not be available at the time of consultation) were less likely to receive these (70%). The similarity in rates of hospitalisation for assessed and unassessed exacerbations suggests that the exacerbation severity in both groups was similar. However, interpretation of the present data is limited by the absence of markers of exacerbation severity in patients not attending clinical assessments, such as symptom diary and peak flow measurements. Although the study did not mandate that FeNO be used to decide on prescription of systemic CS, mean FeNO was significantly lower in those not receiving OCS, and it is possible that this easily measured biomarker was being used to determine the perceived requirement for OCS during exacerbations. Importantly, there was a low observed incidence of treatment failure (defined as requirement for OCS or hospital admission in the 7 days after clinical assessment) when OCS were not prescribed after study clinical assessment. This fits with the findings of a recent study with mepolizumab (and assumed PBE suppression), FeNO measurement emerged as a useful means of discriminating between eosinophilic and non-eosinophilic events, with the latter more likely to be infection driven [20]. An important clinical consideration is the nature of all exacerbation events and specifically whether treatment with OCS is an appropriate treatment in T2 biomarker low events. Describing the clinical and demographic features associated with the T2 biomarker low frequent exacerbator phenotype will allow these patients to be better identified, and given the potential harm caused by regular systemic CS exposure, we believe that the routine use of systemic CS for all exacerbation events in severe asthma requires further study and that clinical assessment and biomarker measurement may be helpful in making more targeted treatment choices. This hypothesis, and specifically the use of FeNO

to guide therapeutic use of OCS during exacerbation needs to be formally tested via controlled clinical trials.

We were particularly interested in the $T2^{LOW}$ phenotype in this cohort and wished to identify clinical and demographic features associated with exacerbation events. Importantly, the $T2^{LOW}$ events had a similar increase in symptoms and fall in lung function compared to those in the $T2^{HIGH}$population, suggesting that the $T2^{LOW}$ events are clinically significant, albeit indistinguishable on the basis of symptoms or lung function. In contrast, measurement of T2 biomarkers showed greater increases in FeNO and PBE from baseline values at the time of exacerbation. In contrast, the T2 $T2^{LOW}$ cohort showed no increase in any T2 biomarker expression, despite similar physiological changes and symptom increase.

A limitation of the current study was the use of spontaneous rather than induced sputum measures, meaning that only a third of patients produced a spontaneous sputum sample at exacerbation. Peripheral biomarkers (PBE and FeNO) were used primarily to determine T2 status, however, as anticipated, the $T2^{LOW}$ cohort as defined by FeNO and PBE, were non-eosinophilic on sputum cytology, whereas the T2-Biology cohort had sputum eosinophilia, consistent with the use of composite T2 biomarker profiling with FeNO and PBE as a surrogate of eosinophilic airways inflammation [29,30]. Exacerbations with sputum data demonstrated a mixed inflammatory profile, with evidence of eosinophilic, neutrophilic and mixed eosinophilic-neutrophilic inflammation on sputum differential cell count, in keeping with previous reports of exacerbation analysis in mild-moderate asthmatic patients [13, 14]. However, this contrasts with observations from the MEX study, which explored exacerbation events on mepolizumab and where it was noted that eosinophilic and non-eosinophilic events were predominantly mutually exclusive [20]. This difference may reflect the greater PBE suppression with mepolizumab, allowing for clearer differentiation of the inflammatory phenotype. It is not yet clear whether the neutrophilic, infection-driven exacerbations seen in patients treated with mepolizumab reflect natural infections, immunosuppression due to biologics-induced reduction in airway eosinophils, or a broader

suppression of the T2-cytokine axis and anti-infective pathways from high dose inhaled corticosteroids.

