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Clinical evaluation of type 2 disease status in a real-world population of difficult to manage asthma using historic electronic healthcare records of blood eosinophil counts

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Abstract

Background: Blood eosinophil measurement is essential for the phenotypic characterization of patients with difficult asthma and in determining eligibility for anti-IL-5/IL-5Rα biological therapies. However, assessing such measures over limited time spans may not reveal the true underlying eosinophilic phenotype, as treatment, including daily oral corticosteroid therapy, suppresses eosinophilic inflammation and asthma is intrinsically variable.

Methods: We interrogated the electronic healthcare records of patients in the Wessex AsThma CoHort of difficult asthma (WATCH) study (UK). In 501 patients being evaluated in this tertiary care centre for difficult to control asthma, all requested full blood count test results in a 10-year retrospective period from the index WATCH assessment were investigated (n = 11,176).

Results: In 235 biological therapy-naïve participants who had 10 or more measures in this time period, 40.3% were eosinophilic (blood eosinophils ≥300 cells/μl) at WATCH enrolment whilst an additional 43.1%, though not eosinophilic at enrolment, demonstrated eosinophilia at least once in the preceding decade. Persistent eosinophilia was associated with worse post-bronchodilator airway obstruction and higher Fractional exhaled Nitric Oxide (FeNO). In contrast, the 16.6% of patients who never demonstrated eosinophilia at this blood eosinophil threshold showed preserved lung function and lower markers of Type 2 inflammation.

Conclusions: This highlights the central role that type 2 inflammation, as indicated by blood eosinophilia, has in difficult asthma and suggests that longitudinal electronic healthcare record analysis can be an important tool in clinical asthma phenotyping, providing insight that may help understand disease progression and better guide more specific treatment approaches.

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KEYWORDS
asthma, eosinophils, epidemiology

1 | INTRODUCTION

Asthma is classically recognized to be a type 2 (T2) inflammatory airway disorder, in which systemic interleukin-5 (IL-5) signalling to the bone marrow increases circulating eosinophils and recruitment to airway tissue.[1] However, it is also recognized that non-eosinophilic forms of asthma constitute a proportion of the asthma population. The measurement of airway eosinophilia in induced sputum is well established as a predictive marker for asthma exacerbations and steroid therapy response[2] but is unsuitable for routine clinical practice or large epidemiological studies due to the practical limitations of undertaking sputum induction in a clinical setting.[3]

Though not perfectly correlated, blood eosinophils are recognized to be a good biomarker for airway eosinophils[4,5] and, in view of their ready accessibility, have been widely adopted into the clinical characterization of asthma patients. The utility of blood eosinophil counts has been demonstrated by large population studies, in which raised baseline blood eosinophil counts are associated with poor asthma control,[6] lung function decline[7] and exacerbations.[8,9] Moreover, they offer theragnostic value by defining a phenotype of severe asthma patients that can be stratified towards newly emerged anti-IL5/ IL-5 receptor alpha (IL-5 Rα) therapies.[10–13]

Eosinophilic inflammation is recognized to fluctuate in both blood and sputum over time[14–17]: few patients are 'eosinophilic' at every measurement.[18,19] This therefore challenges the robustness of translating associations determined by single-measurement cross-sectional study designs,[20] into clinical practice, particularly when full blood counts are among the most commonly requested blood test panels in clinical care.[21,22]

We sought to explore whether interrogation of repeat blood eosinophil count measures provided additional phenotypic information beyond that provided by binary categorization of patients based on a single time point. We have focussed our analysis to the routinely measured full blood count test results extracted by electronic health records (EHR)[23] from patients in the Wessex AsThma CoHort of difficult asthma (WATCH) study, which is drawn from a large catchment area across the South Central England region of the UK.[24]

2 | METHODS

2.1 | Population

WATCH is a prospective observational study of patients managed in a tertiary difficult asthma clinic at University Hospital Southampton with 'high dose therapies' and/or 'continuous or frequent use of oral steroids' according to the BTS (British Thoracic Society) Adult Asthma Management Guidelines 2016. Detailed study methodology has been published elsewhere.[25] The study had ethical approval (REC reference: 14/WM/1226), and all patients provided written informed consent.

Patients were excluded from analysis if they had evidence of other systemic causes for their eosinophilia (eg eosinophilic granulomatosis with polyangiitis). For patients treated with biological asthma therapies, blood tests after therapy start dates were excluded.

