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Abstract: Background Elevated sputum eosinophil counts predict asthma exacerbations and responsiveness to inhaled corticosteroids but are impractical to measure in primary care. We investigated the relationship between blood eosinophil count and prospective annual asthma outcomes for a large UK cohort.
Methods Historical database analysis utilising anonymised medical record data to identify primary care patients with asthma aged 12-80 years with 2 years of continuous data, including 1 year before (baseline) and 1 year after (outcome) their most recent eosinophil count. Negative binomial regression was used to compare outcome exacerbation rates and logistic regression to compare odds of asthma control for patients with blood eosinophils ≤400/μL vs. >400/μL, adjusting for age, sex, body mass index, smoking status, and Charlson comorbidity index.
Findings Overall, 20 929 of 130 248 (16%) of patients had blood eosinophil counts >400/μL. During the outcome year, these patients experienced significantly more severe exacerbations (adjusted rate ratio [RR] 1·42; 95% CI 1·36-1·47) and acute respiratory events (RR 1·28; 1·24-1·33) than those with counts ≤400/μL. They also had significantly lower odds of achieving overall asthma control (OR 0·74; 0·72-0·77), defined as limited reliever use and no asthma-related hospital attendance/admission, acute course of oral corticosteroids, or prescription for antibiotics. Exacerbation rates increased progressively with nine ascending categories of blood eosinophil count as compared with a reference category of ≤200/μL.
Interpretation Patients with asthma and blood eosinophil counts >400/μL experience more severe exacerbations and have poorer asthma control. Furthermore, a count-response relationship exists between blood eosinophil counts and asthma-related outcomes. Blood eosinophil counts could add predictive value to GINA control-based risk assessment.
Funding Teva

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***Reply to Reviewers Comments**

Manuscript reference number: THELANCETRM-D-15-00283R1
Title: Blood eosinophil count and prospective annual asthma disease burden: UK cohort study

Ms. Laura Feetham
Senior Editor, The Lancet Respiratory Medicine
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Dear Ms. Feetham,

Thank you for the second review of our manuscript (THELANCETRM-D-15-00283R1) and for giving us the opportunity to provide a revision.

We have conducted the reanalysis requested by the reviewer and have tested the ELEN index as suggested.The results, summarised below, are now included inthe manuscript and the online supplement.

We have submitted our revised paper as one "clean" copy and one copy where our changes are tracked, as instructed. In addition, we have provided a separate document listing the comments and our replies, point by point (below).

Thank you for reconsidering our submission.

Best wishes,
David B. Price, for the authors

Reviewers' comments:

Reviewer #1:
Reviewer comments: THELANCETRM-D-15-00283R1

Comments:

1. The authors have partially addressed my initial major comment # 1. This study pre-specified testing a binary cutoff of blood eosinophil at >400 cells/uL (which conforms to the RCTs of Teva'sreslizumab) for association with clinical endpoints. Alternatively, GSK initially conducted RCTs of mepolizumab with blood eosinophil cutoff at 300 cells/uL, and later modified the cutoff selection to also allow >150 cells/uL at screening. Various authors in the peer-reviewed literature have recommended other different cutoffs in peripheral blood eosinophils (usually ranging between 200 and 300 cells/uL) as the most accurate for identifying sputum eosinophilic asthmatics. Given the large sample size in this observational study, I believe this particular work has the potential to be a landmark publication for demonstrating an ideal blood eosinophil cutoff for identifying eosinophilic asthmatics in the general clinic to target optimal treatments. That is why I had previously recommended that the authors test associations between the clinical endpoints and blood eosinophil cutoffs at 200, 250, 300, 350, 400 cells/uL, and the ELEN Index. The authors only partially addressed my initial comment.

In this revised submission, the authors tested blood eosinophil cutoffs at 300, 400, and 500 cells/uL to show association with the primary clinical endpoints (Table S3). A trend of increasing exacerbation risk

with increasing levels of blood eosinophils is demonstrated clearly for all tested clinical endpoints. For example, blood eosinophil groups >300, >400, and >500 cells/uL were associated with increased adjusted risk of severe exacerbation by 29, 42, and 58%, respectively. The authors also show an increasing trend of clinical risk by comparing blood eosinophil cutoffs >300 cells/uL to 1000 cells/uL at increments of 100 cells/uL against a reference of =< 200 cells/uL (Fig. 3 A-D). I am not clear about the value of this latter analysis (results shown in Fig. 3 A-D), which 'forces' a 'reference group', which I would argue is unnecessary (the reference group facilitates an indirect comparison to yield only relative differences, whereas direct comparisons can be performed using the clinical endpoints). All binary cutoffs will allow direct comparisons to be made and to show a trend (as in Table S3, if such a trend exists). For example, using a blood eosinophil cutoff of 300 cells vs. 400 cells is associated with an increased risk of severe exacerbation of 29% vs. 42%, respectively, which is quite easy to interpret. A clinician then has to make a call of whether s/he considers a 29% increased risk to be a sufficiently high and clinically meaningful risk (for the potential benefit/cost of a particular treatment). As these are average risks for those groups (not directly attributable to any one individual patient), the clinician will need to also consider the consequences/risks of misdiagnosis (PPV and NPV, i.e., incorrect classification of a patient), knowing that a 42% increased risk will be based only on 16% of the patient population, whereas the 29% increased risk would be relevant for 28% of the patient population. Furthermore, the =< 200 cells/uL cutoff of blood eosinophils used as a "reference' group in this study is discordant with the >150 cells/uL cutoff being proposed to potentially recommend patients for mepolizumab treatment.

Therefore, I would re-encourage the authors to replace the analysis shown in Fig 3 by the recommendation I made earlier (i.e., use cutoffs at 200, 250, 300, 350, 400, and 500 cells/uL) and expand Table S3 (or create new graphs similar to Fig. 3 A-D). I don't believe it will be necessary to analyze data beyond > 500 cells/uL cutoff as the eligible patient population (prevalence) shrinks drastically, and also because the increased risk of severe exacerbations is generally known for patients with very high eosinophil counts. The most uncertainty among researchers and clinicians in agreement on what cutoff in blood eosinophils is ideal for identifying eosinophilic asthmatics seems to fall in between 150 and 400 cells/uL. This interval is where the focus should be in identifying the most appropriate statistically significant and clinically meaningful binary cutoff.

> Response: We agree with the reviewer's suggestion that smaller increments at lower blood eosinophil counts would be relevant. However, we face the problem that a substantial number of blood eosinophil measurements were not recorded at the level of accuracy needed to perform the analysis as suggested. Measurements were frequently expressed at 10^9 cells /litre with only 1 decimal place accuracy. We have now performed a subanalysis using 54,072 (42%) measurements recorded with 2 decimal place accuracy, assuming that this subpopulation is representative of all measurements.
>
> Because these new analyses do not include all patients, we have reported these results in the online appendix, with summary in the main manuscript. The results are depicted inthe new appendix figure S1 and appendix table S4. Table S4 shows that patients with an eosinophil count>300/µL (31% of the population) on average have a 30% increased rate of severe exacerbations compared with patients at lower levels. However, figure S1 shows that most of these patients with values from 301-450/µL (55% of patients with counts>300/µL) do not have a significantly higher risk compared with patients who have counts ≤150/µL.

In regard to the authors' response stating that they were unable to test the ELEN index because of the revision turn-around time of 5 days and that the required neutrophil and lymphocyte data have not been extracted, I accept the justification. However, in their response, the authors also gave a reason that "the ELEN index performed less well than the unadjusted blood eosinophil count as an index of response to Benralizumab (Castro et al. Lancet Respir Med 2014; 2: 879-90)". I do not agree with the second justification. The Benralizumab study was randomized using the ELEN Index as a stratification factor (to control bias). Whereas, the blood eosinophil 300 cells/uL cutoff was a post-hoc analysis performed with post-randomization data, which is subjected to bias. At the 100 mg dose, the ELEN index showed annual asthma exacerbation rate reduction of 41% (from a placebo exacerbation rate of 0.57), whereas the 300 eosinophil cutoff showed a rate reduction of 43% (BUT from a HIGHER placebo exacerbation rate of 0.68). Thus, one cannot say that the blood eosinophil cutoff performed better than the ELEN Index.

> Response:We have added the following text reporting the results when applying the ELEN index to identify patients with sputum eosinophilia: “Applying the ELEN index to the population (calculable for 129 597/130 248 [99.5%] patients), 42 737 (33.0%) were defined as being ELEN index positive. The severe exacerbation rate ratio for these patients, relative to those who were ELEN index negative, was 1·19 (1·16–1·23), and that for acute respiratory events was 1·11 (1·08–1·14). The odds ratios for risk-domain asthma control and overall asthma control were 0·90 (0·87–0·92) and 0·83 (0·81–0·85), respectively.”

2. The authors have satisfactorily addressed my initial major comment # 2 by substituting their original analysis of count data using Poisson regression with the recommended negative binomial (NB) regression. The newly calculated rate ratios, corresponding 95% CIs, and associated p-values are more appropriate for testing the study hypotheses and making inferences given the observed frequency distributions of the count data.

Final comment:

I leave it up to the LRM editor and authors on whether or not they decide to adopt my recommendations in Comment # 1 above. This revised version is significantly improved from the original submission, and I would not object to its publication in its present form. I sincerely believe it would be a stronger and more useful contribution to the asthma clinical and research community if my above recommendation is adopted, especially in light of the large sample size of very well characterized patient data in this study.

Reviewer #2: Dear Editors and Authors,

Thank you for the opportunity to review this revised article. I have re-read the article and author comments. Overall, I am satisfied with their revisions. I only have a few minor comments:

Comments:

1) The authors state "An additional adjustment for the baseline number of lower respiratory consultations treated with antibiotics, an indication for blood count measurements, produced no relevant change in the prospective association between eosinophilia and severe exacerbations,

supporting the concept that selection bias was limited." Adjustment addresses the issue of confounding not selection bias. Perhaps the authors would consider revising this statement.

Response: Thank you; we have revised the sentence accordingly.

2) The authors state "smoking status, and Charlson comorbidity index score." Earlier the Charlson Comorbidity Index was capitalized in the text. Perhaps it is best to be consistent.

Response:We have now made the capitalisation consistent throughout.

3) Well done!

Response: Thank you!

Reviewer #6: The authors have responded appropriately to the issues raised by the reviewers, and the manuscript is much better as a result. The new version of Figure 3 is excellent.

One minor comment: the authors have responded well to my question about the timing of blood sampling in relation to courses of oral steroids, and the effect this might have on suppressing blood eosinophil numbers. The authors may also wish to reflect on the potential for the blood eosinophil count to rise in the period leading up to an exacerbation. A doctor might be more likely to perform a blood count in a patient with worsening asthma, just prior to prescribing oral steroids. Could this inflate the link between high blood eosinophils and asthma exacerbations?

Response: Thank you. We have added the following sentence to the Discussion: “Moreover, it is possible that blood eosinophil count rises in the period preceding an exacerbation, inflating the link between high blood eosinophil count and exacerbations.”

Blood eosinophil count and prospective annual asthma disease burden: UK cohort study

Running head: Eosinophils in asthma and future risk

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Word count: 3376

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Summary

BackgroundElevated sputum eosinophil counts predict asthma exacerbations and responsiveness to inhaled corticosteroids but are impractical to measure in primary care. We investigated the relationship between blood eosinophil count and prospective annual asthma outcomes for a large UK cohort.

MethodsHistorical database analysis utilising anonymised medical record data to identify primary care patients with asthma aged 12–80 years with 2 years of continuous data, including 1 year before (baseline) and 1 year after (outcome) theirmost recent eosinophil count. Negative binomialregression was used to compare outcome exacerbation rates and logistic regression to compare odds of asthma control for patients with blood eosinophils ≤400/μL vs. >400/μL, adjusting for age, sex, body mass index, smoking status, and Charlson comorbidity index.

Findings Overall, 20 929 of 130 248 (16%) of patients had blood eosinophil counts>400/μL. During the outcome year, these patients experienced significantly more severe exacerbations (adjusted rate ratio [RR] 1·42; 95% CI 1·36–1·47) and acute respiratory events (RR 1·28; 1·24–1·33) than those with counts ≤400/μL. They also had significantly lower odds of achieving overall asthma control (OR 0·74; 0·72–0·77), defined as limited reliever use and no asthma-related hospital attendance/admission, acute course of oral corticosteroids, or prescription for antibiotics. Exacerbation rates increasedprogressively with nine ascending categories of blood eosinophil count as compared with a reference category of ≤200/μL.

Interpretation Patients with asthma and blood eosinophil counts >400/μL experience more severe exacerbations and have poorer asthma control. Furthermore, a count-response relationship exists between blood eosinophil counts and asthma-related outcomes. Blood eosinophil counts could add predictive value to GINA control-based risk assessment.

FundingTeva

ClinicalTrials.gov: NCT02140541

Keywords: asthma, control, eosinophils, exacerbations, observational

Introduction

Asthma is a complex and heterogeneous disorder.[1,2] The presence of eosinophils in asthmatic inflammation has been recognised for many years, and eosinophilic asthma is a common phenotype that is usually responsive to corticosteroid therapy.[3] Eosinophilic airway inflammation, as reflected in elevated sputum eosinophils, appears to be closely related to the risk of severe asthma exacerbations and loss of asthma control with inhaled corticosteroid (ICS) withdrawal, although the pathogenetic mechanisms remain undefined.[4–6] The tailoring of asthma therapy based on maintaining sputum eosinophils at ≤2–3% is effective in decreasing asthma exacerbations in patients with severe disease,[4,7] and asthma therapies targeting eosinophils are effective in reducing the incidence of asthma exacerbations and improving markers of asthma control for patients with severe eosinophilic asthma,[8–10] as well as for patients with moderate-to-severe asthma and eosinophilia.[11]

Sputum eosinophil percentages of ≥2% to ≥3% of the total cells, depending on the study,have been used to define eosinophilic asthma.[2,4,11]However, sputum inductionis impractical in non-specialised clinical settings. Instead, peripheral blood eosinophil counts are easily obtained, and their use as a biomarker for increased disease burden or exacerbation risk is a topic of ongoing study. An inverse correlation between blood eosinophil counts and forced expiratory volume (FEV_1) was observed in an earlier small study.[12]In a randomised controlled trial of patients with severe asthma, a progressive increase in risk of exacerbation was found with increasing baseline blood eosinophils,[9] and in another studyof severe asthma blood eosinophil countswere independently associated withboth risk of exacerbation and treatment response to anti-interleukin-5 therapy, whereas sputum eosinophils did not predict response.[13]

Possible associations between blood eosinophil counts and overall disease burden in asthma require further study in the general population of patients with asthma, outside of clinical trials for severe asthma. While a recent validation study reports that blood eosinophils were an accurate biomarker for identifying sputum eosinophilia,[14] other studies report a lack of concordance between presence of sputum eosinophilia and blood eosinophilia.[15,16] Therefore, rather than identifying sputum eosinophils, it is likely more important to determine whether blood eosinophil counts can be used to monitorasthma control/exacerbation risk in clinical practice. In recent observational studies in the US,

elevated blood eosinophil counts have been associated with increased prospective risk of asthma exacerbations and excessive short-acting reliever use[17] as well asincreased historical risk of exacerbations.[18,19]There is a need to replicate these findings in other settings and databases, to study larger numbers of patients, and to examine patient-reported outcomes.

Anonymised data from high-quality electronic primary care records of several million patients are available in the UK, permitting the study of very large, heterogeneous populations of patients with asthma. The primary objective of this historical primary care cohort study was to investigate the relationship between blood eosinophil count and severe asthma exacerbations and asthma control during the subsequent year. A subanalysis was performed to examine the relationship between severe exacerbations and Global Initiative for Asthma (GINA)-defined current clinical control. Secondary objectives were to identify a potential relationship between demographic and clinical characteristics and the prospective risk of elevated eosinophil counts.

Methods

Data sources

These analyses examined data from August 1990 to February 2013 drawn from both the Optimum Patient Care Research Database (OPCRD)[20] and the Clinical Practice Research Datalink (CPRD)[21](see appendix for more detail). Patient data were cross-referenced to avoid duplication of individuals studied.

Patients and study design

Patients aged 12–80 years of age with an asthma diagnostic Read Code, a recorded blood eosinophil count, and 1 year of continuous data before and after their most recent blood eosinophil count (defined as the *index date*) were included in the study (figure 1). A valid eosinophil count was defined as a numeric value in blood eosinophils, recorded at least 1 year before the final data extraction as number of cells $x10^9$/L with 1 or 2 decimals. Values were transformed to blood eosinophils/µL.Patients with chronic obstructive pulmonary disease or any chronic respiratory disease

other than asthma were excluded, as were patients withrecorded eosinophil counts >5000/μL(to avoid extreme outliers) and those lacking information on smoking status.

The study period for each patient comprised 2 sequential years: a 1-year baseline period preceding and including the index date (the date of the last eosinophil count) for patient characterisation and a 1-year outcome period after the index date.

Eligible patients were divided into two cohorts according to blood eosinophil count of≤400/μL or >400/μL, a value representingthe upper limit of the published normal blood eosinophil range (0–400/μL) in UK clinical practice.[22]

Outcome measures

A*severe exacerbation* was defined, as previously described (appendix), according to the American Thoracic Society/European Respiratory Society definition[23]as an asthma-related hospitalisation, attendance at an Accident and Emergency (A&E) department, or a prescription for acute oral corticosteroids. An *acute respiratory event* was defined more broadly as an asthma-related hospital attendance/admission or A&E attendance, prescription for acute oral corticosteroids, or prescription for antibiotics in conjunction with an asthma-related primary care consultation.

Asthma control assessment was based on two measures, previously described (appendix).*Risk-domain asthma control* was defined as the absence of any acute respiratory event (as defined above) or asthma-related outpatient department visit. Criteria were the same for the *overall asthma control measure*, with the additional requirement of an average daily dose of ≤200μg salbutamol or ≤500μg terbutaline (defined as the available dose in prescribed canisters divided by 365).

Both primary diagnosis (asthma) and comorbidities were defined as Read codes recorded in the database at any time. For a subgroup of patients (10%), a measure of GINA-defined current clinical control (2010–2012 definition[24]) was available from information collected via OPCRD questionnaires and GP-recorded data(appendixtable S1).

Statistical analyses

Patient demographic characteristics, comorbidities, severe exacerbations, acute respiratory events, and asthma control (risk-domain, overall, and GINA-defined control) were compared between patients with blood eosinophil count ≤400/μLand >400/μL using the X^2 test for categorical variables. Variables measured on the interval or ratio scale were compared using a *t* test or a Mann–Whitney U-test if the distribution were skewed.

A negative binomialregression model was used to compare rates of severe exacerbations and acute respiratory eventsbetween eosinophil cohorts during the outcome year, and a logistic regression model was used to compare the odds of achieving asthma control. The negative binomial and logistic regression analyses were adjusted for the following confounders: age, sex, body mass index (BMI), smoking status, and the Charlsoncomorbidity index. In addition, we evaluated the potential confounding effect of the following comorbidities recorded ever: non-allergic and allergic rhinitis, eczema, diabetes mellitus, heart failure, ischaemic heart disease, and gastro-oesophageal reflux disease. The "count-response" relationship between the blood eosinophil count and the outcomes was studied by calculating rate ratios and odds ratios for nine ascending categories of blood eosinophil count compared with a reference group of patients with blood eosinophil counts ≤200/μL.