The $T2^{LOW}$ group had a greater number of sputum samples positive for virus at exacerbation, and all of the $T2^{LOW}$ cohort had a virus or bacteria detected on qPCR above threshold, although did not have a concomitant elevated sputum neutrophil count or CRP. The proportion of exacerbations with bacteria identified was similar between both groups at exacerbation, however, the number of sputum samples available at exacerbation are a limiting factor for drawing conclusions on the role of infectious agents being drivers of $T2^{LOW}$ exacerbations. The dynamic nature of inflammatory phenotype seen between the different study visits, (from stable to exacerbation, and from one exacerbation to the next in the same individual), is in keeping with descriptions in the literature [20,31].

The need to elicit the pathways underlying these $T2^{LOW}$ exacerbations is a prerequisite to establishing effective treatments, and minimising the unwanted side effects of treatments that are known to have limited effectiveness, i.e. the use of OCS in $T2^{LOW}$ exacerbations and asthma management more generally. Within the $T2^{LOW}$ cohort, exacerbation risk during the study, was related to higher primary care asthma attendances and rescue systemic CS courses in the previous year, and a higher ACQ-7 score suggesting that these parameters are consistently associated with exacerbation risk in all patients with severe asthma.

The present study demonstrated how asthma exacerbations in patients expressing the $T2^{LOW}$ phenotype are similar physiologically and symptomatically, and occur as frequently as those with underlying T2 biology. $T2^{LOW}$ asthma emerged as an unstable phenotype that needs to be assessed at the time of exacerbation. These data highlight our limited understanding of the underlying pathology and lack of effective, evidence-based management strategies for asthma in the absence of T2 biology, as well as the need for further mechanistic and clinical studies.

ACKKNOWLEDGEMENTS

This study was part of the Medical Research Council UK Refractory Asthma Stratification Programme and programme support was obtained from Hoffman la Roche-Genentech (periostin assay and sample biobanking and Circassia (FeNO measurements – reduced pricing for machines and test kits) for in-kind support within that Consortium. We would like to thank the members of the Trial Steering Committee for all their support and assistance with study delivery; Professor Martyn Partridge (Chair), Professor Mike Morgan, Professor Anne Millar, Mr Mark Stafford-Watson (patient representative – during his tenure on the Trial Steering Committee, Mark sadly passed away and we gratefully acknowledge his significant contribution to this programme and his involvement in other research projects) and Ms Gabriella Cooper. We are grateful Niche Science & Technology Ltd. for assistance with study delivery and to all the patients who volunteered for the study and clinical and research teams at all the participating clinical and academic centres. We thank Sofia Mosesova, Chris Patterson and Nicola Gallagher for statistical advice during study set-up and analysis.

We also thank Amgen Inc. (Thousand Oaks, California, USA), Astra Zeneca (London, UK), Jannsen Research & Development LLC (London, UK), and Vitalograph Inc. (Ennis, Ireland) for supporting the RASP-UK Consortium.