Clinically requested blood tests were processed by the fully accredited hospital pathology laboratory, compliant to ISO142819 standards. 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; differential cell counts on induced sputum were available in a subset of patients (details in supplementary data) from which sputum inflammatory phenotypes were determined using a ≥2% cut-off for sputum eosinophils[26] and ≥61% cut-off for sputum neutrophils.[27] Electronic clinical records were extracted where available to augment comprehensive data capture in a pragmatic fashion.[25]

Patients with multiple blood test results (defined by 10 or more blood test results) over the preceding 10-years leading up to WATCH enrolment were identified. This cut-off was selected since the median number of eosinophil counts in the preceding decade for WATCH cohort enrolled subjects was 10. Patients were categorized as 'never eosinophilic' if they have never demonstrated an eosinophil count ≥300 cells/μl in any of the ten or more blood tests extracted. Those demonstrating at least one eosinophil count of ≥300 cells/μl in the minimum of 10 or more blood tests were categorized as 'eosinophilic'. To further assess how different patterns of eosinophilia might differentially associate with clinical features we subdivided eosinophilic subjects into tertiles determined by the frequency with which their eosinophil counts were ≥300 cells/μl: rare, intermittent and persistent. Blood test metadata were also extracted: date of test, time of test, requester and clinical indication.

2.2 | Statistical analysis

Statistical analysis was performed using SPSS 25, GraphPad Prism 7 and R. Continuous clinical variables are presented as median (IQR) and categorical variables as frequencies (percentages). Between-group differences were assessed by Mann-Whitney, Kruskal-Wallis, chi-squared or Fisher's exact test where appropriate. Pairwise deletion was applied in the case of missing data and correction for multiple testing used where appropriate. Biomarkers were assessed using Receiver Operating Characteristic Curves and Positive Predictive Values calculated from natural frequencies in our cohort. Spearman's rank order correlation was employed to assess associations.

3 | RESULTS

3.1 | Identified patients

A total of 11,176 discrete blood eosinophil count results from the WATCH cohort of 501 patients were identified over the 10-years prior to study enrolment. On exclusion of five patients with a diagnosis of Eosinophilic Granulomatosis with Polyangiitis (EGPA) and blood tests taken after initiation of first biological therapy, 9604 blood eosinophil count results were available from 471 patients (Figure 1).

The earliest extracted blood test was from 05/01/2006 and the latest from 31/07/2019. The median time span for blood test collection was 3,036 (IQR: 3131) days (8.32 years). The majority of blood tests, however, were performed in the last few years, with 49.9% of blood test results derived from the five-year period from 2014 to 2018 (inclusive) (Table S1 and Figure S1). During this period, Omalizumab had already been available in our clinic for a decade (introduced in 2008) and Mepolizumab had only just been introduced (in 2017 and briefly in 2013 through a clinical trial).

Patients excluded from the analysis (n = 25) had a higher FeNO than those with at least one blood test (Table S2) but had no other statistically significant differences in terms of basic demographics, lung function tests, healthcare utilization or asthma control (as measured by ACQ6) between the two broad categories at initial assessment.

The median number of blood tests per patient was 10 (IQR: 18.5). Of the 471 patients, 235 had 10 or more available eosinophil counts. Though broadly comparable in terms of basic demographics, lung function tests, healthcare utilization or asthma control (as measured by ACQ6), patients with ten or more blood tests were slightly older, had a higher BMI and lower total IgE than those with fewer than 10 blood tests (Table S3).

3.2 | Eosinophilic sub-grouping of patients

Of the 235 patients with 10 or more clinical blood test results, 79 (40.3%) were eosinophilic (using a threshold of ≥300 cells/μl) at enrolment to the study. Of the remaining 156 patients who were non-eosinophilic, 117 (75.0%) had historically demonstrated an eosinophilia on at least one occasion whilst just 39 (25.0%) never demonstrated an eosinophilia (Figure 1). Thus, only 16.6% of patients were never eosinophilic, which reduced to 3.4% (n = 8) if the threshold was reduced to ≥200 cells/μl.

3.3 | Never eosinophilic patients

Patients with difficult to treat asthma who never demonstrated eosinophilia were more likely to have less severe post-bronchodilator airflow obstruction, lower fractional exhaled nitric oxide (FeNO)