In a post hoc analysisof the subpopulation of patients who had blood eosinophil counts recorded as number of cells $x10^9$/L with 2 decimal place accuracy, we examined outcomes for eosinophil counts in smaller increments of the lower counts, namely, 200, 250, 300, 350, 400, and >500 eosinophils/μL as cut-points and as compared with a reference group of patients with blood eosinophil counts ≤150/μL.

In addition, we examined outcomes after applying the ELEN index, an algorithm that uses the eosinophil/lymphocyte ratio (ELR) and the eosinophil/neutrophil ratio (ENR), previously described as a means of stratifying patients to predict those with sputum eosinophils ≥2%.[25,26]

To examine potential predictors of peripheral blood eosinophilia, a univariable logistic regression model was used to identify baseline characteristics associated ($p<0·05$) with an eosinophil count >400/μL. A multivariable logistic regression model with stepwise reduction was then used to derive the best-fitting model of non-co-linear predictors ($p<0·05$).

Analyses were conducted using IBM SPSS Statistics version 21 (IBM SPSS Statistics, Feltham, Middlesex, UK). Statistically significant results were defined as $p<0{\cdot}05$.

Role of the funding source

Data acquisition and the analyses were funded by Teva Pharmaceuticals. Access to data from the OPCRD was co-funded by Research in Real-Life Ltd (RiRL, Cambridge, UK). Teva played no role in the collection or analysis of the data or the decision to submit the paper for publication.Teva employees had a role in interpretation of the data and review of the manuscript. The research team at RiRL designed the study, conducted the analyses, and coordinated the writing and revision of the paper in collaboration with all authors. The authors received no funds or honoraria from Teva for participation in the study.

Results

Patients

We identified343 927 patients with asthma and no other chronic respiratory disease diagnosis (figure 1). Patients meeting study eligibility criteriatotalled 248 858, of whom 130 248 (52%) patients had a recorded blood eosinophil count. Those with blood eosinophil counts were older and more likely female than those without (median age 49 vs. 34 years and 68% vs. 45% female, respectively; see appendix table S2); and they had greater asthma burden at baseline (19% vs. 3% experienced one or more severe exacerbations).

The majority of patients in the study cohort were female (68%), of median age 49 years (table 1). The median blood eosinophil count was 200/μL (interquartile range [IQR] 120–340). Sixteen percentof patients (n=20 929) had a blood eosinophil count >400/μL, and 84% (n=109 319) hada count ≤400/μL. The highest eosinophil counts (1501–5000/μL) were recorded for 382 (0·3%) patients. Compared with patients who had a blood eosinophil count ≤400/μL, those with blood eosinophil count >400/μL were more likely to be male, to be younger, to have a slightly lower BMI, and to be a non-smoker (all $p<0{\cdot}001$; table 1). Patients with counts >400/μL had more comorbid rhinitis

andeczema, received more asthma therapy, and experienced higher exacerbation rates during the baseline year than those with counts ≤400/μL (table 1).

Outcome year severe exacerbations, acute respiratory events, and asthma control

During the outcome year, patients with blood eosinophil counts >400/μL were more likely to experience severe exacerbations and acute respiratory events, and less likely toexperience asthma control (both risk-domain and overall control), than those with eosinophil counts ≤400/μL (table 2). The unadjusted rate ratios (95% CIs) for severe exacerbations and acute respiratory events were 1·30 (1·25–1·35) and 1·19 (1·15–1·23), respectively, and the unadjusted odds ratios for risk-domain and overall asthma control were 0·84 (0·82–0·87) and 0·79 (0·76–0·81), respectively (p<0·0001 for all). Adjusted rates of severe exacerbations andof acute respiratory events weresignificantly higher for those with counts >400/μL compared with counts ≤400/μL (severe exacerbation RR 1·42 [1·36–1·47]), and the adjusted odds of achieving risk-domain asthma control and overall asthma control were significantly lower (figure 2). An additional adjustment for the baseline number of lower respiratory consultations treated with antibiotics, an indication for blood count measurements, did not influence the results for severe exacerbations (RR 1·39 [1·34–1·45]). Adjustments for comorbidities, including non-allergic rhinitis, allergic rhinitis, and eczema also had little influence on the association of eosinophil count with severe exacerbations (further details are reported in the appendix).

Using a lower cut-off value to define eosinophilia, the associations became weaker, and using a higher cut-off value the association was greater: namely, at cut-off values of >300, >400, and >500, the RRs for severe exacerbations were 1.30, 1.42, and 1.58, respectively(further details in appendix table S3). Figure 3 depicts outcomes for patients categorised by ascending blood eosinophil count as compared with a reference category of ≤200/μL.Severe exacerbation and acute respiratory event rates increased(figures 3A and 3B) and the odds of asthma control decreased (figures 3C and 3D) progressively with ascending categories of blood eosinophil count as compared with the reference value. Patients with counts ≤300/μL did not show an increased risk of exacerbations, and the risk was marginally increased (<10%) for patients with counts of 301–400/μL.

Blood eosinophil counts to 2 decimal place accuracy were available for 54 072 (42%) patients. For this population, patients with an eosinophil count>300/μL (31% of the subpopulation) on average had a 30% increased rate of severe exacerbations compared with patients at lower counts (appendix table S4). However, most of these patients with countsfrom>300–450/μL (55% of patients with counts >300/μL) did not have a significantly higher risk compared with patients with eosinophil countsof ≤150/μL (appendix figure S1).

Applying the ELEN index to the population (calculable for 129 597/130 248 [99.5%] patients), 42 737 (33.0%) were defined as being ELEN index positive. The severe exacerbation rate ratio for these patients, relative to those who were ELEN index negative, was 1·19 (1·16–1·23), and that for acute respiratory events was 1·11 (1·08–1·14). The odds ratios for risk-domain asthma control and overall asthma control were 0·90 (0·87–0·92) and 0·83 (0·81–0·85), respectively.

Subanalysis: Exacerbations versus GINA current clinical control

GINA current clinical control (symptoms) could be calculated for the 10% of patients in each cohort who had completed an OPCRD asthma questionnaire. Patients with blood eosinophil counts >400/μL vs. ≤400/μL were less likely to achieve GINA complete control (table 3).

Within each severe exacerbation category,there was no significant difference in GINA current clinical control between eosinophil cohorts (≤400/μL vs. >400/μL; figure 4A). However, within the GINA partly controlled and uncontrolled categories, patients with blood eosinophil counts >400/μL experienced significantly more exacerbations than those with eosinophil counts ≤400/μL (figure 4B).

Clinical predictors of elevated blood eosinophil count

Complete univariable results examining potential predictors of index date eosinophil count >400/μL are reported in appendixtable S5.

In the multivariable analysis, baseline year mean blood eosinophil count >400/μL was the main predictor for future elevated eosinophil counts (OR 43·97; 95% CI 41·94–46·09). Excluding prior eosinophil counts from the multivariable model, the following factors were associated with an index date blood eosinophil count >400/μL: younger age, male sex, lower BMI, being a non-smoker,

comorbid eczema or rhinitis (allergic and non-allergic), nasal polyps, and having one or more severe exacerbations in the baseline year (table 4). In addition, as compared with being at British Thoracic Society (BTS) step 1,[27]patients at BTS step 4 had significantly higher odds, and those on no therapy or at BTS step 2 (ICS or leukotriene receptor antagonist as single therapy) had significantly lower odds, of a blood eosinophil count >400/μL (table 4).

Discussion

Of more than 130 000 patients with asthma and no other chronic respiratory disease diagnosis who had a recorded blood eosinophil count in their routine care medical record, we found that 16% had an elevated peripheral blood eosinophil count of >400/μL. The incidence rate of severe asthma exacerbations was 42% higher, and that of acute respiratory events 28% higher, for these patients as compared with the ≤400/μL eosinophil cohort, while the odds of achieving risk-domain asthma control and overall asthma control were, respectively, 22% lower and 26% lower during the subsequent year. In our subanalysis, which included 10% of the study population, eosinophilia was associated with a greaterrate of exacerbations within GINA partly controlled and uncontrolled categories, thus providing independent information on risk and suggesting a mismatch between exacerbations and symptoms as defined by GINA control.These important findings corroborate the findings of prior studies[1,3,9]and suggest that assessment of blood eosinophils could add predictive value to a traditional control-based assessment using the GINA criteria.

A blood eosinophil count measured in the preceding year of >400/μL was a very strong predictor for an elevated blood eosinophil count >400/μL at the index date. Moreover, because the majority of patients with peripheral blood eosinophilia at the index date already had elevated blood eosinophils during the baseline year (see table 1), our data suggest that elevated blood eosinophil countsmay bea stable phenotype, at least over the short term. For this reason we excluded baseline eosinophil count from the multivariable predictive model.

After excluding prior eosinophil count from the model, other predictors identified for a count >400/μL included demographic and clinical characteristics (younger age, male sex, lower BMI, being a non-smoker), comorbidities (allergic and non-allergic rhinitis, nasal polyps, and eczema), and

disease activity (higher baseline exacerbation rates). The presence of atopic conditions and nasal polyps as predictors of eosinophilia is an expected finding.[28]Similarly, lower BMI and non-smoking status as predictors are not unexpected, because the obesity-related asthma phenotype is typically noneosinophilic,[1,2] and current and ex-smokers have lower blood eosinophils than never-smokers.[29] The proportion of patients with peripheral eosinophilia (16%) in the present study was similar to the 18–26% prevalence found among adults with asthma in three recent observational studies using a similar cut-point (<400/μL vs. ≥400/μL).[15,17,19]Zeiger et al[17]in a study of 2392 adults with asthma found that blood eosinophil count ≥400/μL was a risk factor for asthma exacerbation the following year as well as for excessive SABA use (≥4 puffs/day, equivalent to ≥400 μg/day in this study). Wagener et al[14] report different cut-points for cohorts of differing asthma severity and propose that the optimal cut-point may vary by study population. In a recent small study of 164 patients with uncontrolled asthma despite treatment, a cut-point of 260 cells/μL was highly predictive for eosinophilic asthma; a blood eosinophil percentage of 2.7% was the best predictor.[30]In another recent study, blood eosinophil counts were poor predictors of sputum eosinophil percentages[16]; however, the cut-point used (300 cells/μL) was considered possibly too low.[31]

We found a clear and consistent "count-response" relationship between blood eosinophil count and our database-derived measures during the outcome year. Other observational studies have reported similar findings.[18,19]Among subjects in a large NHANES study, the odds of asthma exacerbations the prior year were increased with higher eosinophil counts as compared with <300/μL.[18] Similarly, for 616 patients with severe asthma, a progressive increase in risk of exacerbation with increasing baseline blood eosinophils was reported in the Dose Ranging Efficacy And safety with Mepolizumab in severe asthma (DREAM) trial.[9]The results of oursubanalysis looking at GINA-defined asthma control for 10% of patients indicated that blood eosinophil counts appeared more strongly linked to exacerbation risk than to measures of asthma control and support the view that symptoms and risk/inflammation in asthma are to some extent disassociated.[9]

Strengths of the current analyses include the large study population of patients with physician-diagnosed asthma (n=130 248), much larger than prior observational studies (n<3000 adult patients with asthma).[17–19] Moreover, this study drew on two large, well-maintained databases.[20,21]

Limitations of the current study include the fact that data were not collected prospectively; moreover, the analyses were based on a single blood eosinophil count. The measure of overall asthma control was designed to account for daily symptom management as reflected in reliever use because we were not able to assess asthma symptoms from database records. We were limited to the available data; for example, pack-years of smoking are not available in the databases. In addition, we did not assess whether patients had received a course of oral corticosteroids prescribed within 2 weeks before the blood eosinophil measurement, which could have substantially reduced the blood eosinophil counts. However, this would tend to bias results to the null, and we estimate the percentage of patients to whom this could refer to be small and thus are confident that exclusion of these patients would not have relevantly changed the results. Moreover, we did not assess treatment adherence. While poor adherence to ICS could potentially cause both eosinophilia and poorly controlled asthma, we believe it unlikely that this is the sole explanation for our findings (in patients with COPD, blood eosinophil counts are not very responsive to ICS treatment[32]).We cannot rule out the potential for selection bias:similar to the findings of Zeiger et al,[17]patients who had a recorded blood eosinophil count were more likely to be female, had more comorbidities, and were more likely to have baseline severe exacerbations than those without eosinophil count, possibly limiting the generalisability of our findings. Indeed, we cannot fully exclude that patients with eosinophilia were more likely to be selected, because they have a greater likelihood of having a full blood count measured at an exacerbation, but this would reflect the higher rate of exacerbations associated with blood eosinophilia. Moreover, it is possible that blood eosinophil count rises in the period preceding an exacerbation, inflating the link between high blood eosinophil count and exacerbations. An additional adjustment for the baseline number of lower respiratory consultations treated with antibiotics, an indication for blood count measurements, produced no relevant change in the prospective association between eosinophilia and severe exacerbations, supporting the concept that confounding was limited. Further characterisation of patients with eosinophilia and particularly their response to therapy is needed. While older women were more likely to have a blood eosinophil assessment in UK clinical practice, younger age and male sex were predictors of eosinophilia, suggesting a rationale for checking blood eosinophil count in younger male patients with asthma. Moreover, work is needed to

characterise the different asthma phenotypes and to identify biomarkers that could be used to distinguish them.[2] Clinically, the use of blood eosinophilia as a biomarker for future asthma exacerbations or poor control may enable identification of patients with milder disease who could benefit from higher doses of ICSor, alternatively, the tailoring to specific patients of therapies such as mepolizumab, reslizumab, and other agents that inhibit eosinophilic airway inflammation.

In conclusion, this study demonstrates that, in a cohort of more than 130,000 patients with asthma, blood eosinophil counts >400/μL, as compared with ≤400/μL, are associated with a greater rate of asthma exacerbations and lower odds of achieving asthma control over the subsequent year. Moreover, a clear count-response relationship exists between blood eosinophil count and asthma-related outcomes as defined by our database-derived measures. Expert working groups have recommended that peripheral blood eosinophils be measured in clinical studies as one of the biomarkers to characterise study populations.[33]Our findings suggest there could be benefit in performing full blood counts with differential as a routine assessment in clinical practice for patients with asthma. Moreover, our data suggest that patients with asthma and high blood eosinophil count are potentially at elevated risk of future exacerbations regardless of current GINA control status and should be counselled and monitored accordingly.

Panel: Research in context

Evidence before this study

We searched PubMed for papers published from 2000-2015 investigating the relationship between blood eosinophil count and asthma outcomes, including asthma control and exacerbations, for adult patients with asthma in the general population. We used various combinations of the following search terms: "asthma", "eosinophils/eosinophilia", "exacerbation rate/risk", "asthma control". We reviewed the PubMed search results and reference lists of relevant papers to identify observational studies not limited to patients with severe or uncontrolled asthma. We identified four observational studies reporting the association between elevated blood eosinophil count and increased historical or prospective risk of asthma exacerbations in general populations of patients with asthma.[15,17–19]

Added value of this study

The results of our study support and extend the findings of three of these prior studies to a ≥40 times larger general population of over 130,000 patients with asthma in the UK. (The study of Schleich and coworkers[15] reported results for four cohorts according to blood [≥400 cells/mm^3] and sputum [≥3%] eosinophils and thus could not be directly compared with our findings.) The large cohort size enabled assessment of the prevalence of raised blood eosinophils among patients with asthma, and availability of questionnaire data for 10% of patients enabled us to look at risk in relation to GINA current clinical control. We found that patients with elevated eosinophil counts experienced more severe exacerbations and had poorer asthma control (more disease burden) over a subsequent year than those with a blood eosinophil count ≤400/μL; moreover, we detected a clear and consistent "count-response" relationship between blood eosinophil count and our database-derived measures during the outcome year. Our subanalysisfinding that eosinophilia was associated with an increased risk of exacerbations within GINA partly controlled and uncontrolled categories provides independent information on risk and is in line with earlier clinical trial findings of a dissociation between symptoms and risk of exacerbations for patients with severe asthma.[9]

Implications of all the available evidence

Our findings, together with those of prior studies, suggest that patients seen in primary care with

asthma and blood eosinophilia are potentially at elevated risk of future exacerbations regardless of current GINA control status and should be counselled and monitored accordingly. The question remains whether the elevated blood eosinophil phenotype is stable, and further research is needed to examine blood eosinophil counts in relation to timing of oral corticosteroid bursts, therapy with oral corticosteroids, and therapy/adherence with inhaled corticosteroids, again in the wider general population of patients with asthma.

Contributors

DBP and AR led the study design process; and all authors contributed to the design review. AR, AB, and MK are responsible for the data acquisition and analyses; EVH developed the first draft of the manuscript. All authors contributed to the interpretation of the data, reviewed and edited drafts of the manuscript, and approved the final draft of the manuscript for submission.

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Declaration of interests

DBP has Board Membership with Aerocrine, Almirall, Amgen, AstraZeneca, BoehringerIngelheim, Chiesi,Meda, Mundipharma,Napp, Novartis, and Teva. Consultancy: Almirall, Amgen, AstraZeneca, BoehringerIngelheim, Chiesi, GlaxoSmithKline, Meda, Mundipharma, Napp, Novartis, Pfizer, and Teva; Grants and unrestricted funding for investigator-initiated studies fromUK National Health Service, British Lung Foundation, Aerocrine,AKL Ltd, Almirall, AstraZeneca, BoehringerIngelheim, Chiesi, Eli Lilly, GlaxoSmithKline, Meda, Merck, Mundipharma,Napp, Novartis, Orion, Pfizer, Respiratory Effectiveness Group, Takeda, Teva, and Zentiva; Payments for lectures/speaking: Almirall, AstraZeneca, BoehringerIngelheim, Chiesi, Cipla, GlaxoSmithKline, Kyorin, Meda, Merck,Mundipharma, Novartis, Pfizer, SkyePharma, Takeda, and Teva; Payment for manuscript preparation: Mundipharma and Teva; Patents (planned, pending or issued): AKL Ltd.; Payment for the development of educational materials: GlaxoSmithKline, Novartis; Stock/Stock options: Shares in AKL Ltd which produces phytopharmaceuticals and owns 80% of Research in Real Life Ltd and its subsidiary social enterprise Optimum Patient Care; received Payment for travel/accommodations/meeting expenses from Aerocrine, BoehringerIngelheim, Mundipharma, Napp, Novartis, and Teva; Funding for patient enrolment or completion of research: Almirral, Chiesi, Teva, andZentiva.

At the time of the study, AR, VLA, AB, and MKwere employees of RiRL, which has conducted paid research in respiratory disease on behalf of the following organizations in the past 5 years: Aerocrine,

AKL Ltd, Almirall, BoehringerIngelheim, Chiesi, GlaxoSmithKline, Meda, Mundipharma, Napp, Novartis, Orion, Takeda, Teva, Zentiva.

JDC reports consultancy or research grants with: Amgen, Astellas, AstraZeneca, Bayer, BoehringerIngelheim, Mallinckrodt, Teva, Research in Real Life Ltd., and Respiratory Effectiveness Group.