REFERENCES

1. Chung KF, Wenzel SE, Brozek JL, Bush A, Castro M, Sterk PJ, Adcock IM, Bateman ED, Bel EH, Bleecker ER, Boulet LP, Brightling C, Chanez P, Dahlen SE, Djukanovic R, Frey U, Gaga M, Gibson P, Hamid Q, Jajour NN, Mauad T, Sorkness RL, Teague WG. International ERS/ATS guidelines on definition, evaluation and treatment of severe asthma. Eur Respir J. 2014 Feb;43(2):343-73
2. GINA. GLOBAL INITIATIVE FOR ASTHMA A GINA Pocket Guide For Health Professionals DIFFICULT-TO-TREAT & SEVERE ASTHMA in Adolescent and Adult Patients Diagnosis and Management A GINA Pocket Guide For Health Professionals DIFFICULT-TO-TREAT & SEVERE ASTHMA in Adole.; 2019. www.ginasthma.org. Accessed March 13, 2020
3. Sweeney J, Patterson CC, Menzies-Gow A, et al. Comorbidity in severe asthma requiring systemic corticosteroid therapy: cross-sectional data from the Optimum Patient Care Research Database and the British Thoracic Difficult Asthma Registry. Thorax. 2016;71(4):339-346. doi:10.1136/thoraxjnl-2015-207630
4. O'Neill S, Sweeney J, Patterson CC, et al. The cost of treating severe refractory asthma in the UK: An economic analysis from the British Thoracic Society Difficult Asthma Registry. Thorax. 2015;70(4):376-378. doi:10.1136/thoraxjnl-2013-204114
5. Sarnes E, Crofford L, Watson M, Dennis G, Kan H, Bass D. Incidence and US Costs of Corticosteroid-Associated Adverse Events: A Systematic Literature Review. Clin Ther. 2011;33(10):1413-1432. doi:10.1016/j.clinthera.2011.09.009
6. Malinovschi A, Fonseca ao A, Jacinto T, Alving K, Janson C. Exhaled nitric oxide levels and blood eosinophil counts independently associate with wheeze and asthma events in National Health and Nutrition Examination Survey subjects. J Allergy Clin Immunol. 2013;132:821-827.e5. doi:10.1016/j.jaci.2013.06.007
7. Mogensen I, Alving K, Bjerg A, et al. Simultaneously elevated exhaled nitric oxide and serum-eosinophil cationic protein relate to recent asthma events in asthmatics in a cross-sectional population-based study. Clin Exp Allergy. 2016;46(12):1540-1548. doi:10.1111/cea.12792

8. Wagener AH, De Nijs SB, Lutter R, et al. External validation of blood eosinophils, FENOand serum periostin as surrogates for sputum eosinophils in asthma. Thorax. 2015;70(2):115-120. doi:10.1136/thoraxjnl-2014-205634
9. Castro M, Corren J, Pavord ID, et al. Dupilumab Efficacy and Safety in Moderate-to-Severe Uncontrolled Asthma. N Engl J Med. 2018;378(26):2486-2496. doi:10.1056/NEJMoa1804092
10. Castro M, Zangrilli J, Wechsler ME, et al. Reslizumab for inadequately controlled asthma with elevated blood eosinophil counts: Results from two multicentre, parallel, double-blind, randomised, placebo-controlled, phase 3 trials. Lancet Respir Med. 2015;3(5):355-366. doi:10.1016/S2213-2600(15)00042-9
11. FitzGerald JM, Bleecker ER, Nair P, et al. Benralizumab, an anti-interleukin-5 receptor α monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. Lancet. 2016;388(10056):2128-2141. doi:10.1016/S0140-6736(16)31322-8
12. Ortega HG, Liu MC, Pavord ID, et al. Mepolizumab Treatment in Patients with Severe Eosinophilic Asthma. N Engl J Med. 2014;371(13):1198-1207. doi:10.1056/NEJMoa1403290
13. Turner M, Hussack P, Sears MR, Dolovich J, Hargreave FE, Hussack M R Sears J Dolovich F E Hargreave TP. Exacerbations of asthma without sputum eosinophilia. 2020. doi:10.1136/thx.50.10.1057
14. Fahy J V, Woo Kim K, Liu J, Boushey HA, Francisco S. Prominent Neutrophilic Inflammation in Sputum from Subjects with Asthma Exacerbation. J Allergy Clin Immunol 1995 Apr;95(4):843-52. 1995
15. Heaney LG, Busby J, Hanratty CE, et al. Composite type-2 biomarker strategy versus a symptom–risk-based algorithm to adjust corticosteroid dose in patients with severe asthma: a multicentre, single-blind, parallel group, randomised controlled trial. Lancet Respir Med 2020; 9: 57–68 doi:10.1016/S2213-2600(20)30397-0
16. Heaney LG, Perez de Llano L, Al-Ahmad M, Backer V, Busby J, Canonica GW, Christoff GC, Cosio BG, FitzGerald JM, Heffler E, Iwanaga T, Jackson DJ, Menzies-Gow AN, Papadopoulos NG, Papaioannou AI, Pfeffer PE, Popov TA, Porsbjerg CM, Rhee CK, Sadatsafavi M, Tohda Y,