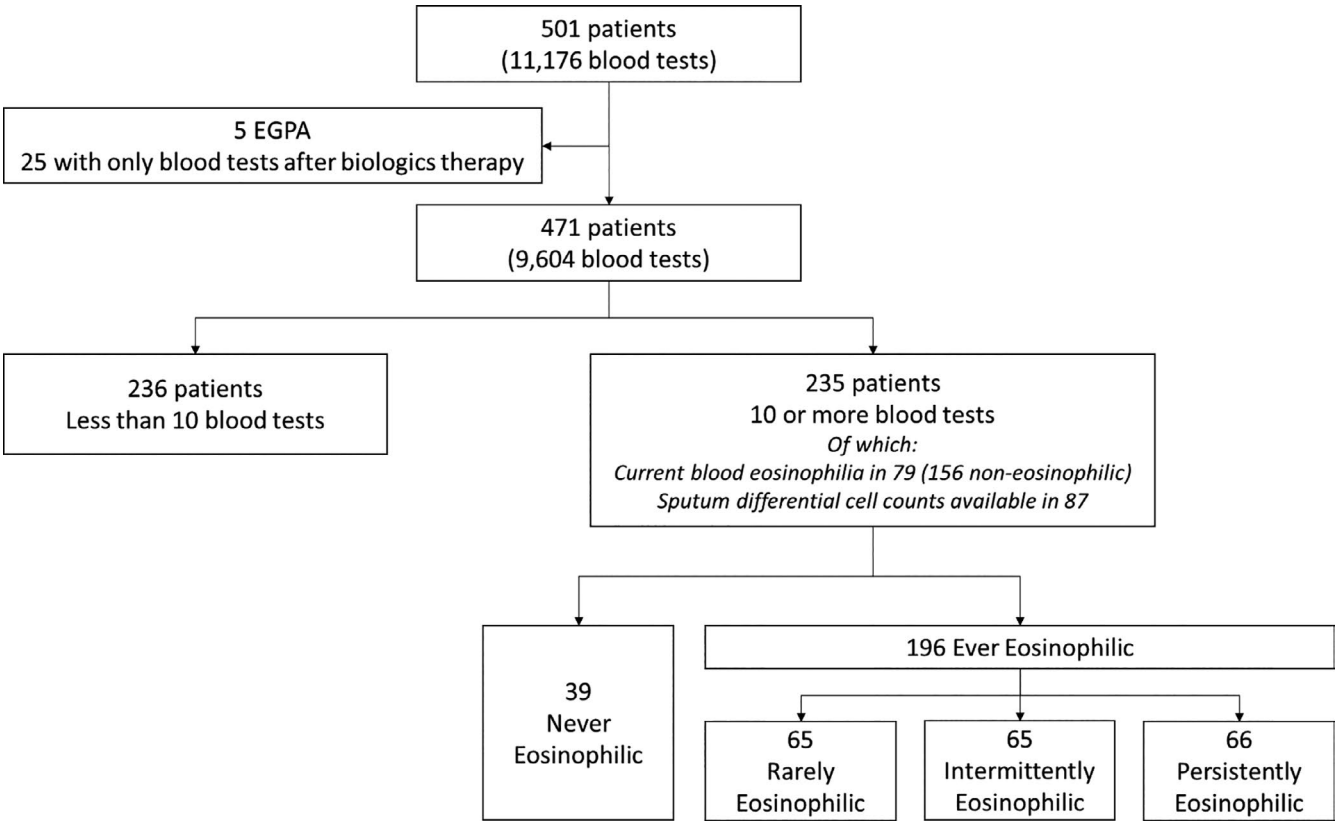


FIGURE 1 Consort diagram of participants recruited to the study and included in this analysis

and lower total serum immunoglobulin E (IgE) levels than the ever eosinophilic (historical) group (Table 1). Nine (23.1%) of these patients subsequently received anti-IgE monoclonal antibody therapy. By comparison, 43.4% of ever eosinophilic patients subsequently commenced biologic therapy. Other than ABPA/SAFS (Allergic Bronchopulmonary Aspergillosis/ Severe Asthma with Fungal Sensitization), which was not seen in the never eosinophilic patients, there were no differences in the prevalence of common co-morbidities between these groups (Table 2). Presence of nasal polyposis on CT and sputum differential cell counts were available in only a small subset of patients, in whom investigation was clinically relevant.

In those patients not currently demonstrating a blood eosinophilia, concurrently measured FeNO was no different between never eosinophilic and historically eosinophilic patients. However, in such patients, serum total IgE was significantly higher in historically eosinophilic patients (median 64.85, IQR: 190.8) compared to never eosinophilic patients (median 11.60, IQR: 79.8), U = 861.5, $p < .001$ (Figure 2). The AUC for serum total IgE in discriminating between these groups was 0.698, $p < .001$; the AUC for FeNO was not statistically significant (Figure 2).

3.4 | Frequency of eosinophilia

'Eosinophilic' patients were divided into equal tertiles based upon percentage of eosinophil counts ≥300 cells/μl, thereby dividing patients into four groups based on frequency of that degree of

TABLE 1 Comparison of asthma characteristics between patients with no evidence of blood eosinophilia and those with eosinophilia on at least one occasion in the past decade