ERB: The following relationships with commercial interests and the NIH may be related to this presentation existed during the past year:

- Industry-sponsored grants are all administered through my employer, Wake Forest School of Medicine and include studies with: AstraZeneca-MedImmune, Boehringer-Ingelheim-Pfizer, Teva, Forest, Genentech, GlaxoSmithKline, Novartis.
- I have served as a consultant with AstraZeneca-MedImmune, Boehringer-Ingelheim-Pfizer, GlaxoSmithKline, Forest, Novartis, Regeneron, Sanofi
- My NIH grants include the following: Severe Asthma Research Program (SARP); AsthmaNet; Spiromics; Pharmacogenetics of Asthma Treatment; Genetic Studies in Populations of African Descent, Genomics of Lung Function and Asthma Severity in African Americans.

CJC has received travel/accommodation/meeting expenses from BoehringerIngelheim, Stallergenes, Diagenics and Allergy Therapeutics, has acted as a consultant for Novartis and collaborated with Novartis in clinical studies and has received payment for lectures and symposia from Allergy Therapeutics and AstraZeneca.

Neither MT nor any member of his close family has any shares in pharmaceutical companies. In the last 3 years he has received speaker's honoraria for speaking at sponsored meetings or satellite symposia at conferences from the following companies marketing respiratory and allergy products: Aerocrine, AstraZeneca, BoehringerInglehiem, GSK, Teva. He has received honoraria for attending advisory panels with Aerocrine, Almirall, AstraZeneca, BI, Chiesi, GSK, MSD, Novartis. He has received sponsorship to attend international scientific meetings from: GSK, AstraZeneca, Mundipharma. He has received funding for research projects from: GSK, Almirall. He is chief medical adviser to the charity Asthma UK, a member of the BTS SIGN Asthma guideline group and the NICE Asthma guideline group.

SEW has received consulting fees from GSK and AstraZeneca. She has performed clinical trials for GSK, Sanofi-Regeneron, Genentech and AstraZeneca.

AMW has no competing interests to declare.

MBS and GG were employees of Teva Pharmaceuticals, Frazer, PA, US, at the time of this study.

EVH is a consultant to RiRL and has received fees from Merck for writing and editorial support.

IDP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, BoehringerInglehiem, Aerocrine, Almirall, Novartis, and GSK and a payment for organising an educational event for SPRs from AZ. He has received honoraria for attending advisory panels with

Almirall, Genentech, Regeneron, AstraZeneca, BoehringerIngelheim, GSK, MSD, Schering-Plough, Novartis, Dey, Napp and Respivert. He has received sponsorship to attend international scientific meetings from BoehringerIngelheim, GSK, AstraZeneca and Napp.

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Figure legends

Figure 1:Flow diagram depicting the identification of eligible patients in the two databases. A valid eosinophil count was defined as a numeric value for blood eosinophils recorded at least 1 year before last data extraction as number of cells $x10^9$/L with 1 or 2 decimals.

CPRD=Clinical Practice Research Datalink. OPCRD=Optimum Patient Care Research Database.

Figure 2:Adjusted rate ratios for severe exacerbations and acute respiratory events, and odds ratios for asthma control, for patients with peripheral blood eosinophil count >400/μL (vs. ≤400/μL) during 1 outcome year

*Adjusted for age, sex, body mass index, smoking status, and Charlson comorbidity index score.p<0·0001 for all comparisons.

OR=odds ratio; RR=rate ratio

Figure 3:Adjusted rate ratios for severe exacerbations (A) and acute respiratory events (B), and odds ratios for risk-domain asthma control (C) and overall asthma control (D), for patients assigned tonine ascending eosinophil count categories as compared with a reference category of peripheral blood eosinophil count ≤200/μL (n=68 407) during 1 outcome year (adjusted for age, sex, body mass index, smoking status, and Charlson comorbidity index score).

Figure 4:Comparison between number of severe exacerbations and GINA current clinical control (A) and GINA current clinical control and severe exacerbations (B) among patients with blood eosinophil counts >400/μL (n=2197) and ≤400/μL (n=11 355). (p values based on X^2 test)

Table 1:Baseline demographic and clinical characteristics by blood eosinophil count on the index date

	Total	Blood eosinophil cohort		p value*
	n=130 248	≤400/µl	>400/µl	
		n=109 319 (84%)	n=20 929 (16%)	
Peripheral blood eosinophil count [cells/µL], median (IQR)	200 (120–340)	200 (100–300)	580 (500–700)	n/a
Sex, n (%) male	42 067 (32·3)	33 895 (31·0)	8172 (39·0)	<0·001
Age [years], median (IQR)	49 (36–63)	50 (37–63)	45 (31–61)	<0·001†
BMI [kg/m^2], median (IQR)‡	27 (24–32)	28 (24–32)	27 (23–31)	<0·001†
Smoking Status, n (%)				
Non-smokers	72 552 (55·7)	59 966 (54·9)	12 586 (60·1)	
Current smokers	24 443 (18·8)	20 998 (19·2)	3445 (16·5)	<0·001
Ex-smokers	33 253 (25·5)	28 355 (25·9)	4898 (23·4)	
Percent predicted FEV_1 or PEF, median (IQR)‡	84 (71–96)	84 (71–96)	83 (70–96)	<0·001†
Comorbid rhinitis, n (%)				
None	79 457 (61·0)	68 426 (62·6)	11 031 (52·7)	
Allergic	37 548 (28·8)	30 775 (28·2)	6773 (32·4)	<0·001
Non-allergic	7659 (5·9)	6424 (5·9)	1235 (5·9)	
Nasal polyps	5584 (4·3)	3694 (3·4)	1890 (9·0)	
Comorbid eczema, n (%)	42 065 (32·3)	34 136 (31·2)	7929 (37·9)	<0·001
Comorbid diabetes, n (%)	25 859 (19·9)	21 933 (20·1)	3926 (18·8)	<0·001
Charlsoncomorbidity index, n (%)				
0	95 709 (73·5)	80 541 (73·7)	15 168 (72·5)	
1-4	28 310 (21·7)	23 390 (21·4)	4920 (23·5)	<0·001
≥ 5	6229 (4·8)	5388 (4·9)	841 (4·0)	
Mean baseline blood eosinophil count >400/µL	24 429 (18·8)	7809 (7·1)	16 620 (79·4)	<0·001
BTS therapy steps, n (%)§				
No therapy	13 488 (10·4)	11 714 (10·7)	1774 (8·5)	
1	14 563 (11·2)	12 220 (11·2)	2343 (11·2)	
2	41 978 (32·2)	35 498 (32·5)	6480 (31·0)	<0·001
3	29 868 (22·9)	24 966 (22·8)	4902 (23·4)	
4	29 218 (22·4)	23 980 (21·9)	5238 (25·0)	

5	1133 (0·9)	941 (0·9)	192 (0·9)	
Asthma therapy, n (%)				
None	13 492 (10·4)	11 716 (10·7)	1776 (8·5)	
SABA ± SAMA	14 579 (11·2)	12 230 (11·2)	2349 (11·2)	
LABA ± LAMA	588 (0·5)	509 (0·5)	79 (0·4)	
LTRA ± LABA ± LAMA	360 (0·3)	309 (0·3)	51 (0·2)	
ICS	50 485 (38·8)	42 786 (39·1)	7699 (36·8)	<0·001
ICS + LABA ± LAMA	44 439 (34·1)	36 698 (33·6)	7741 (37.0)	
ICS + LTRA ± LABA ± LAMA	6252 (4·8)	5024 (4·6)	1228 (5·9)	
Other	53 (0·0)	47 (0·0)	6 (0·0)	
Daily dose of ICS [µg/day], median (IQR)	219 (55– 575)	219 (55–548)	241 (55–592)	0.66
Severe exacerbations, n (%)[¶]				
0	105 283 (80·8)	89 114 (81·5)	16 169 (77·3)	
1	15 962 (12·3)	13 108 (12·0)	2854 (13·6)	
2-3	6438 (4·9)	5095 (4·7)	1343 (6·4)	<0·001
≥ 4	2565 (2·0)	2002 (1·8)	563 (2·7)	
Acute respiratory events, n (%)[¶]				
0	93 221 (71·6)	78 886 (72·2)	14 335 (68·5)	
1	23 359 (17·9)	19 408 (17·8)	3951 (18·9)	
2-3	10 354 (7·9)	8432 (7·7)	1922 (9·2)	<0·001
≥ 4	3314 (2·5)	2593 (2·4)	721 (3·4)	
Risk-domain asthma control, n (%) uncontrolled	38,960 (29·9)	32 075 (29·3)	6885 (32·9)	<0·001
Overall asthma control, n (%) uncontrolled	77,255 (59·3)	63 966 (58·5)	13 289 (63·5)	<0·001
Courses of acute OCS, n (%)[**]				
0	105 696 (81·1)	89453 (81·8)	16243 (77·6)	
1	14 191 (10·9)	11589 (10·6)	2602 (12·4)	<0·001
≥2	10 361 (8·0)	8277 (7·6)	2084 (10·0)	
Courses of antibiotics for LRTI, n (%)				
0	109 448 (84·0)	91 955 (84·1)	17 493 (83·6)	
1	15 491 (11·9)	12 918 (11·8)	2573 (12·3)	0·129
≥ 2	5309 (4·1)	4446 (4·1)	863 (4·1)	

BMI=body mass index. BTS=British Thoracic Society. FEV_1=forced expiratory volume in 1 second. ICS=inhaled corticosteroid. LABA=long-acting β2 agonist. LAMA=long-acting muscarinic antagonist. LTRA=leukotriene receptor

antagonist. OCS=oral corticosteroids. Other=Theophylline or OCS. PEF=peak expiratory flow. SABA=short-acting β2 agonist. SAMA=short-acting muscarinic antagonist.

*χ^2 except as noted

†Mann-Whitney U-test

‡Patients with BMI data numbered 123 352 (95%) overall, including 103 986 (95%) for ≤400/μL, and 19 366 (93%) for >400/μL cohorts; and patients with FEV_1 or PEF data (if FEV_1 were missing) numbered 98 248 (75%) overall, and 82 239 (75%) and 16 009 (76%), respectively

§BTS steps were defined as follows (±SABA at steps 2–5): step 1, SABA only; step 2, ICS or LTRA; step 3, ICS+LABA or high-dose ICS (≥ 800 μg/day of beclomethasone-equivalent); step 4, ICS+LABA+[LTRA or theophylline] or high-dose ICS+[LABA or LTRA or theophylline]; step 5, high-dose ICS+OCS+[LABA or LTRA or theophylline]

¶Two events occurring within a 2-week span were considered to be the result of the same severe exacerbation/acute respiratory event and counted only once

**Acute OCS were courses where dosing instructions suggest exacerbation treatment or unlikely to be maintenance therapy with a code for asthma or lower respiratory tract infection.

Table 2:Severe exacerbations, acute respiratory events, and asthma control during the outcome year by blood eosinophil cohort

	Blood eosinophil cohort	
	≤400/µl	>400/µl
Outcome	*N*=109 319 (84%)	*N*=20 929 (16%)
Severe exacerbation, n (%)		
0	90 290 (82·6)	16 338 (78·1)
1	12 437 (11·4)	2762 (13·2)
2–3	4669 (4·3)	1305 (6·2)
≥ 4	1923 (1·8)	524 (2·5)
Acute respiratory event, n (%)		
0	81 114 (74·2)	14 771 (70·6)
1	18 306 (16·7)	3734 (17·8)
2–3	7456 (6·8)	1787 (8·5)
≥ 4	2443 (2·2)	637 (3·0)
Risk-domain asthma control, n (%)	78 976 (72·2)	14 369 (68·7)
Overall asthma control, n (%)	46 953 (43·0)	7 785 (37·2)

Table 3: GINA current clinical control by blood eosinophil cohort

GINA control, n (%)	Total n=13 552	Blood eosinophil cohort		p value*
		≤400/µl n=11 355 (84%)	>400/µl n=2197 (16%)	
Controlled	1481 (10·9)	1282 (11·3)	199 (9·1)	
Partially controlled	8128 (60·0)	6763 (59·6)	1365 (62·1)	0·005
Uncontrolled	3943 (29·1)	3310 (29·2)	633 (28·8)	

GINA=Global Initiative for Asthma.[23]

*χ^2 †GINA control data were available for 10·4% of the overall patient population of 130 248.

Table 4:Multivariable analysis of baseline factors associated with having a blood eosinophil count >400/μL on the index date.

	Reference category	Category	Odds ratio (95% CI)	*P* value	Overall p-value
Age	Per year of age		0·987 (0·986–0·988)	<0·001	
Sex	Female	Male	1·39 (1·35–1·44)	<0·001	
BMI	Per kg/m^2		0·981 (0·979–0·984)	<0·001	
Smoking status	Non-smoker	Current smoker	0·76 (0·73–0·79)	<0·001	<0·001
		Ex-smoker	0·90 (0·86–0·93)	<0·001	
Comorbid rhinitis	None	Allergic	1·19 (1·14–1·23)	<0·001	<0·001
		Non-allergic	1·18 (1·11–1·27)	<0·001	
		Nasal polyps	3·05 (2·87–3·25)	<0·001	
Comorbid eczema	None	Yes	1·23 (1·19–1·27)	<0·001	
BTS therapy steps*	1	No therapy	0·81 (0·76–0·87)	<0·001	<0·001
		2	0·92 (0·87–0·97)	0·003	
		3	1·01 (0·96–1·07)	0·70	
		4	1·13 (1·07–1·20)	0·004	
		5	1·01 (0·85–1·20)	0·91	
Severe exacerbations†	0	1	1·18 (1·12–1·23)	<0·001	<0·001
		2–3	1·41 (1·32–1·51)	<0·001	
		≥ 4	1·54 (1·39–1·70)	<0·001	

A&E=Accident & Emergency. BMI=body mass index. BTS=British Thoracic Society.

*Replaceable with daily short-acting β-agonist dose (see appendix text for results). BTS steps were defined as follows (±SABA at steps 2–5): step 1, SABA only; step 2, ICS or LTRA; step 3, ICS+LABA or high-dose ICS (≥ 800 μg/day of beclomethasone-equivalent); step 4, ICS+LABA+[LTRA or theophylline] or high-dose ICS+[LABA or LTRA or theophylline]; step 5, high-dose ICS+OCS+[LABA or LTRA or theophylline]

†Replaceable with acute oral corticosteroid courses plus asthma-related A&E visits, withacute respiratory events,or with absence of risk-domain asthma control at baseline.

Figure 1

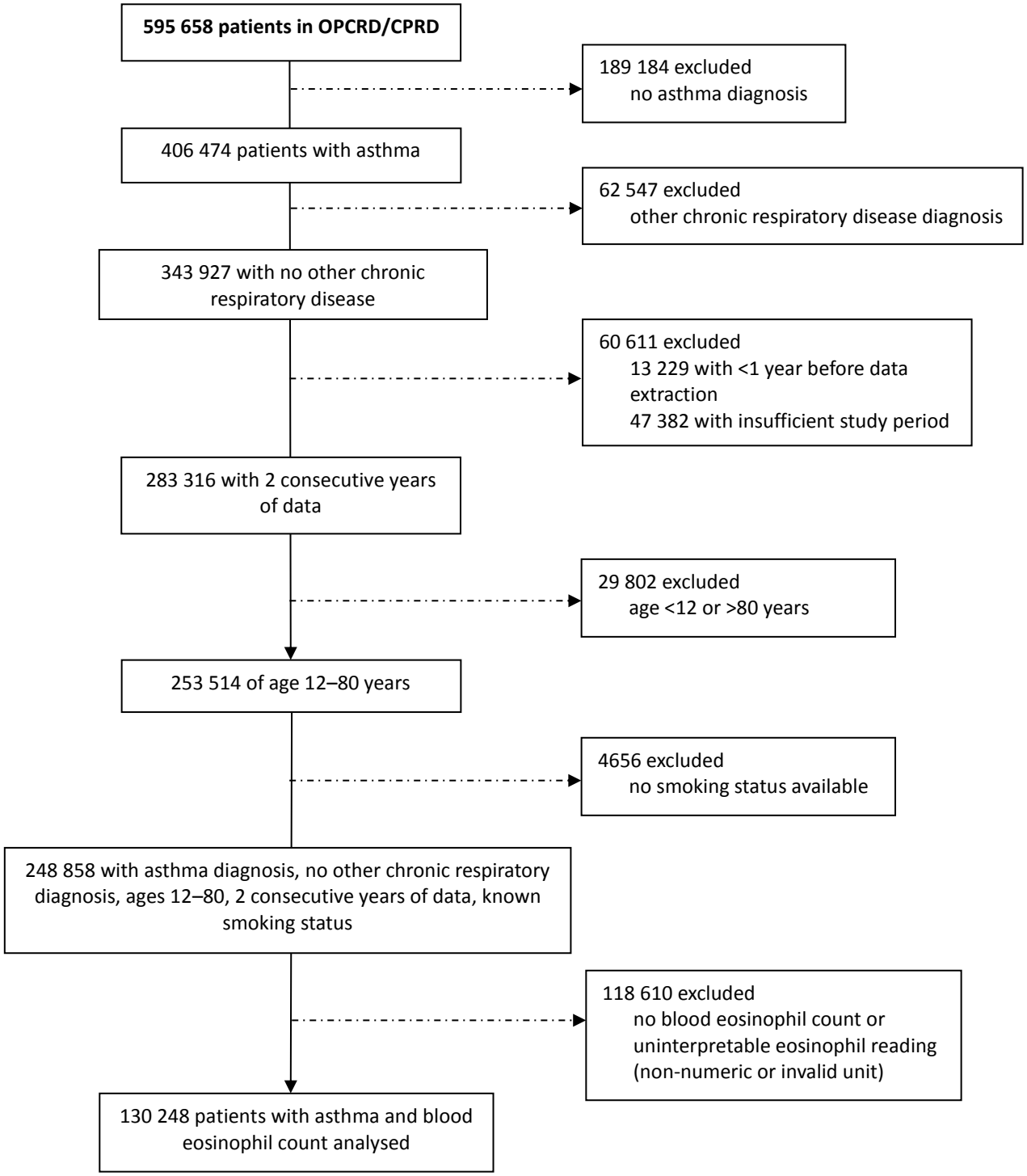

595 658 patients in OPCRD/CPRD
189 184 excluded
no asthma diagnosis
406 474 patients with asthma
62 547 excluded
other chronic respiratory disease diagnosis
343 927 with no other chronic respiratory disease
60 611 excluded
13 229 with <1 year before data extraction
47 382 with insufficient study period
283 316 with 2 consecutive years of data
29 802 excluded
age <12 or >80 years
253 514 of age 12–80 years
4656 excluded
no smoking status available
248 858 with asthma diagnosis, no other chronic respiratory diagnosis, ages 12–80, 2 consecutive years of data, known smoking status
118 610 excluded
no blood eosinophil count or uninterpretable eosinophil reading (non-numeric or invalid unit)
130 248 patients with asthma and blood eosinophil count analysed