Wang E, Wechsler ME, Alacqua M, Altraja A, Bjermer L, Björnsdóttir US, Bourdin A, Brusselle GG, Buhl R, Costello RW, Hew M, Koh MS, Lehmann S, Lehtimäki L, Peters M, Taillé C, Taube C, Tran TN, Zangrilli J, Bulathsinhala L, Carter VA, Chaudhry I, Eleangovan N, Hosseini N, Kerkhof M, Murray RB, Price CA, Price DB. Eosinophilic and Noneosinophilic Asthma: An Expert Consensus Framework to Characterize Phenotypes in a Global Real-Life Severe

17. Jackson D, Busby J, Pfeffer P, et al Characterisation of patients with severe asthma in the UK Severe Asthma Registry in the biologic era. Thorax 2021;76;220-227 doi.org/10.1136/thoraxjnl-2020-215168
18. Fitzpatrick AM, Chipps BE, Holguin F, Woodruff PG. T2-"Low" Asthma: Overview and Management Strategies, Journal Allergy Clinical Immunol: In Practice, 2020: 8 (2): 452-463. https://doi.org/10.1016/j.jaip.2019.11.006.
19. Kyriakopoulos C, Gogali A, Bartziokas K, Kostikas K. Identification and treatment of T2-low asthma in the era of biologics. ERJ Open Research 2021 7: 00309-2020; DOI: 10.1183/23120541.00309-2020
20. McDowell PJ, Diver S, Yang F et al. The inflammatory profile of exacerbations in patients with severe refractory asthma receiving mepolizumab (the MEX study): a prospective observational study. Lancet Respir Med 2021 2021; 9; 1174-1184
21. Hanratty CE, Matthews JG, Arron JR, et al. A randomised pragmatic trial of corticosteroid optimization in severe asthma using a composite biomarker algorithm to adjust corticosteroid dose versus standard care: Study protocol for a randomised trial. Trials. 2018;19(1). doi:10.1186/s13063-017-2384-7
22. Quanjer PH, Stanojevic S, Cole TJ, et al. Multi-ethnic reference values for spirometry for the 3-95-yr age range: The global lung function 2012 equations. Eur Respir J. 2012;40(6):1324-1343. doi:10.1183/09031936.00080312
23. Bafadhel M, McKenna S, Terry S, et al. Acute Exacerbations of Chronic Obstructive Pulmonary Disease. Am J Respir Crit Care Med. 2011;184(6):662-671. doi:10.1164/rccm.201104-0597OC

24. Bafadhel M, Haldar K, Barker B, et al. Airway bacteria measured by quantitative polymerase chain reaction and culture in patients with stable COPD: Relationship with neutrophilic airway inflammation, exacerbation frequency, and lung function. Int J COPD. 2015;10:1075-1083. doi:10.2147/COPD.S80091
25. Bateman ED, Reddel HK, Eriksson G, Peterson S, Östlund O, Sears MR, et al. Overall asthma control: The relationship between current control and future risk. J Allergy Clin Immunol 2010; 125: 600-608.e6, doi.org/10.1016/j.jaci.2009.11.033
26. Yang F, Busby J, Heaney LG, Menzies-Gow A, Pfeffer PE, Jackson DJ, Mansur AH, Siddiqui S, Brightling CE, Niven R, Thomson NC, Chaudhuri R; UK Severe Asthma Registry. Factors Associated with Frequent Exacerbations in the UK Severe Asthma Registry. J Allergy Clin Immunol Pract. 2021 Jul;9(7):2691-2701.e1.
27. Jackson D, Busby J, Pfeffer P, et al Characterisation of patients with severe asthma in the UK Severe Asthma Registry in the biologic era. Thorax 2021;76;220-227 doi.org/10.1136/thoraxjnl-2020-215168
28. Moore W, Meyers D, Wenzel S, et al. Identification of asthma phenotypes using cluster analysis in the Severe Asthma Research Program. Am J Respir Crit Care Med 2010; 181: 315-323 DOI: 10.1164/rccm.200906-0896OC
29. Wagener A, Nijs S, Lutter R et al, External validation of blood eosinophils, FeNO, and serum periostin as surrogates for sputum eosinophils in asthma. Thorax 2015; 70;115-120 doi.org/10.1136/thoraxjnl-2014-205634
30. Westerhof G, Korevaar D, Amelink M et al, Biomarkers to identify sputum eosinophilia in different adult asthma phenotypes. Eur Respir J 2015;: 688-696
31. Kupczyk M, Dahlen D, Sterk P et al. Stability of phenotypes defined by physiological variables and biomarkers in adults with asthma. Allergy 2014; 69: 1198-1204