	Never Eosinophilic (39)	Missing	Ever Eosinophilic (196)	Missing	*p* value
Age (at enrolment; years)	56.0 [37.0,63.0]	0	55.5 [41.8,64.0]	0	Ns
BMI	31.5 [28.8,36.0]	0	30.3 [26.4,36.6]	0	Ns
Sex (female)	31 (79.5%)	0	129 (65.8%)	0	Ns
Smoker (ever)	16 (41.0%)	0	101 (51.5%)	0	Ns
Age of Asthma Diagnosis (years)	20.0 [7.8,44.5]	3 (7.7%)	22.0 [5.8,43.0]	12 (6.1%)	Ns
Current Inhaled Corticosteroid Dose (BDP equivalent, μg)	2050.0 [1625.0,3000.0]	9	3000.0 [2000.0,3000.0]	31	Ns
Subsequently started on Asthma Biologics[a]	9 (23.1%)[b]	0	85 (43.4%)	0	$p < .05$
4 or more OCS courses in past year	11 (30.6%)	3 (7.69%)	67 (38.3%)	21 (10.71%)	Ns
Maintenance OCS	10 (25.6%)	1 (2.6%)	56 (28.3%)	5 (2.5%)	Ns
Hospitalized for asthma in past year	14 (35.9%)	0	67 (34.2%)	0	Ns
Ever Intubated for asthma	5 (12.8%)	0	25 (12.6%)	0	Ns
Atopic (positive SPT to any aeroallergen)	19 (59.4%)	7 (17.95%)	91 (65.0%)	56 (28.57%)	Ns
FeNO	13.0 [5.7,19.0]	14 (35.9%)	18.4 [9.3,34.2]	46 (23.2%)	$p < .05$
Post-BD FEV_1% predicted	83.8 [67.9,92.3]	13 (33.3%)	74.4 [59.4,92.7]	68 (34.3%)	Ns
Post-BD FEV_1/FVC ratio	76.5 [64.5,82.0]	13 (33.3%)	66.0 [55.0,77.0]	68 (34.3%)	$p < .05$
Post-BD FEF_{25-75}% predicted	69.2 [34.5,96.7]	13 (33.3%)	41.6 [27.1,72.1]	68 (34.3%)	Ns
Total IgE	11.6 [0.0,64.3]	7 (17.9%)	71.4 [19.6,331.0]	53 (26.8%)	$p < .001$
ACQ6	2.7 [1.7,3.7]	6 (15.4%)	2.5 [1.5,3.5]	15 (7.6%)	Ns
Number of Full Blood Counts in Past 10-years	17.0 [13.5,26.0]	0	24.0 [15.0,46.2]	0	$p < .05$

Continuous variables expressed as median [Q1, Q3] with differences measured by Mann-Whitney U test.

Categorical variables expressed as *n* (%) with differences measured by chi-square test.

Abbreviations: ACQ, asthma control questionnaire; Body mass index; BDP, beclomethasone dose equivalent; BD, bronchodilator; FEF25-75, forced expiratory flow at 25% to 75% of FVC; FeNO, fraction of nitric oxide in exhaled breath; FEV1, forced expiratory volume in 1 s; FVC, Forced vital capacity; IgE, immunoglobulin E; pred, predicted; OCS, oral corticosteroids; SPT, skin prick test.

aDetermined clinically using evidence of relevant serological and radiological information guided by conventional clinical diagnostic criteria.

bDetermined clinically based on compatible history of reaction on exposure and consistent clinical phenotypes.

TABLE 2 Comparison of co-morbidities between patients with no evidence of blood eosinophilia and those with eosinophilia on at least one occasion

	Never eosinophilic 39	Missing	Ever eosinophilic 196	Missing	*p* value
Rhinitis	24 (70.6%)	5	114 (65.9%)	23	Ns
Nasal Polyposis (on CT)	0	35	7 (25.9%)	169	Ns
Eczema	9 (23.1%)	0	48 (25.0%)	4	Ns
ABPA or SAFS[a]	0	0	20 (10.4%)	3	$p < .05$
Bronchiectasis	3 (7.7%)	0	34 (17.5%)	2	Ns
GORD	29 (76.3%)	1	132 (69.8%)	7	ns
Depression	13 (37.1%)	4	69 (38.1%)	15	Ns
Anxiety	9 (26.5%)	5	64 (35.6%)	16	Ns
Dysfunctional Breathing	21 (55.3%)	1	95 (50.3%)	7	Ns
Vocal Cord Dysfunction	4 (11.4%)	4	31 (17.6%)	20	Ns
Clinical Sulphite Sensitivity[b]	2 (5.1%)	0	12 (6.2%)	2	Ns
Clinical Salicylate Sensitivity[b]	8 (20.5%)	0	43 (22.3%)	3	Ns
Sleep Apnoea	1 (2.6%)	0	14 (7.3%)	4	Ns

Categorical variables expressed as n (%) with differences measured by chi-square test.

Abbreviations: ABPA, Allergic Bronchopulmonary Aspergillosis; CT, Computed Tomography; GORD, Gastro-oesophageal Reflux Disease; SAFS, Severe Asthma with Fungal Sensitization.

aDetermined clinically using evidence of relevant serological and radiological information guided by conventional clinical diagnostic criteria.

bDetermined clinically based on compatible history of reaction on exposure and consistent clinical phenotypes.

eosinophilia: Never, Rare, Intermittent and Persistent. Broadly, patients demonstrated a blood eosinophilia in 1 in 10 blood results in the Rare group; 1 in 3 results in the Intermittent group; and 3 in 4 results in the Persistent group. Table 3). Only one patient, with at least 10 blood eosinophil results, registered an eosinophil count of ≥300 cells/μl in all of their test results.

The clinical features of these four groups are shown in Table S4. In general, as the frequency of eosinophilia increased so too did the surrogate biomarkers FeNO and total IgE and co-morbidity with ABPA and bronchiectasis. Increasing eosinophilia was also associated with worsening lung function, particularly FEV_1/FVC ratio and $FEF_{25-75\%}$ (Figure 3, data in Table S5).