Figure 3A

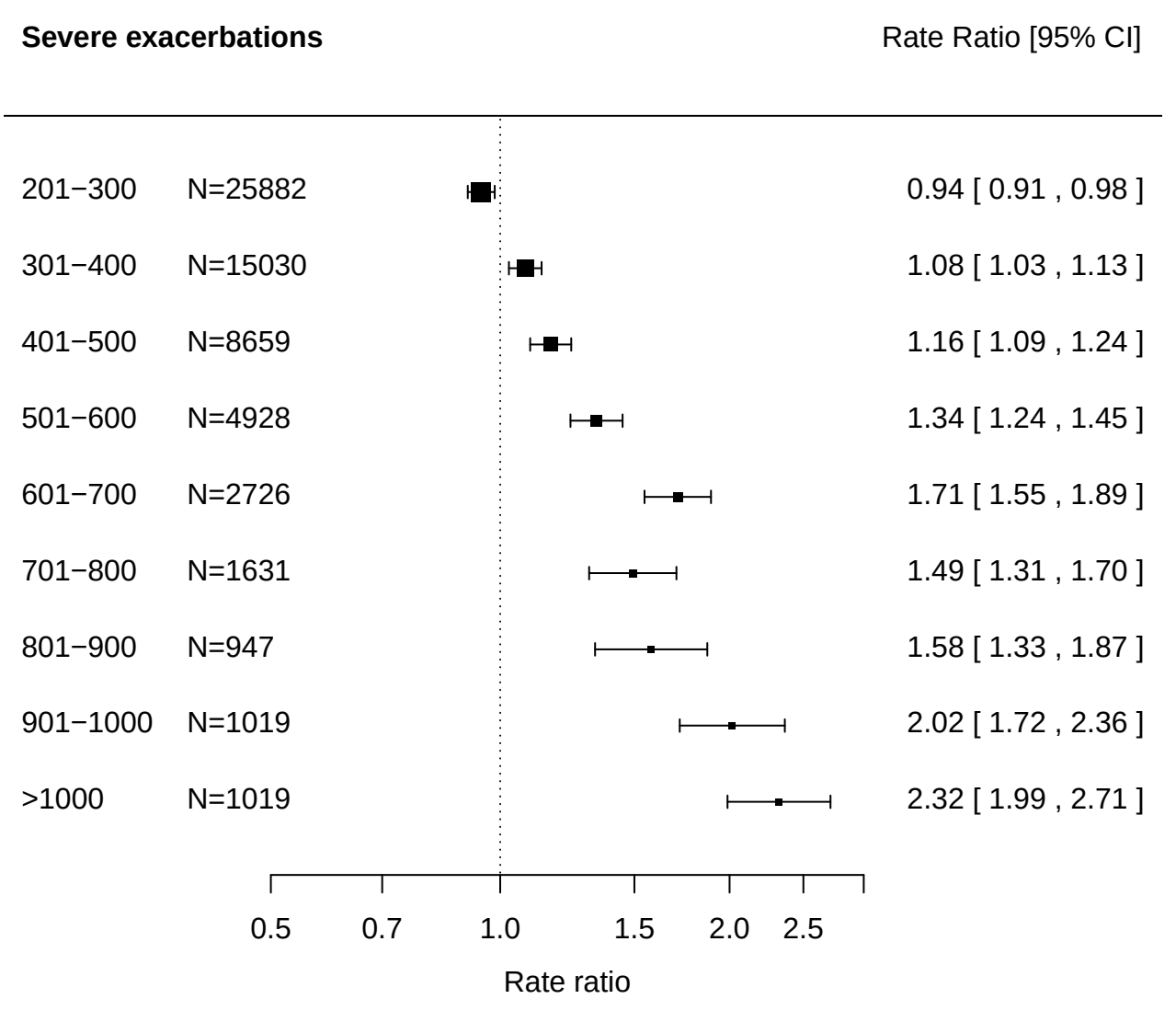

Severe exacerbations
Rate Ratio [95% CI]
201−300 N=25882 0.94 [0.91 , 0.98]
301−400 N=15030 1.08 [1.03 , 1.13]
401−500 N=8659 1.16 [1.09 , 1.24]
501−600 N=4928 1.34 [1.24 , 1.45]
601−700 N=2726 1.71 [1.55 , 1.89]
701−800 N=1631 1.49 [1.31 , 1.70]
801−900 N=947 1.58 [1.33 , 1.87]
901−1000 N=1019 2.02 [1.72 , 2.36]
>1000 N=1019 2.32 [1.99 , 2.71]
0.5
0.7
1.0
1.5
2.0
2.5
Rate ratio

Figure 3B

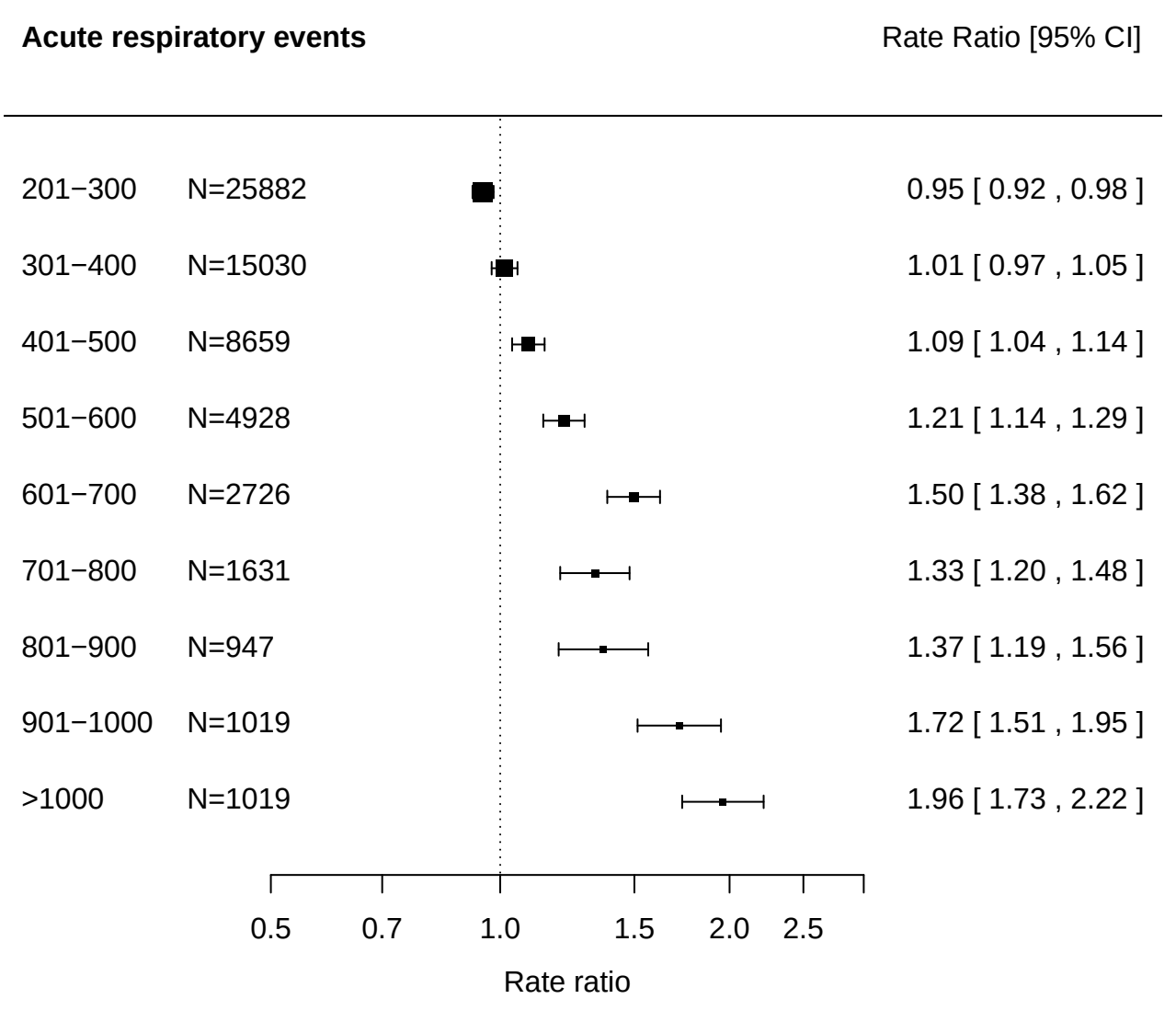

Acute respiratory events
Rate Ratio [95% CI]
201−300 N=25882 0.95 [0.92 , 0.98]
301−400 N=15030 1.01 [0.97 , 1.05]
401−500 N=8659 1.09 [1.04 , 1.14]
501−600 N=4928 1.21 [1.14 , 1.29]
601−700 N=2726 1.50 [1.38 , 1.62]
701−800 N=1631 1.33 [1.20 , 1.48]
801−900 N=947 1.37 [1.19 , 1.56]
901−1000 N=1019 1.72 [1.51 , 1.95]
>1000 N=1019 1.96 [1.73 , 2.22]
0.5
0.7
1.0
1.5
2.0
2.5
Rate ratio

Figure 3C

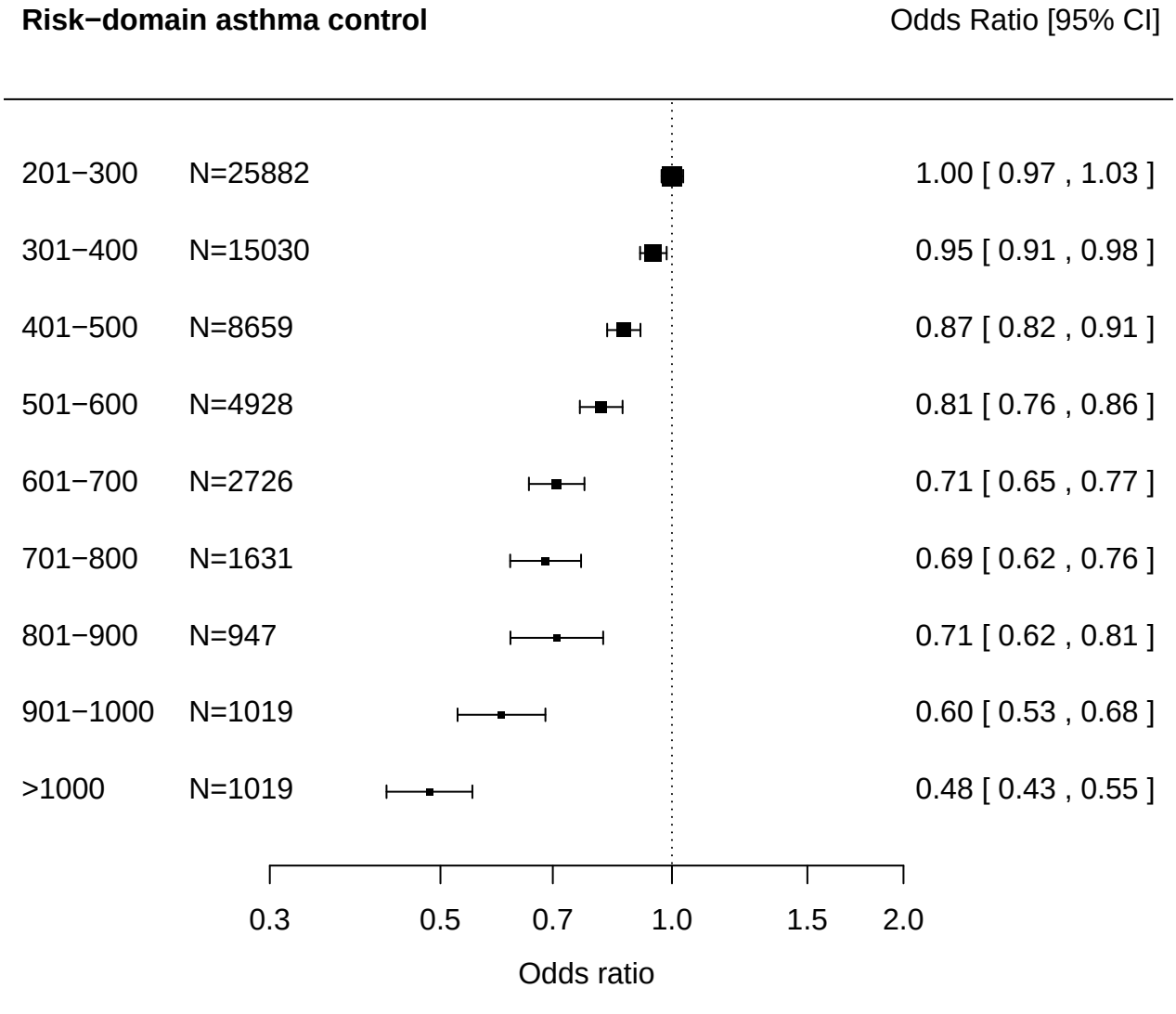
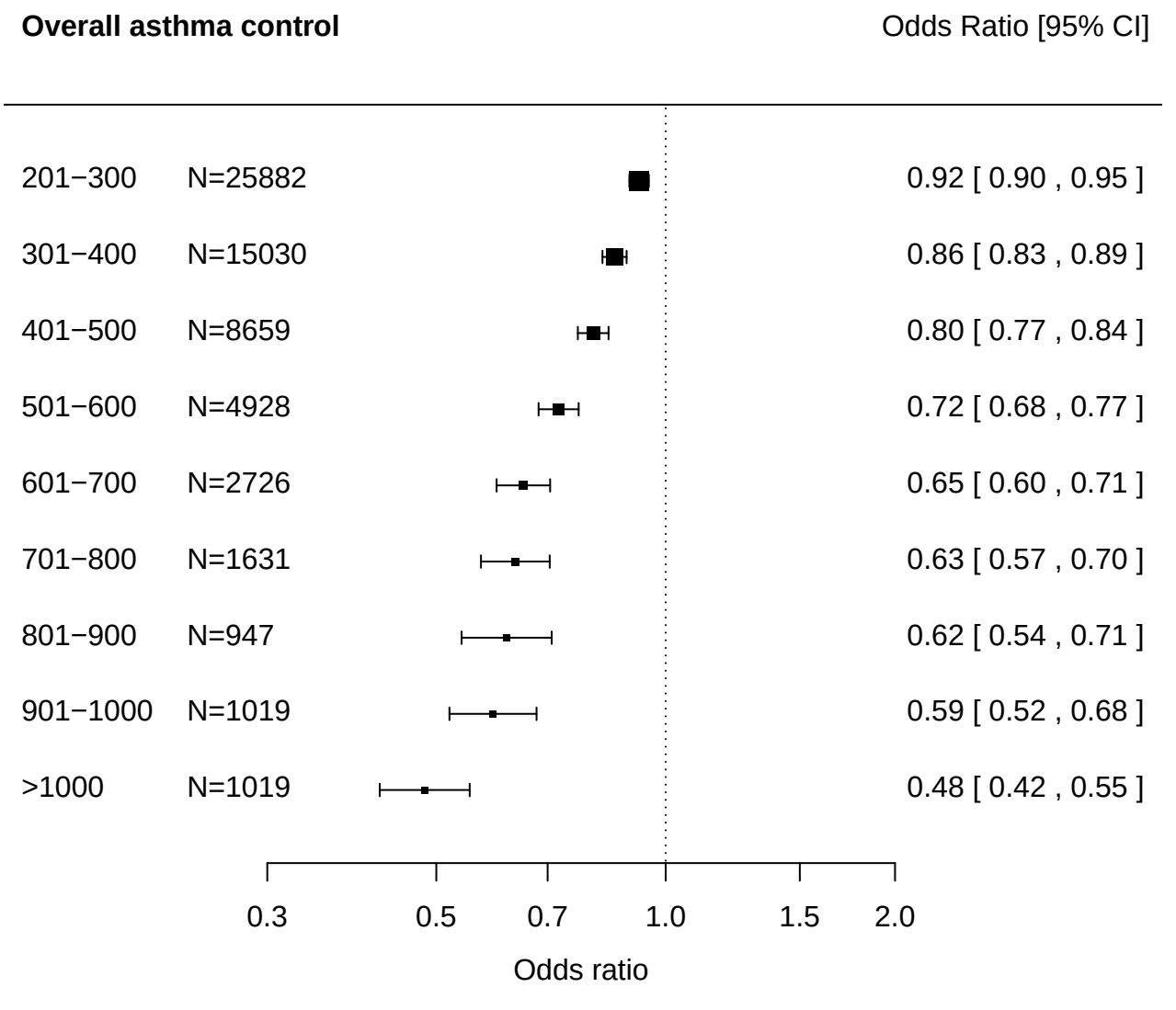
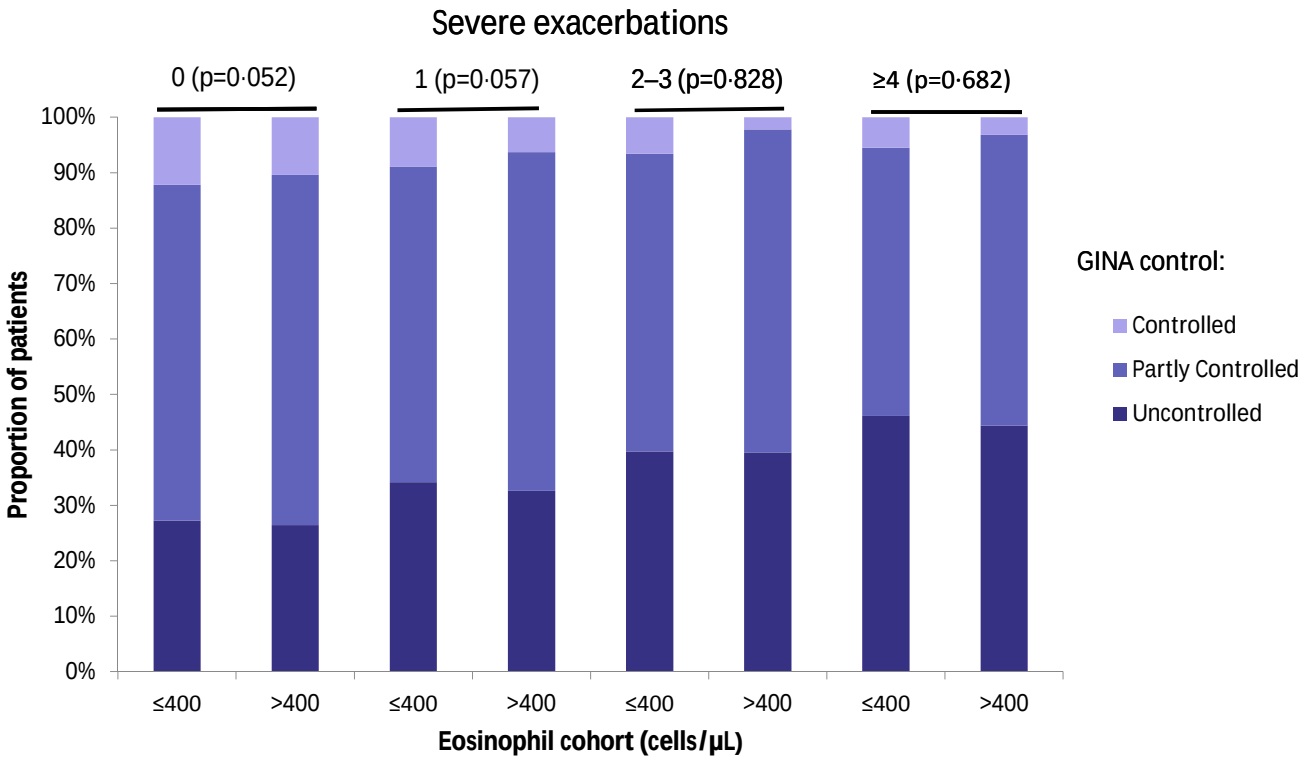
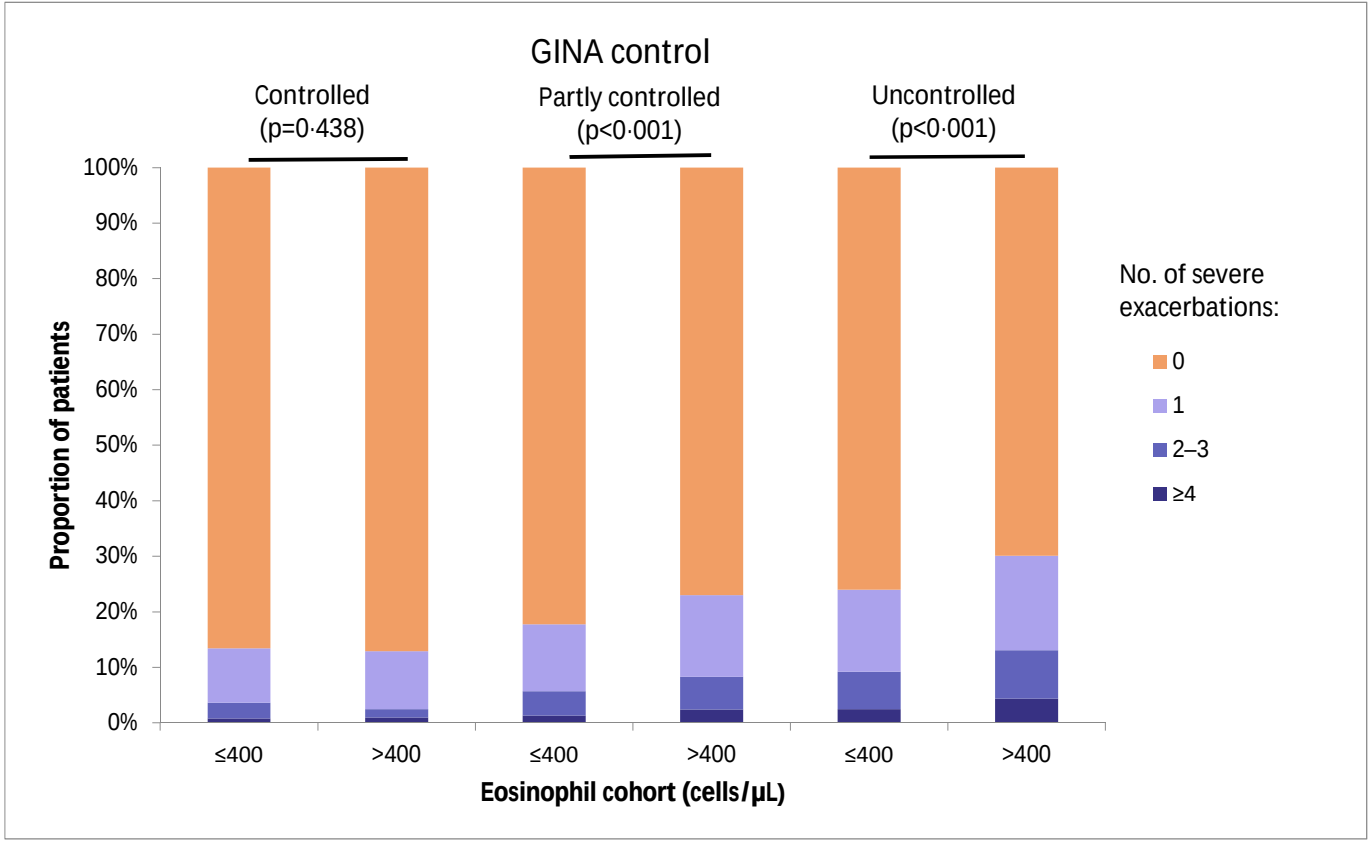