Table 1. Table of baseline characteristics of study exacerbators versus non-exacerbators (N=301).

		No Exacerbations (n=118)	≥1 Exacerbation (n=183)	P-value
Number of Exacerbations 0 1 2 3 4 5 6		118 (100%)	0 (0.0%) 77 (42.1%) 47 (25.7%) 33 (18.0%) 15 (8.2%) 6 (3.3%) 5 (2.7%)	
Age At Study Enrolment (Y)	**N=301**	56.5 (13.1)	55.2 (13.2)	0.41
Gender Female Male	**N=301**	 65 (55.1%) 53 (44.9%)	 129 (70.5%) 54 (29.5%)	0.0064
BMI (kg/m2)	**N=300**	29.4 (5.4)	33.1 (7.8)	<0.0001
Smoking Status Never Smoked Ex-Smoker	**N=301**	83 (70.3%) 35 (29.7%)	141 (77.0%) 42 (23.0%)	0.19
Atopic Disease	**N=300**	81 (68.6%)	126 (69.2%)	0.91
Hospital Admissions for Asthma In Last Year	**N=301**	0.0 (0.0,0.0)	0.0 (0.0,0.0)	0.087
ED Visits in Last Year	**N=301**	0.0 (0.0,0.0)	0.0 (0.0,0.0)	0.23
GP Visits for Asthma in The Last Year	**N=301**	0.0 (0.0,2.0)	1.0 (0.0,4.0)	<0.0001
Rescue Courses of Oral Steroids In The Last Year	**N=301**	1.0 (0.0,3.0)	3.0 (1.0,4.0)	<0.0001
Prior Admission for Asthma to HDU/ICU	**N=301**	19 (16.1%)	45 (24.6%)	0.079
Number Of Prior Admission for Asthma to HDU/ ICU	**N=63**	1.0 (1.0,1.0)	1.0 (1.0,3.0)	0.027
Ever Been Ventilated	**N=63**	6 (31.6%)	25 (56.8%)	0.066
ACQ7 Score	**N=301**	1.7 (1.1)	2.1 (1.1)	0.0043
AQLQ Total Score	**N=291**	5.1 (1.3)	4.8 (1.4)	0.039
FEV_1 (L)	**N=301**	2.4 (0.7)	2.0 (0.7)	0.0002
% Predicted FEV_1	**N=301**	80.1 (18.8)	72.5 (19.0)	0.0008
FVC (L)	**N=301**	3.6 (0.9)	3.1 (0.8)	<0.0001
% Predicted FVC	**N=301**	96.1 (16.9)	87.9 (16.1)	<0.0001
FEV_1/FVC	**N=301**	0.66 (0.11)	0.65 (0.12)	0.83
PEFR (L/min)	**N=298**	403.8 (135.7)	358.4 (119.6)	0.0026
Sputum Eosinophils (%)	**N=123**	2.3 (1.0,8.0)	1.0 (0.3,8.0)	0.043
Sputum Neutrophils (%)	**N=123**	64.8 (35.5,79.3)	58.3 (31.0,78.0)	0.67
FeNO (ppb)	**N=301**	22 (13,30)	19 (13,28)	0.17
Blood Eosinophils (10^9/L)	**N=301**	0.20 (0.12,0.35)	0.21 (0.10,0.33)	0.39
Periostin (ng/mL)	**N=298**	55.2 (16.7)	51.4 (15.8)	0.052
OCS User	**N=301**	35 (29.7%)	76 (41.5%)	0.037
OCS Dose	**N=111**	10 (6,10)	10 (5,10)	0.71
~ICS Dose (BDP*)	**N=301**	2207 (681)	2256 (739)	0.56
T2 Status at study enrolment T2-LOW T2-BIOLOGY	**N=301**	 24 (20.3%) 94 (79.7%)	 47 (25.7%) 136 (74.3%)	0.29