3.5 | Sputum eosinophilia

Sputum differential counts were performed in a subset (n = 87) of patients at a single time point following non-biologics asthma treatment optimization, as part of their workup in the regional difficult asthma clinic at University Hospital Southampton.

Sputum eosinophil counts were higher in those patients with persistent blood eosinophilia (median 4.4%, IQR: 10.6) than patients never eosinophilic (median 0.4%, IQR: 1.1), $p < .001$ by Kruskal-Wallis corrected for multiple comparisons) (Figure 4A). Accordingly, patients less frequently eosinophilic on blood tests showed a tendency to paucicellular or neutrophilic sputum profiles whilst those that showed more frequent blood eosinophilia had a tendency to demonstrate eosinophilic sputum profiles (Tables S6 and S7). However, increasing persistence of blood eosinophil counts was also associated with an increase in sputum neutrophilia (Table S6).

We compared the predictive value for current and historical evidence for blood eosinophilia in determining the sputum eosinophilia (sputum eosinophils ≥2%). The positive predictive value (PPV) for a single contemporaneous blood eosinophil count to predict sputum eosinophilia was 60.71% (95%CI: 45.94 to 73.76); superior to evidence of any retrospectively noted historical eosinophilia 42.25% (95%CI: 38.64 to 45.95). Whilst the negative predictive value (NPV) of a contemporaneous blood eosinophil count was 71.11% (95% CI: 61.20 to 79.35), never demonstrating a blood eosinophilia across multiple historical counts improved the NPV to 90.91% (95% CI: 57.35% to 98.67%) (Figure 4B, contingency tables described in Table S8).

4 | DISCUSSION

The measurement of clinical and biological features in large cohorts has clearly demonstrated the heterogenous nature of severe asthma. However, the chronic and dynamic nature of severe asthma is poorly represented in clusters derived from cross-sectional study designs,

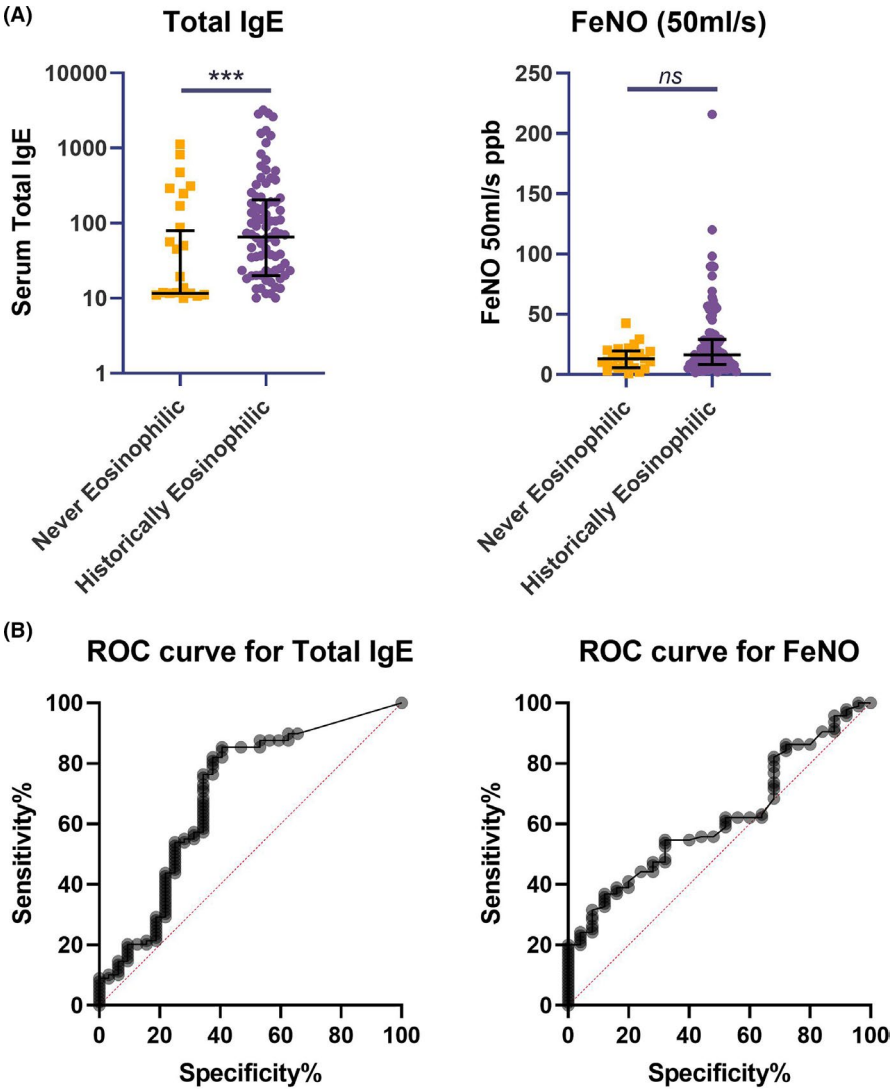


FIGURE 2 A: Boxplots comparing Total IgE and FeNO between "never eosinophilic" and "historically eosinophilic" (at least one blood eosinophil count >300 cells/μL but not currently)". B: Receiver Operating Characteristic Curve for Total IgE and FeNO in predicting "historically eosinophilic (but not currently)" from "never eosinophilic".