Risk−domain asthma control
Odds Ratio [95% CI]
201−300 N=25882 1.00 [0.97 , 1.03]
301−400 N=15030 0.95 [0.91 , 0.98]
401−500 N=8659 0.87 [0.82 , 0.91]
501−600 N=4928 0.81 [0.76 , 0.86]
601−700 N=2726 0.71 [0.65 , 0.77]
701−800 N=1631 0.69 [0.62 , 0.76]
801−900 N=947 0.71 [0.62 , 0.81]
901−1000 N=1019 0.60 [0.53 , 0.68]
>1000 N=1019 0.48 [0.43 , 0.55]
0.3
0.5
0.7
1.0
1.5
2.0
Odds ratio

Figure 3D


Overall asthma control
Odds Ratio [95% CI]
201−300 N=25882 0.92 [0.90 , 0.95]
301−400 N=15030 0.86 [0.83 , 0.89]
401−500 N=8659 0.80 [0.77 , 0.84]
501−600 N=4928 0.72 [0.68 , 0.77]
601−700 N=2726 0.65 [0.60 , 0.71]
701−800 N=1631 0.63 [0.57 , 0.70]
801−900 N=947 0.62 [0.54 , 0.71]
901−1000 N=1019 0.59 [0.52 , 0.68]
>1000 N=1019 0.48 [0.42 , 0.55]
0.3
0.5
0.7
1.0
1.5
2.0
Odds ratio


Severe exacerbations
0 (p=0·052)
1 (p=0·057)
2–3 (p=0·828)
≥4 (p=0·682)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Proportion of patients
≤400
>400
≤400
>400
≤400
>400
≤400
>400
Eosinophil cohort (cells/µL)
GINA control:
Controlled
Partly Controlled
Uncontrolled


GINA control
Controlled
(p=0·438)
Partly controlled
(p<0·001)
Uncontrolled
(p<0·001)
100%
90%
80%
70%
60%
50%
40%
30%
20%
10%
0%
Proportion of patients
≤400
>400
≤400
>400
≤400
>400
Eosinophil cohort (cells/µL)
No. of severe exacerbations:
0
1
2–3
≥4

Online suppl-revised-clean

Click here to download Necessary Additional Data: THELANCETRM-D-15-00283R1-Supp-revised-clean.pdf

Blood eosinophil count and prospective annual asthma disease burden: UK cohort study

Running head: Eosinophils in asthma and future risk

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Word count: 3376

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Summary

BackgroundElevated sputum eosinophil counts predict asthma exacerbations and responsiveness to inhaled corticosteroids but are impractical to measure in primary care. We investigated the relationship between blood eosinophil count and prospective annual asthma outcomes for a large UK cohort.

MethodsHistorical database analysis utilising anonymised medical record data to identify primary care patients with asthma aged 12–80 years with 2 years of continuous data, including 1 year before (baseline) and 1 year after (outcome) theirmost recent eosinophil count. Negative binomialregression was used to compare outcome exacerbation rates and logistic regression to compare odds of asthma control for patients with blood eosinophils ≤400/μL vs. >400/μL, adjusting for age, sex, body mass index, smoking status, and Charlson comorbidity index.

Findings Overall, 20 929 of 130 248 (16%) of patients had blood eosinophil counts>400/μL. During the outcome year, these patients experienced significantly more severe exacerbations (adjusted rate ratio [RR] 1·42; 95% CI 1·36–1·47) and acute respiratory events (RR 1·28; 1·24–1·33) than those with counts ≤400/μL. They also had significantly lower odds of achieving overall asthma control (OR 0·74; 0·72–0·77), defined as limited reliever use and no asthma-related hospital attendance/admission, acute course of oral corticosteroids, or prescription for antibiotics. Exacerbation rates increasedprogressively with nine ascending categories of blood eosinophil count as compared with a reference category of ≤200/μL.

Interpretation Patients with asthma and blood eosinophil counts >400/μL experience more severe exacerbations and have poorer asthma control. Furthermore, a count-response relationship exists between blood eosinophil counts and asthma-related outcomes. Blood eosinophil counts could add predictive value to GINA control-based risk assessment.

FundingTeva

ClinicalTrials.gov: NCT02140541

Keywords: asthma, control, eosinophils, exacerbations, observational

Introduction

Asthma is a complex and heterogeneous disorder.[1,2] The presence of eosinophils in asthmatic inflammation has been recognised for many years, and eosinophilic asthma is a common phenotype that is usually responsive to corticosteroid therapy.[3] Eosinophilic airway inflammation, as reflected in elevated sputum eosinophils, appears to be closely related to the risk of severe asthma exacerbations and loss of asthma control with inhaled corticosteroid (ICS) withdrawal, although the pathogenetic mechanisms remain undefined.[4–6] The tailoring of asthma therapy based on maintaining sputum eosinophils at ≤2–3% is effective in decreasing asthma exacerbations in patients with severe disease,[4,7] and asthma therapies targeting eosinophils are effective in reducing the incidence of asthma exacerbations and improving markers of asthma control for patients with severe eosinophilic asthma,[8–10] as well as for patients with moderate-to-severe asthma and eosinophilia.[11]

Sputum eosinophil percentages of ≥2% to ≥3% of the total cells, depending on the study,have been used to define eosinophilic asthma.[2,4,11]However, sputum inductionis impractical in non-specialised clinical settings. Instead, peripheral blood eosinophil counts are easily obtained, and their use as a biomarker for increased disease burden or exacerbation risk is a topic of ongoing study. An inverse correlation between blood eosinophil counts and forced expiratory volume (FEV_1) was observed in an earlier small study.[12]In a randomised controlled trial of patients with severe asthma, a progressive increase in risk of exacerbation was found with increasing baseline blood eosinophils,[9] and in another studyof severe asthma blood eosinophil countswere independently associated withboth risk of exacerbation and treatment response to anti-interleukin-5 therapy, whereas sputum eosinophils did not predict response.[13]

Possible associations between blood eosinophil counts and overall disease burden in asthma require further study in the general population of patients with asthma, outside of clinical trials for severe asthma. While a recent validation study reports that blood eosinophils were an accurate biomarker for identifying sputum eosinophilia,[14] other studies report a lack of concordance between presence of sputum eosinophilia and blood eosinophilia.[15,16] Therefore, rather than identifying sputum eosinophils, it is likely more important to determine whether blood eosinophil counts can be used to monitorasthma control/exacerbation risk in clinical practice. In recent observational studies in the US,

elevated blood eosinophil counts have been associated with increased prospective risk of asthma exacerbations and excessive short-acting reliever use[17] as well asincreased historical risk of exacerbations.[18,19]There is a need to replicate these findings in other settings and databases, to study larger numbers of patients, and to examine patient-reported outcomes.

Anonymised data from high-quality electronic primary care records of several million patients are available in the UK, permitting the study of very large, heterogeneous populations of patients with asthma. The primary objective of this historical primary care cohort study was to investigate the relationship between blood eosinophil count and severe asthma exacerbations and asthma control during the subsequent year. A subanalysis was performed to examine the relationship between severe exacerbations and Global Initiative for Asthma (GINA)-defined current clinical control. Secondary objectives were to identify a potential relationship between demographic and clinical characteristics and the prospective risk of elevated eosinophil counts.

Methods

Data sources

These analyses examined data from August 1990 to February 2013 drawn from both the Optimum Patient Care Research Database (OPCRD)[20] and the Clinical Practice Research Datalink (CPRD)[21](see appendix for more detail). Patient data were cross-referenced to avoid duplication of individuals studied.

Patients and study design

Patients aged 12–80 years of age with an asthma diagnostic Read Code, a recorded blood eosinophil count, and 1 year of continuous data before and after their most recent blood eosinophil count (defined as the *index date*) were included in the study (figure 1). A valid eosinophil count was defined as a numeric value in blood eosinophils~~/μL~~, recorded at least 1 year before the final data extraction as number of cells x10^9/L with 1 or 2 decimals. Values were transformed to blood eosinophils/μL.Patients with chronic obstructive pulmonary disease or any chronic respiratory disease

other than asthma were excluded, as were patients withrecorded eosinophil counts >5000/μL(to avoid extreme outliers) and those lacking information on smoking status.

The study period for each patient comprised 2 sequential years: a 1-year baseline period preceding and including the index date (the date of the last eosinophil count) for patient characterisation and a 1-year outcome period after the index date.

Eligible patients were divided into two cohorts according to blood eosinophil count of≤400/μL or >400/μL, a value representingthe upper limit of the published normal blood eosinophil range (0–400/μL) in UK clinical practice.[22]

Outcome measures

A*severe exacerbation* was defined, as previously described (appendix), according to the American Thoracic Society/European Respiratory Society definition[23]as an asthma-related hospitalisation, attendance at an Accident and Emergency (A&E) department, or a prescription for acute oral corticosteroids. An *acute respiratory event* was defined more broadly as an asthma-related hospital attendance/admission or A&E attendance, prescription for acute oral corticosteroids, or prescription for antibiotics in conjunction with an asthma-related primary care consultation.

Asthma control assessment was based on two measures, previously described (appendix).*Risk-domain asthma control* was defined as the absence of any acute respiratory event (as defined above) or asthma-related outpatient department visit. Criteria were the same for the *overall asthma control measure*, with the additional requirement of an average daily dose of ≤200μg salbutamol or ≤500μg terbutaline (defined as the available dose in prescribed canisters divided by 365).

Both primary diagnosis (asthma) and comorbidities were defined as Read codes recorded in the database at any time. For a subgroup of patients (10%), a measure of GINA-defined current clinical control (2010–2012 definition[24]) was available from information collected via OPCRD questionnaires and GP-recorded data(appendixtable S1).

Statistical analyses

Patient demographic characteristics, comorbidities, severe exacerbations, acute respiratory events, and asthma control (risk-domain, overall, and GINA-defined control) were compared between patients with blood eosinophil count ≤400/μLand >400/μL using the X^2 test for categorical variables. Variables measured on the interval or ratio scale were compared using a *t* test or a Mann–Whitney U-test if the distribution were skewed.

A negative binomialregression model was used to compare rates of severe exacerbations and acute respiratory eventsbetween eosinophil cohorts during the outcome year, and a logistic regression model was used to compare the odds of achieving asthma control. The negative binomial and logistic regression analyses were adjusted for the following confounders: age, sex, body mass index (BMI), smoking status, and the Charlson~~Comorbidity~~ comorbidity ~~Index~~index. In addition, we evaluated the potential confounding effect of the following comorbidities recorded ever: non-allergic and allergic rhinitis, eczema, diabetes mellitus, heart failure, ischaemic heart disease, and gastro-oesophageal reflux disease. The "count-response" relationship between the blood eosinophil count and the outcomes was studied by calculating rate ratios and odds ratios for nine ascending categories of blood eosinophil count compared with a reference group of patients with blood eosinophil counts ≤200/μL. In a post hoc analysisof the subpopulation of patients who had blood eosinophil counts recorded as number of cells x10^9/L with 2 decimal place accuracy, we examinedoutcomes for eosinophil counts in smaller incrementsof the lower counts, namely,200, 250, 300, 350, 400, and >500 eosinophils/μL as cut-points and as compared with a reference group of patients with blood eosinophil counts ≤150/μL. In addition, we examined outcomes after applying the ELEN index, an algorithm that uses the eosinophil/lymphocyte ratio (ELR) and the eosinophil/neutrophil ratio (ENR), previously described as a means of stratifying patients to predict those with sputum eosinophils ≥2%.[25,26]

To examine potential predictors of peripheral blood eosinophilia, a univariable logistic regression model was used to identify baseline characteristics associated (p<0·05) with an eosinophil count >400/μL. A multivariable logistic regression model with stepwise reduction was then used to derive the best-fitting model of non-co-linear predictors (p<0·05).

Analyses were conducted using IBM SPSS Statistics version 21 (IBM SPSS Statistics, Feltham, Middlesex, UK). Statistically significant results were defined as p<0·05.

Role of the funding source

Data acquisition and the analyses were funded by Teva Pharmaceuticals. Access to data from the OPCRD was co-funded by Research in Real-Life Ltd (RiRL, Cambridge, UK). Teva played no role in the collection or analysis of the data or the decision to submit the paper for publication.Teva employees had a role in interpretation of the data and review of the manuscript. The research team at RiRL designed the study, conducted the analyses, and coordinated the writing and revision of the paper in collaboration with all authors. The authors received no funds or honoraria from Teva for participation in the study.

Results

Patients

We identified343 927 patients with asthma and no other chronic respiratory disease diagnosis (figure 1). Patients meeting study eligibility criteriatotalled 248 858, of whom 130 248 (52%) patients had a recorded blood eosinophil count. Those with blood eosinophil counts were older and more likely female than those without (median age 49 vs. 34 years and 68% vs. 45% female, respectively; see appendix table S2); and they had greater asthma burden at baseline (19% vs. 3% experienced one or more severe exacerbations).

The majority of patients in the study cohort were female (68%), of median age 49 years (table 1). The median blood eosinophil count was 200/μL (interquartile range [IQR] 120–340). Sixteen percentof patients (n=20 929) had a blood eosinophil count >400/μL, and 84% (n=109 319) hada count ≤400/μL. The highest eosinophil counts (1501–5000/μL) were recorded for 382 (0·3%) patients. Compared with patients who had a blood eosinophil count ≤400/μL, those with blood eosinophil count >400/μL were more likely to be male, to be younger, to have a slightly lower BMI, and to be a non-smoker (all $p<0·001$; table 1). Patients with counts >400/μL had more comorbid rhinitis andeczema, received more asthma therapy, and experienced higher exacerbation rates during the baseline year than those with counts ≤400/μL (table 1).

Outcome year severe exacerbations, acute respiratory events, and asthma control

During the outcome year, patients with blood eosinophil counts >400/µL were more likely to experience severe exacerbations and acute respiratory events, and less likely toexperience asthma control (both risk-domain and overall control), than those with eosinophil counts ≤400/µL (table 2). The unadjusted rate ratios (95% CIs) for severe exacerbations and acute respiratory events were 1·30 (1·25–1·35) and 1·19 (1·15–1·23), respectively, and the unadjusted odds ratios for risk-domain and overall asthma control were 0·84 (0·82–0·87) and 0·79 (0·76–0·81), respectively (p<0·0001 for all). Adjusted rates of severe exacerbations andof acute respiratory events weresignificantly higher for those with counts >400/µL compared with counts ≤400/µL (severe exacerbation RR 1·42 [1·36–1·47]), and the adjusted odds of achieving risk-domain asthma control and overall asthma control were significantly lower (figure 2). An additional adjustment for the baseline number of lower respiratory consultations treated with antibiotics, an indication for blood count measurements, did not influence the results for severe exacerbations (RR 1·39 [1·34–1·45]). Adjustments for comorbidities, including non-allergic rhinitis, allergic rhinitis, and eczema also had little influence on the association of eosinophil count with severe exacerbations (further details are reported in the appendix).

Using a lower cut-off value to define eosinophilia, the associations became weaker, and using a higher cut-off value the association was greater: namely, at cut-off values of >300, >400, and >500, the RRs for severe exacerbations were 1.30, 1.42, and 1.58, respectively(further details in appendix table S3). Figure 3 depicts outcomes for patients categorised by ascending blood eosinophil count as compared with a reference category of ≤200/µL.Severe exacerbation and acute respiratory event rates increased(figures 3A and 3B) and the odds of asthma control decreased (figures 3C and 3D) progressively with ascending categories of blood eosinophil count as compared with the reference value. Patients with counts ≤300/µL did not show an increased risk of exacerbations, and the risk was marginally increased (<10%) for patients with counts of 301–400/µL.