'maintenance oral corticosteroid ~Inhaled corticosteroid *Beclometasone Dipropionate equivalent ^Corticosteroid

OCS = Oral corticosteroid; CS = corticosteroid; ACQ7 = asthma control questionnaire-7; FEV_1 = forced expiratory volume in 1 second; forced vital capacity; peak expiratory flow rate = PEFR; High dependency unit = HDU; Intensive care unit = ICU

Table 2. Characteristics of features of $T2^{LOW}$ versus $T2^{HIGH}$ exacerbations at first assessed exacerbation (n=71).

		T2-LOW (n=19)	T2-BIOLOGY (n=52)	P-value
Characteristics at study entry				
Age At Study Enrolment	**N=71**	56.3 (12.8)	52.9 (14.0)	0.35
Gender				
Female		15 (78.9%)	33 (63.5%)	
Male	**N=71**	4 (21.1%)	19 (36.5%)	0.22
BMI (kg/m²)	**N=70**	35.8 (10.2)	33.5 (6.7)	0.27
Atopic Disease	**N=71**	15 (78.9%)	36 (69.2%)	0.42
Rescue Courses of Oral Steroids in the Last Year	**N=71**	3.0 (2.0,3.0)	2.5 (2.0,4.0)	0.46
Prior Admission for Asthma To A HDU/ICU	**N=71**	4 (21.1%)	14 (26.9%)	0.61
Number Of Prior Admission for Asthma to HDU/ICU	**N=18**	1.0 (1.0,1.0)	1.0 (1.0,2.0)	0.24
At exacerbation				
FEV_1 (L)	**N=70**	1.7 (0.7)	1.8 (0.8)	0.64
Difference from Baseline in FEV_1 (L)	***N=70***	*-0.2 (0.4)*	*-0.2 (0.3)*	*0.93*
% Predicted FEV_1	**N=70**	64.0 (24.1)	61.4 (17.6)	0.63
Difference from Baseline in % Predicted FEV_1	***N=70***	*-8.5 (12.8)*	*-8.3 (11.4)*	*0.96*
FVC (L)	**N=70**	1.7 (0.7)	1.8 (0.8)	0.64
Difference from Baseline in FVC (L)	***N=70***	*-0.4 (0.7)*	*-0.3 (0.4)*	*0.55*
% Predicted FVC	**N=70**	81.9 (20.1)	78.0 (14.4)	0.37
Difference from Baseline in % Predicted FVC	***N=70***	*-11.6 (22.7)*	*-8.1 (11.4)*	*0.40*
FEV_1/FVC	**N=69**	0.61 (0.15)	0.63 (0.11)	0.60