	Never (39)	Rare (65)	Intermittent (65)	Persistent (66)
% Eos counts ≥0.3	0	11.27 (10.69)	39.61 (16.96)	76.26 (14.71)
Number of tests	17 (14)	27.50 (43)	24.50 (39)	20.50 (23)
Minimum Eos	0 (0)	0 (0)	0 (0)	0 (0.1)
Median Eos	0.1 (0.1)	0.1 (0.0)	0.2 (0.0)	0.4 (0.11)
Maximum Eos	0.2 (0.0)	0.4 (0.2)	0.7 (0.6)	1.1 (0.9)

TABLE 3 Summary Eosinophil count statistics in groups defined by frequency of blood eosinophilia

which are weighted towards measurements that are static (age of onset, atopic status) or the measurement of variables known to fluctuate (eosinophil counts[18,19] and FeNO[28]) at a single time point. Variability in eosinophils has been associated with poor asthma control[17] and lung function decline.[16] Therefore, characterizing patients based on fluctuations in repeated measures offers a novel approach to asthma phenotyping.[29]

Here, we have used electronic health records to stratify patients with difficult to control asthma based upon repeated blood eosinophil counts into clinically intuitive and therefore clinically translatable descriptions. Using a 300 cells/μl cut-off (as per NICE asthma biologic guidelines), consistent with other studies, we found very few patients (0.8%) to be eosinophilic on every measurement.[14,17–19] However, though our cross-sectional data corroborates the statement that T2 inflammation is found in around 50% of patients with severe asthma,[30] our findings demonstrate that in fact, the vast majority (83%) of difficult to treat asthma patients have evidence of eosinophilia on at least one occasion in the past decade. Whilst blood eosinophil counts of 150 cells/μl or greater have been used in severe asthma as a predictor of response to anti-eosinophil biologics,[10,11] and this used to define an eosinophilic phenotype, we have taken the more conservative level of 300 cells/μl. However, we have

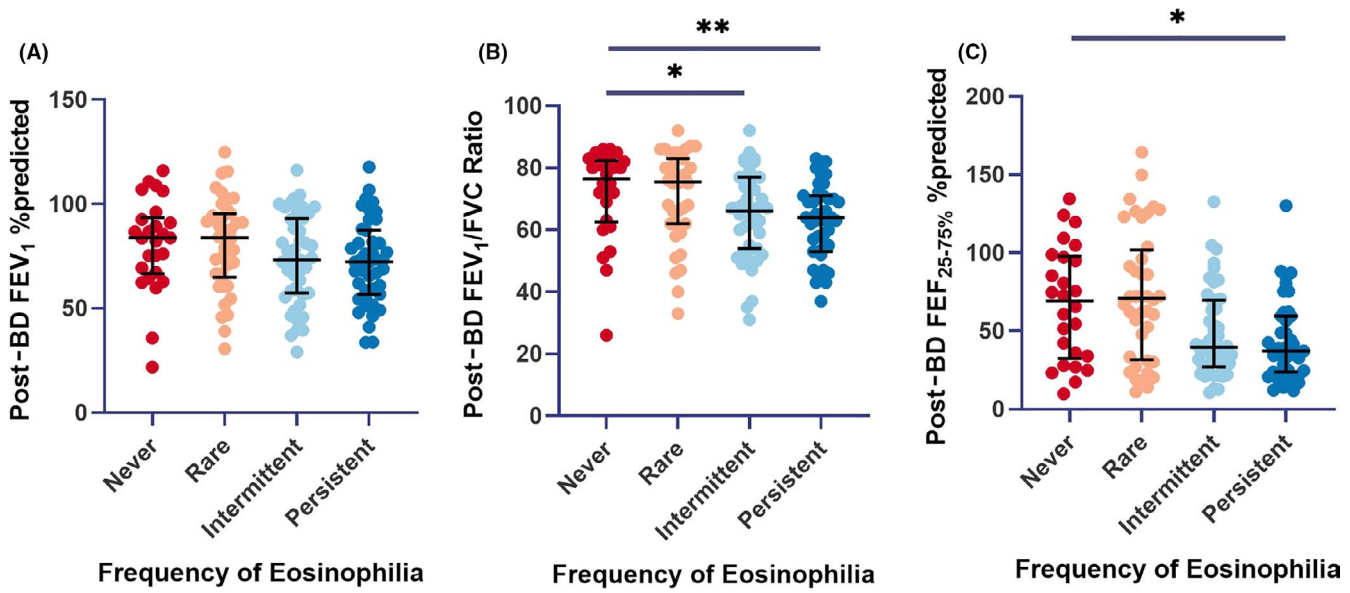
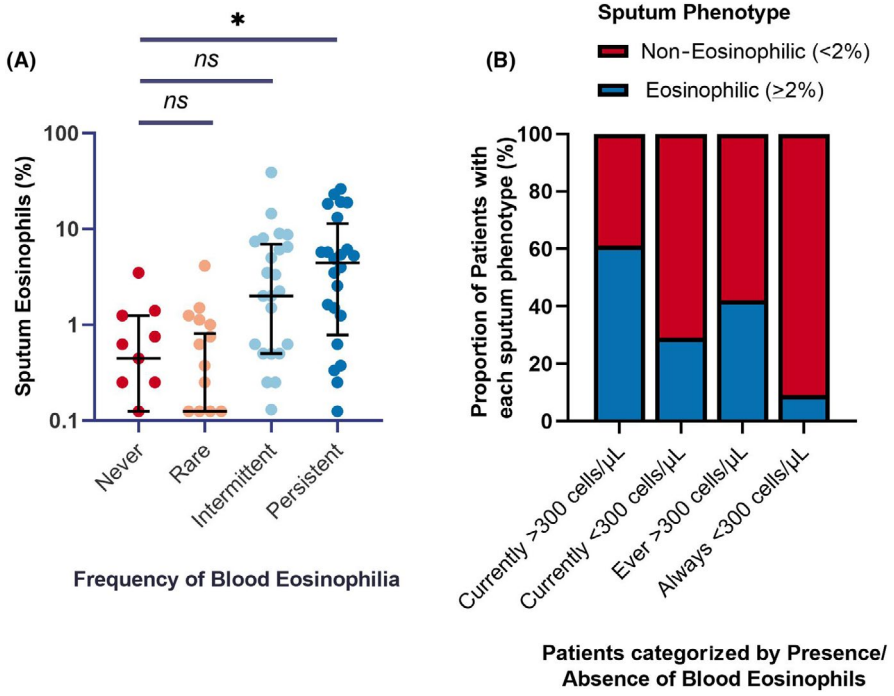