Blood eosinophil counts to 2 decimal place accuracy were available for 54 072 (42%) patients.For this population, patients with an eosinophil count>300/µL (31% of the subpopulation) on average had a 30% increased rate of severe exacerbations compared with patients at lower counts (appendix table

S4). However, most of these patients with countsfrom>300–450/µL (55% of patients with counts >300/µL) did not have a significantly higher risk compared with patients with eosinophil countsof ≤150/µL (appendix figure S1).

Applying the ELEN index to the population (calculable for 129 597/130 248 [99.5%] patients), 42 737 (33.0%) were defined as being ELEN index positive. The severe exacerbation rate ratio for these patients, relative to those who were ELEN index negative, was 1·19 (1·16–1·23), and that for acute respiratory events was 1·11 (1·08–1·14).The odds ratios for risk-domain asthma control and overall asthma control were 0·90 (0·87–0·92) and 0·83 (0·81–0·85), respectively.

Subanalysis: Exacerbations versus GINA current clinical control

GINA current clinical control (symptoms) could be calculated for the 10% of patients in each cohort who had completed an OPCRD asthma questionnaire. Patients with blood eosinophil counts >400/µL vs. ≤400/µL were less likely to achieve GINA complete control (table 3).

Within each severe exacerbation category,there was no significant difference in GINA current clinical control between eosinophil cohorts (≤400/µL vs. >400/µL; figure 4A). However, within the GINA partly controlled and uncontrolled categories, patients with blood eosinophil counts >400/µL experienced significantly more exacerbations than those with eosinophil counts ≤400/µL (figure 4B).

Clinical predictors of elevated blood eosinophil count

Complete univariable results examining potential predictors of index date eosinophil count >400/µL are reported in appendixtable ~~S4~~S5.

In the multivariable analysis, baseline year mean blood eosinophil count >400/µL was the main predictor for future elevated eosinophil counts (OR 43·97; 95% CI 41·94–46·09). Excluding prior eosinophil counts from the multivariable model, the following factors were associated with an index date blood eosinophil count >400/µL: younger age, male sex, lower BMI, being a non-smoker, comorbid eczema or rhinitis (allergic and non-allergic), nasal polyps, and having one or more severe exacerbations in the baseline year (table 4). In addition, as compared with being at British Thoracic Society (BTS) step 1,[~~25~~27]patients at BTS step 4 had significantly higher odds, and those on no therapy

or at BTS step 2 (ICS or leukotriene receptor antagonist as single therapy) had significantly lower odds, of a blood eosinophil count >400/μL (table 4).

Discussion

Of more than 130 000 patients with asthma and no other chronic respiratory disease diagnosis who had a recorded blood eosinophil count in their routine care medical record, we found that 16% had an elevated peripheral blood eosinophil count of >400/μL. The incidence rate of severe asthma exacerbations was 42% higher, and that of acute respiratory events 28% higher, for these patients as compared with the ≤400/μL eosinophil cohort, while the odds of achieving risk-domain asthma control and overall asthma control were, respectively, 22% lower and 26% lower during the subsequent year. In our subanalysis, which included 10% of the study population, eosinophilia was associated with a greaterrate of exacerbations within GINA partly controlled and uncontrolled categories, thus providing independent information on risk and suggesting a mismatch between exacerbations and symptoms as defined by GINA control.These important findings corroborate the findings of prior studies[1,3,9]and suggest that assessment of blood eosinophils could add predictive value to a traditional control-based assessment using the GINA criteria.

A blood eosinophil count measured in the preceding year of >400/μL was a very strong predictor for an elevated blood eosinophil count >400/μL at the index date. Moreover, because the majority of patients with peripheral blood eosinophilia at the index date already had elevated blood eosinophils during the baseline year (see table 1), our data suggest that elevated blood eosinophil countsmay bea stable phenotype, at least over the short term. For this reason we excluded baseline eosinophil count from the multivariable predictive model.

After excluding prior eosinophil count from the model, other predictors identified for a count >400/μL included demographic and clinical characteristics (younger age, male sex, lower BMI, being a non-smoker), comorbidities (allergic and non-allergic rhinitis, nasal polyps, and eczema), and disease activity (higher baseline exacerbation rates). The presence of atopic conditions and nasal polyps as predictors of eosinophilia is an expected finding.[26~~28~~]Similarly, lower BMI and non-smoking

status as predictors are not unexpected, because the obesity-related asthma phenotype is typically noneosinophilic,[1,2] and current and ex-smokers have lower blood eosinophils than never-smokers.[27~~27~~29]

The proportion of patients with peripheral eosinophilia (16%) in the present study was similar to the 18–26% prevalence found among adults with asthma in three recent observational studies using a similar cut-point (<400/μL vs. ≥400/μL).[15,17,19]Zeiger et al[17]in a study of 2392 adults with asthma found that blood eosinophil count ≥400/μL was a risk factor for asthma exacerbation the following year as well as for excessive SABA use (≥4 puffs/day, equivalent to ≥400 μg/day in this study). Wagener et al[14] report different cut-points for cohorts of differing asthma severity and propose that the optimal cut-point may vary by study population. In a recent small study of 164 patients with uncontrolled asthma despite treatment, a cut-point of 260 cells/μL was highly predictive for eosinophilic asthma; a blood eosinophil percentage of 2.7% was the best predictor.[28~~28~~30]In another recent study, blood eosinophil counts were poor predictors of sputum eosinophil percentages[16]; however, the cut-point used (300 cells/μL) was considered possibly too low.[29~~29~~31]

We found a clear and consistent "count-response" relationship between blood eosinophil count and our database-derived measures during the outcome year. Other observational studies have reported similar findings.[18,19]Among subjects in a large NHANES study, the odds of asthma exacerbations the prior year were increased with higher eosinophil counts as compared with <300/μL.[18] Similarly, for 616 patients with severe asthma, a progressive increase in risk of exacerbation with increasing baseline blood eosinophils was reported in the Dose Ranging Efficacy And safety with Mepolizumab in severe asthma (DREAM) trial.[9]The results of oursubanalysis looking at GINA-defined asthma control for 10% of patients indicated that blood eosinophil counts appeared more strongly linked to exacerbation risk than to measures of asthma control and support the view that symptoms and risk/inflammation in asthma are to some extent disassociated.[9]

Strengths of the current analyses include the large study population of patients with physician-diagnosed asthma (n=130 248), much larger than prior observational studies (n<3000 adult patients with asthma).[17–19] Moreover, this study drew on two large, well-maintained databases.[20,21]

Limitations of the current study include the fact that data were not collected prospectively; moreover, the analyses were based on a single blood eosinophil count. The measure of overall asthma control

was designed to account for daily symptom management as reflected in reliever use because we were not able to assess asthma symptoms from database records. We were limited to the available data; for example, pack-years of smoking are not available in the databases. In addition, we did not assess whether patients had received a course of oral corticosteroids prescribed within 2 weeks before the blood eosinophil measurement, which could have substantially reduced the blood eosinophil counts. However, this would tend to bias results to the null, and we estimate the percentage of patients to whom this could refer to be small and thus are confident that exclusion of these patients would not have relevantly changed the results. Moreover, we did not assess treatment adherence. While poor adherence to ICS could potentially cause both eosinophilia and poorly controlled asthma, we believe it unlikely that this is the sole explanation for our findings (in patients with COPD, blood eosinophil counts are not very responsive to ICS ~~treatment~~[30] treatment[32]).We cannot rule out the potential for selection bias:similar to the findings of Zeiger et al,[17]patients who had a recorded blood eosinophil count were more likely to be female, had more comorbidities, and were more likely to have baseline severe exacerbations than those without eosinophil count, possibly limiting the generalisability of our findings. Indeed, we cannot fully exclude that patients with eosinophilia were more likely to be selected, because they have a greater likelihood of having a full blood count measured at an exacerbation, but this would reflect the higher rate of exacerbations associated with blood eosinophilia. Moreover, it is possible that blood eosinophil count rises in the period preceding an exacerbation, inflating the link betweenhigh blood eosinophil count and exacerbations. An additional adjustment for the baseline number of lower respiratory consultations treated with antibiotics, an indication for blood count measurements, produced no relevant change in the prospective association between eosinophilia and severe exacerbations, supporting the concept that ~~selection bias~~confounding was limited.

Further characterisation of patients with eosinophilia and particularly their response to therapy is needed. While older women were more likely to have a blood eosinophil assessment in UK clinical practice, younger age and male sex were predictors of eosinophilia, suggesting a rationale for checking blood eosinophil count in younger male patients with asthma. Moreover, work is needed to characterise the different asthma phenotypes and to identify biomarkers that could be used to

distinguish them.[2] Clinically, the use of blood eosinophilia as a biomarker for future asthma exacerbations or poor control may enable identification of patients with milder disease who could benefit from higher doses of ICSor, alternatively, the tailoring to specific patients of therapies such as mepolizumab, reslizumab, and other agents that inhibit eosinophilic airway inflammation.

In conclusion, this study demonstrates that, in a cohort of more than 130,000 patients with asthma, blood eosinophil counts >400/μL, as compared with ≤400/μL, are associated with a greater rate of asthma exacerbations and lower odds of achieving asthma control over the subsequent year. Moreover, a clear count-response relationship exists between blood eosinophil count and asthma-related outcomes as defined by our database-derived measures. Expert working groups have recommended that peripheral blood eosinophils be measured in clinical studies as one of the biomarkers to characterise study populations.[~~31~~33] Our findings suggest there could be benefit in performing full blood counts with differential as a routine assessment in clinical practice for patients with asthma. Moreover, our data suggest that patients with asthma and high blood eosinophil count are potentially at elevated risk of future exacerbations regardless of current GINA control status and should be counselled and monitored accordingly.

Panel: Research in context

Evidence before this study

We searched PubMed for papers published from 2000-2015 investigating the relationship between blood eosinophil count and asthma outcomes, including asthma control and exacerbations, for adult patients with asthma in the general population. We used various combinations of the following search terms: "asthma", "eosinophils/eosinophilia", "exacerbation rate/risk", "asthma control". We reviewed the PubMed search results and reference lists of relevant papers to identify observational studies not limited to patients with severe or uncontrolled asthma. We identified four observational studies reporting the association between elevated blood eosinophil count and increased historical or prospective risk of asthma exacerbations in general populations of patients with asthma.[15,17–19]

Added value of this study

The results of our study support and extend the findings of three of these prior studies to a ≥40 times larger general population of over 130,000 patients with asthma in the UK. (The study of Schleich and coworkers[15] reported results for four cohorts according to blood [≥400 cells/mm^3] and sputum [≥3%] eosinophils and thus could not be directly compared with our findings.) The large cohort size enabled assessment of the prevalence of raised blood eosinophils among patients with asthma, and availability of questionnaire data for 10% of patients enabled us to look at risk in relation to GINA current clinical control. We found that patients with elevated eosinophil counts experienced more severe exacerbations and had poorer asthma control (more disease burden) over a subsequent year than those with a blood eosinophil count ≤400/μL; moreover, we detected a clear and consistent "count-response" relationship between blood eosinophil count and our database-derived measures during the outcome year. Our subanalysisfinding that eosinophilia was associated with an increased risk of exacerbations within GINA partly controlled and uncontrolled categories provides independent information on risk and is in line with earlier clinical trial findings of a dissociation between symptoms and risk of exacerbations for patients with severe asthma.[9]

Implications of all the available evidence

Our findings, together with those of prior studies, suggest that patients seen in primary care with

asthma and blood eosinophilia are potentially at elevated risk of future exacerbations regardless of current GINA control status and should be counselled and monitored accordingly. The question remains whether the elevated blood eosinophil phenotype is stable, and further research is needed to examine blood eosinophil counts in relation to timing of oral corticosteroid bursts, therapy with oral corticosteroids, and therapy/adherence with inhaled corticosteroids, again in the wider general population of patients with asthma.

Contributors

DBP and AR led the study design process; and all authors contributed to the design review. AR, AB, and MK are responsible for the data acquisition and analyses; EVH developed the first draft of the manuscript. All authors contributed to the interpretation of the data, reviewed and edited drafts of the manuscript, and approved the final draft of the manuscript for submission.

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Declaration of interests

DBP has Board Membership with Aerocrine, Almirall, Amgen, AstraZeneca, BoehringerIngelheim, Chiesi,Meda, Mundipharma,Napp, Novartis, and Teva. Consultancy: Almirall, Amgen, AstraZeneca, BoehringerIngelheim, Chiesi, GlaxoSmithKline, Meda, Mundipharma, Napp, Novartis, Pfizer, and Teva; Grants and unrestricted funding for investigator-initiated studies fromUK National Health Service, British Lung Foundation, Aerocrine,AKL Ltd, Almirall, AstraZeneca, BoehringerIngelheim, Chiesi, Eli Lilly, GlaxoSmithKline, Meda, Merck, Mundipharma,Napp, Novartis, Orion, Pfizer, Respiratory Effectiveness Group, Takeda, Teva, and Zentiva; Payments for lectures/speaking: Almirall, AstraZeneca, BoehringerIngelheim, Chiesi, Cipla, GlaxoSmithKline, Kyorin, Meda, Merck,Mundipharma, Novartis, Pfizer, SkyePharma, Takeda, and Teva; Payment for manuscript preparation: Mundipharma and Teva; Patents (planned, pending or issued): AKL Ltd.; Payment for the development of educational materials: GlaxoSmithKline, Novartis; Stock/Stock options: Shares in AKL Ltd which produces phytopharmaceuticals and owns 80% of Research in Real Life Ltd and its subsidiary social enterprise Optimum Patient Care; received Payment for travel/accommodations/meeting expenses from Aerocrine, BoehringerIngelheim, Mundipharma, Napp, Novartis, and Teva; Funding for patient enrolment or completion of research: Almirral, Chiesi, Teva, andZentiva.

At the time of the study, AR, VLA, AB, and MKwere employees of RiRL, which has conducted paid research in respiratory disease on behalf of the following organizations in the past 5 years: Aerocrine,

AKL Ltd, Almirall, BoehringerIngelheim, Chiesi, GlaxoSmithKline, Meda, Mundipharma, Napp, Novartis, Orion, Takeda, Teva, Zentiva.

JDC reports consultancy or research grants with: Amgen, Astellas, AstraZeneca, Bayer, BoehringerIngelheim, Mallinckrodt, Teva, Research in Real Life Ltd., and Respiratory Effectiveness Group.

ERB: The following relationships with commercial interests and the NIH may be related to this presentation existed during the past year:

- Industry-sponsored grants are all administered through my employer, Wake Forest School of Medicine and include studies with: AstraZeneca-MedImmune, Boehringer-Ingelheim-Pfizer, Teva, Forest, Genentech, GlaxoSmithKline, Novartis.
- I have served as a consultant with AstraZeneca-MedImmune, Boehringer-Ingelheim-Pfizer, GlaxoSmithKline, Forest, Novartis, Regeneron, Sanofi
- My NIH grants include the following: Severe Asthma Research Program (SARP); AsthmaNet; Spiromics; Pharmacogenetics of Asthma Treatment; Genetic Studies in Populations of African Descent, Genomics of Lung Function and Asthma Severity in African Americans.

CJC has received travel/accommodation/meeting expenses from BoehringerIngelheim, Stallergenes, Diagenics and Allergy Therapeutics, has acted as a consultant for Novartis and collaborated with Novartis in clinical studies and has received payment for lectures and symposia from Allergy Therapeutics and AstraZeneca.

Neither MT nor any member of his close family has any shares in pharmaceutical companies. In the last 3 years he has received speaker's honoraria for speaking at sponsored meetings or satellite symposia at conferences from the following companies marketing respiratory and allergy products: Aerocrine, AstraZeneca, BoehringerInglehiem, GSK, Teva. He has received honoraria for attending advisory panels with Aerocrine, Almirall, AstraZeneca, BI, Chiesi, GSK, MSD, Novartis. He has received sponsorship to attend international scientific meetings from: GSK, AstraZeneca, Mundipharma. He has received funding for research projects from: GSK, Almirall. He is chief medical adviser to the charity Asthma UK, a member of the BTS SIGN Asthma guideline group and the NICE Asthma guideline group.

SEW has received consulting fees from GSK and AstraZeneca. She has performed clinical trials for GSK, Sanofi-Regeneron, Genentech and AstraZeneca.

AMW has no competing interests to declare.

MBS and GG were employees of Teva Pharmaceuticals, Frazer, PA, US, at the time of this study.

EVH is a consultant to RiRL and has received fees from Merck for writing and editorial support.

IDP has received speaker's honoraria for speaking at sponsored meetings from AstraZeneca, BoehringerInglehiem, Aerocrine, Almirall, Novartis, and GSK and a payment for organising an educational event for SPRs from AZ. He has received honoraria for attending advisory panels with

Almirall, Genentech, Regeneron, AstraZeneca, BoehringerIngelheim, GSK, MSD, Schering-Plough, Novartis, Dey, Napp and Respivert. He has received sponsorship to attend international scientific meetings from BoehringerIngelheim, GSK, AstraZeneca and Napp.

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Figure legends

Figure 1:Flow diagram depicting the identification of eligible patients in the two databases. A valid eosinophil count was defined as a numeric value for blood eosinophils~~/µL~~ recorded at least 1 year before last data extraction as number of cells x10^9/L with 1 or 2 decimals.

CPRD=Clinical Practice Research Datalink. OPCRD=Optimum Patient Care Research Database.

Figure 2:Adjusted rate ratios for severe exacerbations and acute respiratory events, and odds ratios for asthma control, for patients with peripheral blood eosinophil count >400/µL (vs. ≤400/µL) during 1 outcome year

*Adjusted for age, sex, body mass index, smoking status, and Charlson comorbidity index score.p<0·0001 for all comparisons.

OR=odds ratio; RR=rate ratio

Figure 3:Adjusted rate ratios for severe exacerbations (A) and acute respiratory events (B), and odds ratios for risk-domain asthma control (C) and overall asthma control (D), for patients assigned tonine ascending eosinophil count categories as compared with a reference category of peripheral blood eosinophil count ≤200/µL (n=68 407) during 1 outcome year (adjusted for age, sex, body mass index, smoking status, and Charlson comorbidity index score).