Difference from Baseline in FEV_1/FVC	***N=69***	*-0.01 (0.07)*	*-0.02 (0.06)*	*0.45*
PEFR (L/min)	**N=64**	301.9 (109.5)	305.7 (109.6)	0.90
Difference From Baseline in PEFR (L/min)	***N=63***	*-47.9 (52.7)*	*-46.6 (56.1)*	*0.93*
Sputum Eosinophils (%)*	**N=26**	0.0 (0.0,1.5)	3.3 (1.3,15.8)	0.018
Difference from baseline sputum eosinophils (%)	***N=21***	*-0.3 (-2.0,0.0)*	*2.9 (-3.8,10.3)*	*0.14*
Sputum Neutrophils (%)*	**N=26**	56.2 (25.7,73.2)	53.3 (30.7,78.8)	0.85
Difference from Baseline in Sputum Neutrophils (%)	***N=21***	*-5.6 (-37.3,4.2)*	*-5.1 (-13.8,11.0)*	*0.65*
FeNO (ppb)	**N=71**	11 (9,14)	32 (22,44)	<0.0001
Difference from Baseline in FeNO (ppb)	**N=71**	-3 (-11,0)	9 (-2,20)	0.0005
Blood Eosinophils (109/L)	**N=71**	0.04 (0.02,0.10)	0.21 (0.12,0.41)	<0.0001
Difference From Baseline in Blood Eosinophils (10^9/L)	***N=71***	*-0.15 (-0.19,-0.01)*	*-0.00 (-0.11,0.16)*	*0.0028*
Periostin (ng/mL)	**N=70**	47.2 (14.1)	53.7 (19.5)	0.19
Difference from Baseline in Periostin (ng/mL)	***N=69***	*-1.0 (6.8)*	*1.2 (18.6)*	*0.62*
ACQ7 Score	**N=69**	3.4 (1.1)	3.7 (0.9)	0.24
Difference From Baseline in ACQ7 Score	***N=69***	*1.4 (0.8)*	*1.3 (0.8)*	*0.72*
Temperature (C)	**N=69**	37.0 (36.6,37.4)	36.7 (36.3,36.9)	0.028
Difference From Baseline in Temperature (C)	***N=68***	*0.3 (-0.1,0.7)*	*0.1 (-0.1,0.4)*	*0.072*
CRP (mg/L)	**N=55**	4.8 (2.0,9.3)	7.7 (5.0,11.0)	0.31
Any Virus (PCR)	**N=24**	8 (88.9%)	6 (40.0%)	0.019
Any Bacteria (Spec qPCR)	**N=23**	4 (50.0%)	6 (40.0%)	0.65
Any Bacteria or Virus (Spec qPCR)	**N=23**	8 (100.0%)	10 (66.7%)	0.065
Oral/IV CS	**N=71**	12 (63.2%)	40 (76.9%)	0.25
ABX	**N=71**	12 (63.2%)	21 (40.4%)	0.089
Oral/IV CS & ABX	**N=71**	10 (52.6%)	19 (36.5%)	0.22