FIGURE 3 Post-bronchodilator Spirometry differences between groups of patients defined by frequency of blood eosinophilia. * $p < .05$, ** $p < .01$

FIGURE 4 (A) Differences in Percentage of Sputum Eosinophils in Cell Differential Count of Induced Sputum in Patients between groups of patients defined by frequency of blood eosinophilia. Between-group differences assessed by Kruskal-Wallis with pairwise comparisons against never eosinophilic groups by Dunn's correction for multiple comparisons * $p < .05$. (B) Proportion of patients with a sputum eosinophilia (≥2%) according to demonstration of blood eosinophila at cross-section or retrospectively



also evaluated the impact of taking a lower threshold of 200 cells/uL, used as the laboratory in Southampton only reports results in centiles and so cannot define a 150 cells/uL threshold. At the blood eosinophil threshold of 200 cells/μl, only 3% of severe asthmatics were never eosinophilic. This may have significant relevance to clinical practice of prescribing a rapidly expanding portfolio of biologic drugs whose use is partly governed by meeting a qualifying blood eosinophilia as demonstration of having eosinophilic/ T2 asthma.

Transient or intermittent blood eosinophilia is likely to reflect varying levels of T2 suppressing treatment such as corticosteroids and the inherent variability in disease severity within this exacerbation prone population. As such, contemporaneously taken measures have greater relevance to understanding the airway biology[4] of patients as part of their workup in a severe asthma clinic. Nevertheless, subjects with persistently uncontrolled eosinophilic expression (never and rare vs intermittent and persistent) over the 10-year observational period had by the end of the observation period more sputum eosinophilia, more severe airflow obstruction and worse small airways disease pattern, as assessed by FEF_{25-75}. Eosinophilic inflammation has been implicated in airway remodelling in asthma,[31] a process that alters airway wall thickness and has been linked to reduced lung function and loss of reversibility. The reported

rates of lung decline in a severe asthma population have varied at around 30 ml/year[16,32,33]; however, the nature of our EHR data means that there are few spirometry data pre-dating the blood test data to allow for assessment of change over time. Similarly, in the absence of detailed contemporaneous medication data, it is difficult to describe the proportion of blood eosinophil count fluctuations that occur independently of changes in acute or maintenance treatments. As the WATCH longitudinal cohort study continues, these data can be collected prospectively, potentially allowing further stratification of the variable blood eosinophil sub-group. Nevertheless, the present findings are consistent with the established association of eosinophilic inflammation and lung function decline[14,16,34,35] and support the rationale of a treatment strategy to control eosinophilic inflammation in asthma,[2] a rationale further evidenced by the lack of lung function decline in those who are rendered exacerbation free with mepolizumab therapy.[36]