Figure 4:Comparison between number of severe exacerbations and GINA current clinical control (A) and GINA current clinical control and severe exacerbations (B) among patients with blood eosinophil counts >400/µL (n=2197) and ≤400/µL (n=11 355). (p values based on X^2 test)

Table 1:Baseline demographic and clinical characteristics by blood eosinophil count on the index date

	Total	Blood eosinophil cohort		p value*
	n=130 248	≤400/µl	>400/µl	
		n=109 319 (84%)	n=20 929 (16%)	
Peripheral blood eosinophil count [cells/µL], median (IQR)	200 (120–340)	200 (100–300)	580 (500–700)	n/a
Sex, n (%) male	42 067 (32·3)	33 895 (31·0)	8172 (39·0)	<0·001
Age [years], median (IQR)	49 (36–63)	50 (37–63)	45 (31–61)	<0·001†
BMI [kg/m^2], median (IQR)‡	27 (24–32)	28 (24–32)	27 (23–31)	<0·001†
Smoking Status, n (%)				
Non-smokers	72 552 (55·7)	59 966 (54·9)	12 586 (60·1)	
Current smokers	24 443 (18·8)	20 998 (19·2)	3445 (16·5)	<0·001
Ex-smokers	33 253 (25·5)	28 355 (25·9)	4898 (23·4)	
Percent predicted FEV_1 or PEF, median (IQR)‡	84 (71–96)	84 (71–96)	83 (70–96)	<0·001†
Comorbid rhinitis, n (%)				
None	79 457 (61·0)	68 426 (62·6)	11 031 (52·7)	
Allergic	37 548 (28·8)	30 775 (28·2)	6773 (32·4)	<0·001
Non-allergic	7659 (5·9)	6424 (5·9)	1235 (5·9)	
Nasal polyps	5584 (4·3)	3694 (3·4)	1890 (9·0)	
Comorbid eczema, n (%)	42 065 (32·3)	34 136 (31·2)	7929 (37·9)	<0·001
Comorbid diabetes, n (%)	25 859 (19·9)	21 933 (20·1)	3926 (18·8)	<0·001
Charlson~~Comorbidity~~ comorbidity index, n (%)				
0	95 709 (73·5)	80 541 (73·7)	15 168 (72·5)	
1-4	28 310 (21·7)	23 390 (21·4)	4920 (23·5)	<0·001
≥ 5	6229 (4·8)	5388 (4·9)	841 (4·0)	
Mean baseline blood eosinophil count >400/µL	24 429 (18·8)	7809 (7·1)	16 620 (79·4)	<0·001
BTS therapy steps, n (%)§				
No therapy	13 488 (10·4)	11 714 (10·7)	1774 (8·5)	
1	14 563 (11·2)	12 220 (11·2)	2343 (11·2)	
2	41 978 (32·2)	35 498 (32·5)	6480 (31·0)	<0·001
3	29 868 (22·9)	24 966 (22·8)	4902 (23·4)	
4	29 218 (22·4)	23 980 (21·9)	5238 (25·0)	

5	1133 (0·9)	941 (0·9)	192 (0·9)	
Asthma therapy, n (%)				
None	13 492 (10·4)	11 716 (10·7)	1776 (8·5)	
SABA ± SAMA	14 579 (11·2)	12 230 (11·2)	2349 (11·2)	
LABA ± LAMA	588 (0·5)	509 (0·5)	79 (0·4)	
LTRA ± LABA ± LAMA	360 (0·3)	309 (0·3)	51 (0·2)	
ICS	50 485 (38·8)	42 786 (39·1)	7699 (36·8)	<0·001
ICS + LABA ± LAMA	44 439 (34·1)	36 698 (33·6)	7741 (37.0)	
ICS + LTRA ± LABA ± LAMA	6252 (4·8)	5024 (4·6)	1228 (5·9)	
Other	53 (0·0)	47 (0·0)	6 (0·0)	
Daily dose of ICS [µg/day], median (IQR)	219 (55– 575)	219 (55–548)	241 (55–592)	0.66
Severe exacerbations, n (%)[¶]				
0	105 283 (80·8)	89 114 (81·5)	16 169 (77·3)	
1	15 962 (12·3)	13 108 (12·0)	2854 (13·6)	<0·001
2-3	6438 (4·9)	5095 (4·7)	1343 (6·4)	
≥ 4	2565 (2·0)	2002 (1·8)	563 (2·7)	
Acute respiratory events, n (%)[¶]				
0	93 221 (71·6)	78 886 (72·2)	14 335 (68·5)	
1	23 359 (17·9)	19 408 (17·8)	3951 (18·9)	<0·001
2-3	10 354 (7·9)	8432 (7·7)	1922 (9·2)	
≥ 4	3314 (2·5)	2593 (2·4)	721 (3·4)	
Risk-domain asthma control, n (%) uncontrolled	38,960 (29·9)	32 075 (29·3)	6885 (32·9)	<0·001
Overall asthma control, n (%) uncontrolled	77,255 (59·3)	63 966 (58·5)	13 289 (63·5)	<0·001
Courses of acute OCS, n (%)[**]				
0	105 696 (81·1)	89453 (81·8)	16243 (77·6)	
1	14 191 (10·9)	11589 (10·6)	2602 (12·4)	<0·001
≥2	10 361 (8·0)	8277 (7·6)	2084 (10·0)	
Courses of antibiotics for LRTI, n (%)				
0	109 448 (84·0)	91 955 (84·1)	17 493 (83·6)	
1	15 491 (11·9)	12 918 (11·8)	2573 (12·3)	0·129
≥ 2	5309 (4·1)	4446 (4·1)	863 (4·1)	

BMI=body mass index. BTS=British Thoracic Society. FEV_1=forced expiratory volume in 1 second. ICS=inhaled corticosteroid. LABA=long-acting β2 agonist. LAMA=long-acting muscarinic antagonist. LTRA=leukotriene receptor

antagonist. OCS=oral corticosteroids. Other=Theophylline or OCS. PEF=peak expiratory flow. SABA=short-acting β2 agonist. SAMA=short-acting muscarinic antagonist.

*χ^2 except as noted

†Mann-Whitney U-test

‡Patients with BMI data numbered 123 352 (95%) overall, including 103 986 (95%) for ≤400/μL, and 19 366 (93%) for >400/μL cohorts; and patients with FEV_1 or PEF data (if FEV_1 were missing) numbered 98 248 (75%) overall, and 82 239 (75%) and 16 009 (76%), respectively

§BTS steps were defined as follows (±SABA at steps 2–5): step 1, SABA only; step 2, ICS or LTRA; step 3, ICS+LABA or high-dose ICS (≥ 800 μg/day of beclomethasone-equivalent); step 4, ICS+LABA+[LTRA or theophylline] or high-dose ICS+[LABA or LTRA or theophylline]; step 5, high-dose ICS+OCS+[LABA or LTRA or theophylline]

¶Two events occurring within a 2-week span were considered to be the result of the same severe exacerbation/acute respiratory event and counted only once

**Acute OCS were courses where dosing instructions suggest exacerbation treatment or unlikely to be maintenance therapy with a code for asthma or lower respiratory tract infection.

Table 2:Severe exacerbations, acute respiratory events, and asthma control during the outcome year by blood eosinophil cohort

	Blood eosinophil cohort	
	≤400/µl	>400/µl
Outcome	*N*=109 319 (84%)	*N*=20 929 (16%)
Severe exacerbation, n (%)		
0	90 290 (82·6)	16 338 (78·1)
1	12 437 (11·4)	2762 (13·2)
2–3	4669 (4·3)	1305 (6·2)
≥ 4	1923 (1·8)	524 (2·5)
Acute respiratory event, n (%)		
0	81 114 (74·2)	14 771 (70·6)
1	18 306 (16·7)	3734 (17·8)
2–3	7456 (6·8)	1787 (8·5)
≥ 4	2443 (2·2)	637 (3·0)
Risk-domain asthma control, n (%)	78 976 (72·2)	14 369 (68·7)
Overall asthma control, n (%)	46 953 (43·0)	7 785 (37·2)

Table 3: GINA current clinical control by blood eosinophil cohort

GINA control, n (%)	Total n=13 552	Blood eosinophil cohort		p value*
		≤400/µl n=11 355 (84%)	>400/µl n=2197 (16%)	
Controlled	1481 (10·9)	1282 (11·3)	199 (9·1)	
Partially controlled	8128 (60·0)	6763 (59·6)	1365 (62·1)	0·005
Uncontrolled	3943 (29·1)	3310 (29·2)	633 (28·8)	

GINA=Global Initiative for Asthma.[23]

*χ^2 †GINA control data were available for 10·4% of the overall patient population of 130 248.

Table 4:Multivariable analysis of baseline factors associated with having a blood eosinophil count >400/μL on the index date.

	Reference category	Category	Odds ratio (95% CI)	*P* value	Overall p-value
Age	Per year of age		0·987 (0·986–0·988)	<0·001	
Sex	Female	Male	1·39 (1·35–1·44)	<0·001	
BMI	Per kg/m^2		0·981 (0·979–0·984)	<0·001	
Smoking status	Non-smoker	Current smoker	0·76 (0·73–0·79)	<0·001	<0·001
		Ex-smoker	0·90 (0·86–0·93)	<0·001	
Comorbid rhinitis	None	Allergic	1·19 (1·14–1·23)	<0·001	<0·001
		Non-allergic	1·18 (1·11–1·27)	<0·001	
		Nasal polyps	3·05 (2·87–3·25)	<0·001	
Comorbid eczema	None	Yes	1·23 (1·19–1·27)	<0·001	
BTS therapy steps*	1	No therapy	0·81 (0·76–0·87)	<0·001	<0·001
		2	0·92 (0·87–0·97)	0·003	
		3	1·01 (0·96–1·07)	0·70	
		4	1·13 (1·07–1·20)	0·004	
		5	1·01 (0·85–1·20)	0·91	
Severe exacerbations†	0	1	1·18 (1·12–1·23)	<0·001	<0·001
		2–3	1·41 (1·32–1·51)	<0·001	
		≥ 4	1·54 (1·39–1·70)	<0·001	

A&E=Accident & Emergency. BMI=body mass index. BTS=British Thoracic Society.

*Replaceable with daily short-acting β-agonist dose (see appendix text for results). BTS steps were defined as follows (±SABA at steps 2–5): step 1, SABA only; step 2, ICS or LTRA; step 3, ICS+LABA or high-dose ICS (≥ 800 μg/day of beclomethasone-equivalent); step 4, ICS+LABA+[LTRA or theophylline] or high-dose ICS+[LABA or LTRA or theophylline]; step 5, high-dose ICS+OCS+[LABA or LTRA or theophylline]

†Replaceable with acute oral corticosteroid courses plus asthma-related A&E visits, withacute respiratory events,or with absence of risk-domain asthma control at baseline.

Online suppl-revisions

Click here to download Necessary Additional Data: THELANCETRM-D-15-00283R1-Supp-revisions.pdf

Research in Real Life
Clinical profile of patients with raised eosinophilic asthma.
Version Date: 16th April 2013

Draft protocol

Clinical profile of patients with raised blood eosinophils.

The clinical and economic burden of raised eosinophilic asthma

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Contents

OBJECTIVES

This study aims to answer three main research questions:

1. What is the relationship between elevated blood-eosinophil counts, number of exacerbations and asthma control?
2. What are the clinical predictors of an elevated blood eosinophil count? How do different patterns of rhinitis, the presence of co-morbid eczema and asthma therapy step affect eosinophil counts?
3. What is the cost and burden of uncontrolled high risk asthma patients in association with a high eosinophil count and eosinophil predictors?

BACKGROUND

Worldwide studies of asthma control report that many asthma patients achieve only suboptimal asthma control as defined by the Global Initiative for Asthma (GINA).[i,ii,iii] One of the hallmarks of allergic disease is the increased presence of eosinophils in tissues due to the inflammatory response. Asthma results from an improper Th2 immune response and eosinophilic inflammation is often identified in asthmatic lungs. Suppression of eosinophillic infiltration into the tissue by glucocorticoids has been shown to lead to improved asthma symptoms for the majority of asthma patients[iv].

Allergic rhinitis is strongly linked to asthma, showing similar epidemiology and a high incidence of co-morbidity. In a review of 830 patients receiving inhaler steroids for asthma, 83% were shown to have co-morbid rhinitis[v]. Rhinitis also shows similar immunology, involving Th2 helper cells and eosinophilic inflammation. Inflammatory changes in the nasal mucosa have been shown to be correlated with similar changes in the bronchial mucosa in asthma patients, showing the relatedness of the two diseases.[vi]

Current guidelines for asthma treatment focus on the use of inhaled corticosteroids (ICS) and long-acting β-agonists to reduce inflammation[vii]. This therapy provides treatment for the lower airways, but not for any upper airway inflammation. It is also not useful for patients who have become resistant to ICS, or who are non-responders to the treatment. Current levels of suboptimal asthma control indicate that there may be a need for further therapies.

Alternative therapies for asthma treatment target the resulting eosinophilia, and include interleukin suppressors such as anti-IL5. IL5 is produced by Th2 helper cells and eosinophils in order to mediate eosinophil activation. A randomized, double-blind placebo-controlled parallel-group study of the anti-IL5 therapy Mepolizumab in eosinophilic asthma patients showed Mepolizumab was associated with significantly fewer exacerbations than placebo over 50 weeks, with a relative risk of 0.57 and 95% confidence intervals 0.32 to 0.92 (p=0.02). Mepolizumab also showed significant improvements in quality of life scores.[viii]

The primary aim of the current study is to estimate the relationship between high blood-eosinophil counts and future number of exacerbations and asthma control. The planned analysis will also investigate the relationship between elevated eosinophil counts and asthma therapy stage as well as co-morbidities including allergic and non-allergic rhinitis and eczema. The study design will facilitate an exploration of the potential medical benefits of an asthma therapy targeting elevated eosinophil counts.

Previous research from Research in Real Life showed that the costs of asthma are disproportionally incurred by uncontrolled patients with ≥2 exacerbations per year[ix]. A

secondary aim of this study is therefore to examine the burden of illness and annual average costs per patient associated with uncontrolled/severe asthmatic patients with high eosinophil counts. This will help to identify potential future sources of savings in reducing the exacerbation levels.

DATASOURCE

This study will use the Optimum Patient Care Research Database (OPCRD) which comprises anonymous data extracted from practices in order to perform reviews of their chronic respiratory services. Two types of anonymised patient data are typically collected:

(1) Routine clinical data

• OPC software interfaces with primary care practice management systems and extracts disease coding and prescribing information.

(2) Questionnaires

• Patients identified as recipients of the respiratory service under review are invited to complete validated disease assessment questionnaires to better understand their current health status (and/or possible reasons for sub-optimal status).

• Anonymised questionnaires are assigned a unique code to aid matching routine data to questionnaire results.

The OPCRD has been approved by Trent Multi Centre Research Ethics Committee for clinical research use. The anonymous, longitudinal patient data offers a high-quality data source for use in clinical, epidemiological and pharmaceutical research. It enables research to be carried out across a broad-range of respiratory areas.

Where necessary, data will be supplemented with information from the The Clinical Practice Research Database (CPRD) including:

- Large computerised primary care database.
- De-identified, longitudinal data from 5 million active medical records from more than 600 subscribing practices throughout the UK.
- Practice-based quality marker providing “up-to-standard date”, is generated by the CPRD for each subscribing practice and data subsequent to the practice up-to-standard date.
- The CPRD is well-validated and used frequently for medical and health research.

STUDY DESIGN

The study will be a retrospective database analysis of asthma patients, consisting of a (one year) baseline period, a (one year) outcome period and an index date, defined as the date of the last eosinophil count at which asthma patients will be split into the following groups:

- Patients with blood-eosinophil count ≤ 400 $x10^6$/L
- Patients with blood-eosinophil count > 400 $x10^6$/L

400 $x10^6$/L represents the upper limit of what is generally regarded as the normal blood eosinophil range (0-400 $x10^6$/L) in UK clinical practice[x].
The baseline period will be one year prior to and including the index date and it will be used for confounder definition, such as demographics, predictors of an elevated eosinophil count,

baseline asthma therapy and asthma control status. The outcome period will be one year following the index date.

There are three main aims to the study:

1) Determining the relationship between eosinophil counts (≤ 400 x10^6/L vs. >400 x10^6/L), future number of exacerbations (0, 1, 2-3, 4+) and asthma control status (Risk Domain Asthma Control/ Overall Asthma Control - Risk & Impairment as well as GINA control for a sub-group of patients with readable GINA control data from the OPCRD).
2) Determining whether asthma co-morbidities (such as eczema and different patterns of rhinitis) or asthma therapy step are clinical predictors of high eosinophil levels and exploring their relationship with the outcomes listed in aim 1.
3) Determining the direct annual mean costs per patient by number of exacerbations (0, 1, 2-3, 4+) and asthma control status for patients with eosinophil count either ≤ 400 x10^6/L or >400 x10^6/L . Asthma control will be defined as Risk Domain Asthma Control / Overall Asthma Control - Risk & Impairment and GINA control will be calculated for a sub-group of patients with readable GINA control data from the OPCRD.

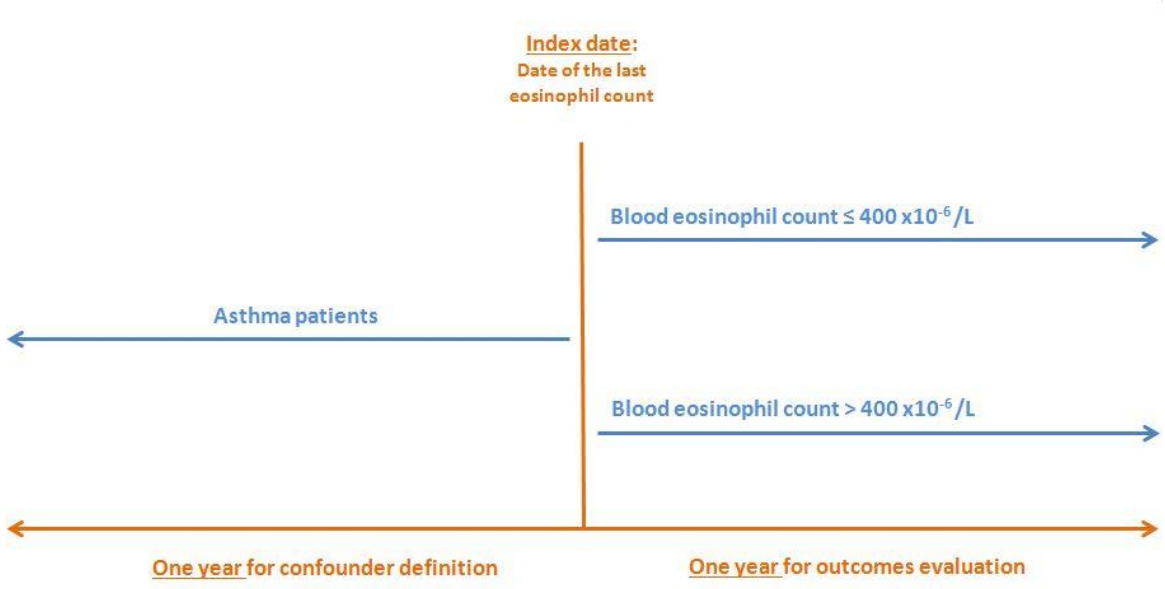


Figure 1: Study design

Demographics (gender, age, weight, height, BMI, smoking status) will be calculated at (or closest to) the date of last eosinophil count.

For the year prior to and for the year after last eosinophil count, the following will be calculated:

- Total number of exacerbations (ATS/ clinical definition)
- Asthma control status (Risk Domain Asthma Control and Overall Asthma Control - Risk & Impairment). GINA control will be calculated for the year after the last eosinophil count for the sub-group of patients with the available data.
- BTS asthma management step
- Asthma related costs including Drug Costs, Primary care costs (GP consultation costs), Secondary Care Costs (respiratory-related inpatient admissions, A&E attendances and OPD visits).

Co-morbidities rhinitis (allergic and non-allergic) and eczema will be examined regardless of when diagnosis relative to the index date was made.