CS = corticosteroid; ACQ7 = asthma control questionnaire-7; FEV_1 = forced expiratory volume in 1 second; forced vital capacity; peak expiratory flow rate = PEFR; High dependency unit = HDU; Intensive care unit = ICU; IV = Intravenous; ABX = antibiotics, PCR= Polymerase chain reaction. qPCR=quantitative polymerase chain reaction. Spec= specific bacteria threshold of $\geq 10^6$ genome copies/ml on quantitative PCR. *Sputum differential cell count at first exacerbation (n=26), 9/26 were $T2^{LOW}$, 17/26 were $T2^{HIGH}$.

Table 3. $T2^{LOW}$ **cohort at study entry: factors associated with those who proceeded to exacerbation, and those who did not.**

		No Exacerbation (N=24)	≥1 exacerbation during study (N=47)	P-value
Age At Inclusion	**N=71**	56.5 (12.5)	50.7 (12.5)	0.06
Gender Female Male	**N=71**	16 (66.7%) 8 (33.3%)	32 (68.1%) 15 (31.9%)	0.90
BMI (kg/m^2)	**N=71**	30.7 (6.4)	32.4 (6.3)	0.29
Atopic Disease	**N=71**	17 (70.8%)	34 (72.3%)	0.89
Hospital Admissions for Asthma in Last Year	**N=71**	0.0 (0.0,0.0)	0.0 (0.0,1.0)	0.06
A&E Visits in Last Year	**N=71**	0.0 (0.0,0.0)	0.0 (0.0,1.0)	0.16
GP Visits for Asthma in the Last Year	**N=71**	0.5 (0.0,4.0)	3.0 (1.0,4.0)	0.050
Rescue Courses of Oral Steroids in the Last Year	**N=71**	2.0 (0.0,3.5)	3.0 (2.0,5.0)	0.012
Prior Admission for Asthma to HDU or ICU	**N=71**	4 (16.7%)	19 (40.4%)	0.043
Number of Prior Admission for Asthma to HDU or ICU	**N=22**	1.0 (1.0,2.0)	1.0 (1.0,4.0)	0.71
Ever Been Ventilated	**N=22**	1 (25.0%)	9 (50.0%)	0.36
ACQ7 Score	**N=71**	1.7 (0.8)	2.4 (1.1)	0.010
AQL Total Score	**N=69**	5.0 (1.1)	4.5 (1.3)	0.14
FEV_1 (L)	**N=71**	2.3 (0.7)	2.2 (0.8)	0.47
% Predicted FEV_1	**N=71**	82.7 (19.6)	74.8 (20.5)	0.12
FVC (L)	**N=71**	3.4 (0.9)	3.2 (0.8)	0.25
% Predicted FVC	**N=71**	94.3 (17.4)	86.7 (15.1)	0.06
FEV1/FVC	**N=71**	0.69 (0.11)	0.69 (0.14)	0.81
PEFR (L/min)	**N=70**	387.8 (136.7)	380.2 (132.3)	0.82
Sputum Eosinophils (%)	**N=24**	1.0 (0.0,2.8)	0.3 (0.0,0.8)	0.35
Sputum Neutrophils (%)	**N=24**	60.8 (43.0,83.0)	72.0 (53.4,87.8)	0.55
FeNO (ppb)	**N=71**	13 (10,17)	12 (9,16)	0.71
Blood Eosinophils (10^9/L)	**N=71**	0.08 (0.05,0.11)	0.08 (0.03,0.12)	0.73
Periostin (ng/mL)	**N=70**	49.1 (14.6)	44.1 (11.7)	0.12
OCS User	**N=71**	8 (33.3%)	26 (55.3%)	0.08
OCS Dose	**N=34**	10 (8,13)	10 (10,12)	0.63
ICS Dose (BDP)	**N=71**	2208 (655)	2221 (726)	0.94
CS Study Change Reduce Maintain Increase	**N=56**	13 (65.0%) 6 (30.0%) 1 (5.0%)	20 (55.6%) 8 (22.2%) 8 (22.2%)	0.24

A&E = Accident and Emergency; OCS = oral corticosteroid; ACQ7 = asthma control questionnaire-7; FEV_1 = forced expiratory volume in 1 second; FVC = forced vital capacity; peak expiratory flow rate = PEFR; FeNO = Fractional excretion of nitrous oxide; BMI = Body mass index; High dependency unit = HDU; Intensive care unit = ICU

Table 4A. Stability of inflammatory phenotype from baseline study entry to first assessed exacerbation.

	First Exacerbation		
Baseline	**$T2^{LOW}$**	**$T2^{HIGH}$**	**Total**
$T2^{LOW}$	6	11	17
$T2^{HIGH}$	13	45	58
Total	19	56	75
McNemars (P-value) = 0.84, Kappa = 0.12			

Table 4B. Stability of inflammatory phenotype from first to second assessed exacerbation.

	Second Exacerbation		
First Exacerbation	**$T2^{LOW}$**	**$T2^{HIGH}$**	**Total**
$T2^{LOW}$	2	3	5
$T2^{HIGH}$	4	16	20
Total	6	19	25
McNemars (P-value) = 1.00, Kappa = 0.19			

Figure 1: Cohort flow diagram of all exacerbations during the study. A 'first exacerbation' was the initial exacerbation assessed as a clinical study visit for any individual patient.