At the other end of the spectrum, retrospective interrogation of blood eosinophil counts better identifies a group of 'never eosinophilic' patients than considering contemporaneous blood tests alone. Strictly, these patients should be more accurately termed 'patients with no evidence of prior eosinophilia'. It is possible, for example, that many such patients do in fact have an 'eosinophilic phenotype' but that it has never been captured by intermittent snapshot testing or was masked by oral corticosteroid treatment. Comparison of never and historically eosinophilic patients suggests that this might be especially true of patients with a raised serum IgE. Patients with no evidence of prior eosinophilia underwent fewer blood tests results than patients with evidence of eosinophilia and so it is possible that if they had had additional blood tests that they might reveal eosinophilia. However, as they showed a tendency to have paucicellular or neutrophilic, rather than eosinophilic, sputum phenotypes this would argue against this and favour them being truly non-eosinophilic. Furthermore, the clinical features of these patients with no evidence of eosinophilia are also distinct: they have preserved lung function and have lower levels of FeNO, total IgE and sputum eosinophilia but otherwise similar levels of poor asthma control and healthcare utilization (in the past 12 months). This is consistent with other cluster analyses of secondary care asthma population[37] and severe asthma populations.[38] It is possible that these difficult-to-treat asthma patients represent a distinctive phenotype of patients with heightened symptom perception that is discordant to their airway pathophysiology or a group with other distinct biology. Future research should focus on further assessing their mechanistic nature.

Though patients with no evidence of historical blood eosinophilia are unlikely to demonstrate airway eosinophilia, the direct opposite is not necessarily true. Patients with persistent blood eosinophilia remain heterogenous in terms of airway inflammation and also demonstrate an increase in sputum neutrophilia. Accepting the temporal dissociation between blood and sputum sampling, a number of mechanisms may be responsible for this finding. Firstly, blood eosinophil counts are a biomarker of the entire respiratory tract rather than just central airways that are described by induced sputum.[39] Confirmation of nasal polyposis by CT was only available in a small proportion of patients (Table S4); however, it is likely that the upper airways also contribute to the recorded blood eosinophilia.[40] Alternatively, airway neutrophilia is associated with an altered airway microbiome,[41] which may induce corticosteroid resistance through TAK1 (Transforming growth factor-β–associated kinase-1)/MAPK (mitogen-activated protein kinase) activation, for example.[42] This would favour persistent type 2 disease expression despite high dose steroid therapy and contribute to persistent blood eosinophilia. Both of these proposed mechanisms reinforce the importance of interpreting eosinophil blood test results in the context of disease state, co-morbidities and treatment being taken[43] and explain why a single cross-sectional measure may be an imperfect biomarker for T2 high asthma[44] or anti-IL5 therapy response.[45]

Inherent to the EHR system analysis described here are a number of biases, such as patient behaviour, clinician behaviour and healthcare processes. We have included all full blood counts from the past ten years indiscriminately. Blood tests were requested for asthma purposes, such as during an acute exacerbation or as part of a characterization process, but some were requested for any other purpose, ranging from routine (eg annual diabetes check, pre-operative assessment) to emergent (eg chest pain). Perhaps the most salient reason for a spike in eosinophilia is an asthma exacerbation but it is worth considering different patients will have different thresholds for seeking medical attention: for the same severity of exacerbation, some patients may self-manage their asthma, whilst others may present to a primary care service and others to the Emergency Department. Each presentation harbours varying likelihoods of performing blood tests in relation to commencing steroid treatment (ranging from no blood test to blood test on rescue steroids). Similarly, whilst all the patients included in the study were treated with high dose asthma therapies, a proportion of blood tests predate asthma treatment optimization, diagnosis or even symptom onset.

The impact of patient behaviour, clinician behaviour and healthcare processes on EHR data mean that any inferences to underlying mechanisms are purely hypothesis generating, but this form of sampling bias is not exclusively undesirable. The purposive sampling towards clinical events mean that the occurrence of a blood test, independent of its result, might itself be significant.[46] Moreover, these data are truly representative of clinical practice, and the findings described herein are therefore highly translational and relevant to the clinical setting.

5 | CONCLUSIONS

Here, we demonstrate that the longitudinal perspective facilitated by the interrogation of electronic healthcare records provides an opportunity to stratify patients beyond the binary classification of eosinophilia. This additional phenotypic perspective allows appreciation of the multifactorial contributions to severe eosinophilic asthma as well as the identification of a small but distinct non-eosinophilic

phenotype. Future studies should prioritize longitudinal perspectives on asthma characterization, as these are likely to better guide stratified patient management.

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CONFLICT OF INTERESTS

PHH declares that he has employment though GSK. AA, CN, CB, ZL, MH, DK, AF, WCGF, PD, HMH, RD and RK declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

AUTHOR CONTRIBUTIONS

AA contributed to study design, data collection, analysis and drafted the initial manuscript. CN, MH, DK, CB, AF, WGCF, PD, HMH contributed to study design, undertook longitudinal data collection and contributed to manuscript preparation. RD and PH contributed to study design, and manuscript preparation. RK contributed to study design, data collection, manuscript preparation and acts as guarantor for the paper.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section.

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