Inclusion Criteria

In order to be included in the study, patients must:

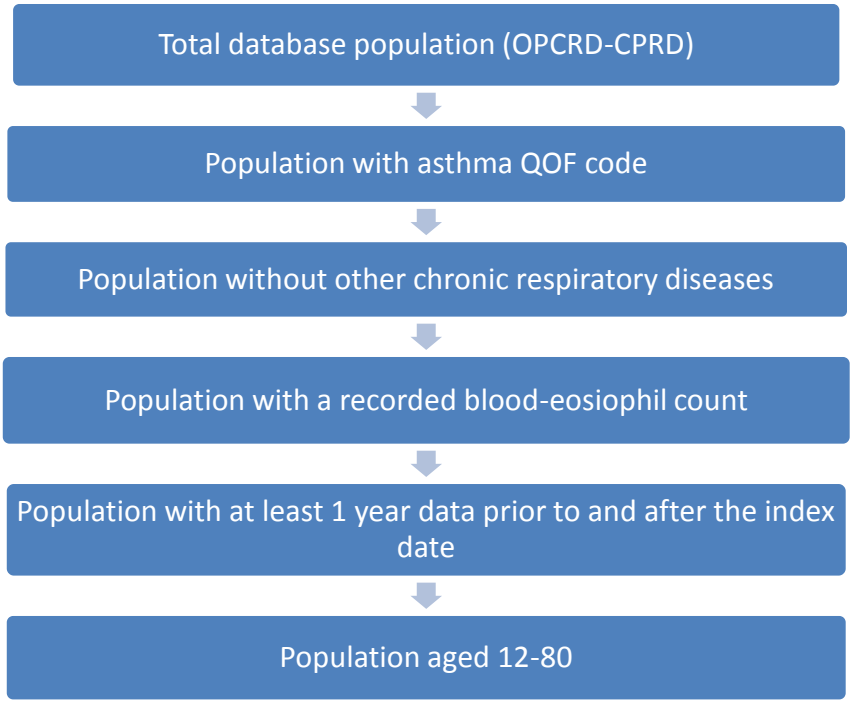
1. Be aged 12-80 years old;
2. Have an asthma diagnostic code;
3. Have one year of continuous data prior to and after the last eosinophil count date; and
4. Have a recorded blood-eosinophil count.

Exclusion Criteria

The following patients will be excluded from the study

1. Any patients with any other chronic respiratory disease (including COPD) other than asthma.

Consort Diagram



Objective 1: Relationship between elevated eosinophil counts, future exacerbations (ATS definition or clinical definition) and asthma control status (Risk Domain Asthma Control/ Overall Asthma Control - Risk & Impairment)

Two definitions of asthma exacerbations will be used:

ATS Exacerbation defined as the occurrence of:

- Asthma-related[1]
  - Hospital admissions OR
  - A&E attendance OR
- Use of acute oral steroids[2]

Clinical exacerbations defined as the occurrence of:

- Asthma-related[1]
  - Hospital attendance / admissions OR
  - A&E attendance OR
  - Out of hours attendance
- GP consultations for lower respiratory tract infection OR
- Use of acute oral steroids[2]

The number of exacerbations will be categorised as: 0, 1, 2-3, 4+ to cover upcoming changes to the NICE guidelines.

Two definitions of asthma control (yes/no) will be used:

Risk Domain Asthma Control. Control defined as absence of:

- Asthma-related1:
  - Hospital attendance / admissions OR
  - A&E attendance OR
  - Out of hours attendance OR
  - Out-patient Department attendance
- GP consultations for lower respiratory tract infection OR
- Use of acute oral steroids2

Overall Asthma Control (Risk & Impairment) control defined as the absence of:

- Asthma-related1:
  - Hospital attendance / admissions OR
  - A&E attendance OR
  - Out of hours attendance OR
  - Out-patient Department attendance
- GP consultations for lower respiratory tract infection OR
- Use of acute oral steroids2
- Average prescribed daily dose of albuterol of ≤200mg.

[1] Asthma-related includes all events with a **lower respiratory code**, i.e. including all asthma codes, and lower respiratory tract infection codes

[2] Where:

- ≥1 oral steroid prescription occurs within 2 weeks of another, or
- ≥1 hospitalisation occurs within 2 weeks of another, or
- ≥1 hospitalisation occurs within 2 weeks of an oral steroid prescription

These events will be considered to be the result of the same exacerbation (and will only be counted once).

The additional measure of Overall Asthma Control (Risk & Impairment) provides a more sensitive measure of asthma control, taking into account daily symptom management as well.

Sub-analysis 1: Exacerbations vs. GINA control

For a sub-group of patients, a measure of GINA control will be available from information collected via OPCRD questionnaires and GP-recorded data. Lung function data is also included in GP medical records – about 80-90% of patients will have a peak-flow-based measure of lung function which will be incorporated into the GINA status definition.

GINA control is defined as per the table below:

Characteristic	Controlled	Partly controlled	Uncontrolled
Daytime symptoms	None (twice or less/week)	More than twice per week	Three or more features of "partly controlled" asthma present in any week
Limitations of activities	None	Any	
Nocturnal symptoms/awakening	None	Any	
Need for reliever/rescue treatment	None (twice or less per week)	More than twice per week	
Lung function PEF or FEV_1	Normal	<80% predicted or personal best (if known)	

Table 2: Definition of GINA control

A table of exacerbations vs. GINA control will be produced for patients with either blood-eosinophil count ≤ 400 $x10^6$/L or blood-eosinophil count >400 $x10^6$/L

	Number of Exacerbations			
GINA Control n(%)	0	1	2-3	4+
Controlled				
Partly Controlled				
Uncontrolled				

Table 1: GINA control vs. Exacerbations

The table will be replicated both for the ATS and the clinical definition of exacerbations.

Sub-analysis 2: Control by blood-eosinophil counts

If enough data for continuous numerical blood-eosinophil data is present, this may be plotted against asthma control to produce a curve:

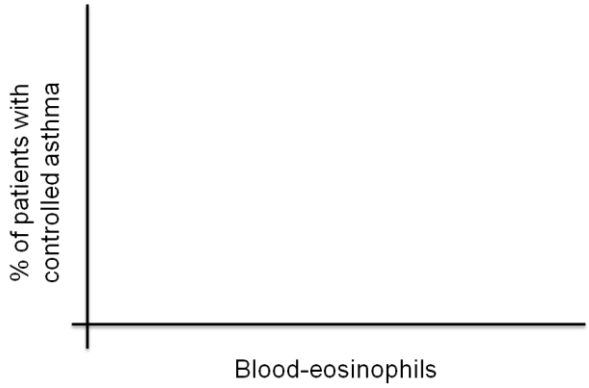


Figure 2: Plot for blood-eosinophil counts vs. asthma control

Objective 2:
Potential clinical predictors of eosinophil count.

Blood-eosinophil count

The following diagnostic Read codes will be used to identify patients with normal or raised eosinophil readings:

Read Code	Diagnosis
424..00	Full blood count – FBC
4241	Full blood count normal
4242	Full blood count borderline
4243	Full blood count abnormal
424Z.00	Full blood count NOS
42K.00	Eosinophil count
42K1.00	Eosinophil count normal
42K2.00	Eosinopenia
42K3.00	Eosinophil count raised
42KZ.00	Eosinophil count NOS

Eosinophil levels may be captured in two ways:

- the actual numerical blood-eosinophil count split as ≤ 400 x10^6/L and > 400 x10^6/L; and/or
- the physician coded record of “raised” or “normal”.

Objective 3:
Burden of Illness and costs impact

For patients meeting the inclusion criteria of our study, the average annual asthma-related costs will be calculated after the date of last eosinophil count. Total asthma-related costs will include:

1. Asthma-related Drug Costs[3],
2. Primary care costs (GP consultation costs)[4]
3. Secondary Care Costs[5] (respiratory-related inpatient admissions, A&E attendances and OPD visits).

The mean annual direct asthma-related cost per patient will be provided for patients with both blood-eosinophil count ≤ 400 $x10^6$/L and blood-eosinophil count > 400 $x10^6$/L, split by number of exacerbations and asthma control status.
We will also provide the percentage of total direct annual asthma-related costs attributable to high risk/uncontrolled patients obtained from OPCRD and CPRD data. Costs will be scaled up to give estimates of total annual direct asthma-related costs to the NHS across the UK for high risk/uncontrolled patients. We are not expecting disproportional costs for high risk/uncontrolled patients since in the UK, patients are less likely to be hospitalized for an exacerbation compared with the US, considerably decreasing the costs associated with these patients.

Sub-analysis 3: Mean annual direct asthma-related costs by GINA control

For the subgroup of patients with GINA control data, costs will also be provided for patients with both blood-eosinophil count ≤ 400 $x10^6$/L and blood-eosinophil count > 400 $x10^6$/L using the table below:

	Number of Exacerbations			
GINA Control n(%)	0	1	2-3	4+
Controlled				
Partly Controlled				
Uncontrolled				

Table 2: Mean annual direct asthma-related costs to be provided split by GINA control and exacerbations

The table will be replicated for the ATS and clinical definition of exacerbations.

DEFINING RHINITIS PATTERNS

The study will distinguish between two types of rhinitis:

Allergic rhinitis: Patients with allergic rhinitis/ hay fever/ seasonal rhinitis / animal allergies as defined as follows.

Non-allergic rhinitis: Patients with perennial rhinitis / chronic rhinosinusitis / nasal polyps or with other rhinitis read codes as defined as follows.

[3] **Drug unit costs** will be based on BNF costs.
[4] ***Unit costs for GP consultations*** have been accessed from the Personal Social Services Research Unit (PSSRU) report: Unit costs of Health and Social Care.
[5] ***Hospital usage costs*** will be accessed from the National Health Service's Reference Costs

1. **Allergic rhinitis**: Patients who have one of the following diagnostic Read codes ever:

ALLERGIC RHINITIS CODES	
READ CODE	**READ TERM**
Hyu2100	[X]Other allergic rhinitis
H17z.00	Allergic rhinitis NOS
H171.00	Allerg.rhinit.-other allergens
H17..00	Allergic rhinitis
H172.00	Allergic rhinitis-unsp allerg

2. **Animal allergies:** Patients who have one of the following diagnostic Read codes ever:

ANIMAL ALLERGIES READ CODES	
READ CODE	**READ TERM**
H171.11	Cat allergy
H171.12	Dander (animal) allergy
H171000	Allergy to animal
H171100	Dog allergy
14M4.00	H/O: cat allergy

3. **Hay fever:** Patients who have one of the following diagnostic Read codes ever:

HAY FEVER READ CODES	
READ CODE	**READ TERM**
H170.00	Allergic rhinitis - pollens
H170.11	Hay fever - pollens
H171.14	Hay fever - other allergen
H172.11	Hay fever - unspec allergen
Hyu2000	[X]Oth seasonal allergic rhinitis

4. **Perennial rhinitis:** Patients with a hay fever code ever recorded, receiving at least two prescriptions during the study period, at least one of which is prescribed outside hay-fever season (1st March – 31st August).

5. **Seasonal rhinitis**: Patients with a hay fever code ever recorded, receiving at least one prescription during the study period but only in hay-fever season.

6. **Nasal polyps:** Patients who have one of the following diagnostic Read codes ever:

NASAL POLYP READ CODES	
READ CODE	**READ TERM**
H11..00	Nasal polyps
H11y.11	Nasal sinus polyps
2D33.00	O/E - nasal polyp present
H11z.00	Nasal polyp NOS
7406000	Nasal polypectomy
H110z00	Polyp of nasal cavity NOS
7416F00	FESS - polypectomy nasal sinus
H110z00	Polyp of nasal cavity NOS
H11y100	Polyp of ethmoidal sinus
7402900	Excision polyp nasal septum
7402911	Nasal septum polypectomy

7. **Chronic rhinosinusitis:** Patients who have one of the following diagnostic Read codes ever:

CHRONIC RHINOSINUSITIS READ CODES	
READ CODE	**READ TERM**
H13..00	Chronic sinusitis
H13..11	Chronic rhinosinusitis
H130.00	Chronic maxillary sinusitis
H135.00	Recurrent sinusitis
H13z.00	Chronic sinusitis NOS

8. **Other Rhinitis Read codes**:

OTHER RHINITIS READ CODES	
READ CODE	**READ TERM**
H120700	Chronic fibrinous rhinitis
H120z00	Chronic rhinitis NOS
H120500	Chronic ulcerative rhinitis
H120600	Chronic membranous rhinitis
H17..11	Perennial rhinitis
H120400	Chronic infective rhinitis
H18..00	Vasomotor rhinitis
H00..16	Rhinitis - acute
H120.00	Chronic rhinitis
H120000	Chronic simple rhinitis
H120300	Chronic atrophic rhinitis
H120200	Chronic hypertrophic rhinitis
H120100	Chronic catarrhal rhinitis

STATISTICAL ANALYSIS

Summary statistics will be calculated for the following variables by blood-eosinophil count ≤ 400 x10^6/L and >400 x10^6/L and compared:

- Demographics examined at (or closest to) the relevant index date (including age, gender, BMI, smoking status);
- Co-morbidities examined regardless of when they occurred relative to the index date (including Eczema, Rhinitis diagnosis: allergic and non-allergic) and CCI score examined in the year before the index date and in the year after;
- Number of exacerbations in the year before the index date and in the year after (ATS and clinical definition);
- Proportion of patients achieving Asthma Control in the year before the index date and in the year after (Risk Domain & Overall; also GINA control);
- Mean Annual Direct Costs examined in the year before the index date and in the year after.

For variables measured on the interval or ratio scale, summary statistics produced will be:

- Sample size (n)
- Percentage non missing
- Mean
- Variance/standard deviation
- Range (minimum- maximum)
- Median
- Inter-quantile range (25^{th} and 75^{th} percentile)

For categorical variable the summary statistics will include:

- Sample size (n)
- Range (if applicable)
- Count and percentage by category (distribution)

Outcomes will be compared using a Mann Whitney U test/Chi squared Test (for variables measured on the interval or ratio scale/ categorical variables respectively.)

For objective 1, we will estimate whether an eosinophil count >400 x10^6/L can predict asthma control and future asthma exacerbations. For asthma control a logistic regression model will be used, while the expected number of exacerbations will be modelled with a Poisson regression model. Significant predictors of eosinophil count >400 x10^6/L will be included in the model, as well as other potential baseline confounders.

For objective 2, we will estimate whether clinical proxies (such as pattern of rhinitis - allergic and non-allergic, eczema, prior exacerbations, step of asthma therapy, asthma control-Risk Domain & Overall) predict eosinophil count >400 x10^6/L with a logistic regression model. Adjustments will be made for potential baseline confounders.

For objective 3, average asthma-related annual direct costs will be estimated using a generalised linear model with a log link and gamma distribution, adjusting for baseline costs and other potential baseline confounders.

In order to validate that the GP-entered Read code for raised/normal eosinophil counts corresponds to the correct blood-eosinophil count level (≤ 400 $x10^6$/L vs. > 400 $x10^6$/L) a McNemar test will used and, if possible, a ROC curve will be plotted.

Statistically significant results will be defined as $p < 0.05$ and trends as $0.05 \leq p < 0.10$.

SENSITIVITY ANALYSIS

Differences exist in inclusion/exclusion criteria between Reslizumab Phase III study and this planned study due to two main reasons:

1. While the Reslizumab Phase III study is examining the efficacy of treatment in patients with raised blood eosinophil counts, this study is examining the relationship between raised blood-eosinophil counts, number of exacerbations and asthma control in a real-life setting.
2. While the design of real-world studies usually does not exclude smokers, or patients presenting co-morbities, in clinical trials these exclusion criteria are often used to ensure internal validity: in a clinical trial they try to create an ideal context to isolate the efficacy of the drug from confounding factors.

In order to take these differences into account, we will carry out a sensitivity analysis based on the inclusion/exclusion criteria of the Reslizumab Phase III study (full criteria shown in appendix A):

The analysis will be repeated for the sub-group of patients with the following inclusion criteria:

- Patients aged 12-75
- Patients with at least 1 asthma exacerbation during the 12 months prior to the index date.
- Patients with a current blood eosinophil counts of at least 400 $x10^6$/L / with a current blood eosinophil level lower than 400 $x10^6$/L
- Patients taking inhaled steroid at a dosage of at least 800 µg (FP equivalent) daily.
- Patients with airway reversibility of at least 12% to beta-agonist administration (to be calculated from maximum and minimum peak flow data as well as recorded reversibility)
- Uncontrolled patients (GINA control measure, equivalent to ACQ≥1.5)[6]

Based on the exclusion criteria of the Reslizumab Phase III studies, the following patients will be excluded from the sensitivity analysis subgroup:

- Current smokers (in 6 months period prior the index date)
- Patients having a history of concurrent immunodeficiency - human immunodeficiency virus [HIV] or acquired immunodeficiency syndrome or congenital immunodeficiency.
- Patients with pulmonary conditions with symptoms of asthma and blood eosinophilia (eg, Churg-Strauss syndrome, allergic bronchopulmonary aspergillosis).
- Any patient with a Read code for hypereosinophilic syndrome
- Any patients with cystic fibrosis
- Any patients with lung cancer

[6] O'Byrne P.M., Measuring asthma control: a comparison of three classification systems. Eur Respir J. 2010 Aug;36 (2):269-76.

TIMELINE, DELIVERY AND COSTINGS

This section redacted for journal submission – confidential information

APPENDIX A: CRITERIA FOR TEVA PHASE II CLINICAL TRIAL

This section redacted for journal submission – confidential information

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[x]http://www.pathology.leedsth.nhs.uk/pathology/ClinicalInfo/Haematology/FullBloodCount.aspx

STROBE Statement—Checklist of items that should be included in reports of ***cohort studies*** –**Price et al. THELANCETRM-D-15-00283**

	Item No	Recommendation	Reported on page No
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	1 & 2
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	2
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	3-4
Objectives	3	State specific objectives, including any prespecified hypotheses	4
Methods			
Study design	4	Present key elements of study design early in the paper	4-5
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	4-5 +appendix
Participants	6	(*a*) Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up	4-5
		(*b*) For matched studies, give matching criteria and number of exposed and unexposed	n/a
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	4-6 +appendix
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	4-5 +appendix
Bias	9	Describe any efforts to address potential sources of bias	6 +appendix
Study size	10	Explain how the study size was arrived at	7 +figure 1
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	6
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	6 +appendix
		(*b*) Describe any methods used to examine subgroups and interactions	4-6
		(*c*) Explain how missing data were addressed	6, 9, +appendix
		(*d*) If applicable, explain how loss to follow-up was addressed	n/a
		(*e*) Describe any sensitivity analyses	n/a
Results			
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	7 +figure 1
		(b) Give reasons for non-participation at each stage	7 +figure 1
		(c) Consider use of a flow diagram	Figure 1

Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	7 + table 1
		(b) Indicate number of participants with missing data for each variable of interest	Table 1
		(c) Summarise follow-up time (eg, average and total amount)	5
Outcome data	15*	Report numbers of outcome events or summary measures over time	Multiple tables +figs
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	7-8, tables, figures
		(*b*) Report category boundaries when continuous variables were categorized	Tables, figures
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	n/a
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	8-9, figures, +appendix
Discussion			
Key results	18	Summarise key results with reference to study objectives	9-10
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	11-12
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	11-12
Other information			**10-12**
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	6-7 +15

*Give information separately for exposed and unexposed groups.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at http://www.strobe-statement.org.