Patient characteristics, biomarkers and exacerbation risk in severe, uncontrolled asthma

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A pooled analysis of placebo data from seven randomised controlled trials identified exacerbation risk factors in patients with severe, uncontrolled asthma and revealed a prognostic role for persistence of elevations in type 2 inflammation biomarkers https://bit.ly/3sWCKVd


Abstract

Background Greater precision in asthma exacerbation risk prediction may improve outcomes. We sought to identify clinical characteristics and biomarkers associated with elevated exacerbation risk in patients with severe, uncontrolled asthma.

Methods Data were pooled from seven similarly designed phase II and III randomised controlled clinical trials of biologic therapies for the treatment of severe, uncontrolled asthma that enrolled comparable patient populations. Annualised asthma exacerbation rates (AAERs) for patients randomised to placebo were assessed by baseline clinical characteristics, and by biomarker concentrations at baseline and over the study duration.

Results The AAER for the 2016 patients in the combined placebo group was 0.91 (95% CI 0.84–0.98). Baseline characteristics associated with greater AAER were frequent or severe exacerbations within the prior 12 months, nasal polyposis, maintenance oral corticosteroid use, Asian race and Asian or Western European region. AAER increased with baseline blood eosinophil counts and exhaled nitric oxide fraction (\(F_{\text{ENO}}\)) concentration, with the greatest AAER occurring for patients with eosinophils \(\geq 300\) cells·\(\mu\)L\(^{-1}\) and \(F_{\text{ENO}}\) \(\geq 50\) ppb. No relationship was observed between baseline serum IgE concentration and AAER. Combining type 2 inflammation criteria for eosinophils and \(F_{\text{ENO}}\) had greater prognostic value than either biomarker alone. Persistent eosinophil and \(F_{\text{ENO}}\) elevations throughout the study period were associated with greater AAER.

Conclusions Exacerbation history, maintenance corticosteroid use, nasal polyposis, Asian race, geographic region, and elevations in blood eosinophil counts and \(F_{\text{ENO}}\) concentrations (particularly when combined and/or persistently achieving type 2 inflammation criteria) were associated with increased exacerbation risk in patients with severe, uncontrolled asthma.

Introduction

Asthma, which affects approximately 339 million people worldwide [1], is a chronic inflammatory airway disease punctuated by recurring flare-ups (exacerbations or attacks) of symptom resurgence [2]. Exacerbations occur across the spectrum of disease severity, yet patients with inadequate symptom control or who have more severe disease are particularly prone to more frequent and severe exacerbations [3, 4]. Approximately 4% of patients with asthma have severe disease that is uncontrolled despite use of, and adherence to, the standard of care for asthma management [5].
Asthma exacerbations are debilitating for patients [6] and exact a heavy toll on the healthcare system [7]. Approximately 8–12% of patients with asthma experience ≥1 exacerbations per year, the frequency of which increases with disease severity [3]. Asthma exacerbations are a driver of disease-related morbidity, negatively influence health-related quality of life and are associated with progressive loss of lung function [7, 8].

The ability to predict exacerbation risk based on patient-related and clinical factors may aid the development of newer asthma treatment strategies. The placebo arm of randomised controlled trials for biologic therapies provides a unique population of patients who meet defined moderate-to-severe asthma criteria, have available data for demographic and clinical characteristics at baseline, and are subject to regular clinical assessments and defined criteria for asthma exacerbations. Moreover, these patients are generally required to remain on stable background asthma management regimens throughout the study period.

This analysis was performed to identify demographics, clinical characteristics and biomarkers associated with asthma exacerbation risk. These factors were obtained at baseline for patients with severe, uncontrolled asthma using a large, pooled dataset from seven clinical trials of biologic therapies. Together these trials included more than 5000 patients, 2016 of whom were randomised to placebo. In addition to baseline factors, risk for exacerbations was also evaluated for patients who received placebo and had persistent versus fluctuating type 2 (T2) inflammation status during the studies, as defined by Global Initiative for Asthma (GINA) criteria [2]. Some of the results of these analyses have been previously reported in the form of an abstract [9].

**Methods**

**Study design and participants**

Patient-level data were pooled from seven multicentre, randomised, double-blind, placebo-controlled clinical trials registered at ClinicalTrials.gov, including two phase III studies (SIROCCO (NCT01928771) and CALIMA (NCT01914757)) and one phase Ib study (NCT01238861) of benralizumab, two phase III studies (STRATOS 1 (NCT02161757) and STRATOS 2 (NCT02194699)) and one phase Ib study (NCT01402986) of tralokinumab, and one phase II study (PATHWAY (NCT02054130)) of tezepelumab [10–15]. Studies selected for inclusion were 48–56 weeks in duration; included biologic therapy and placebo treatment arms; had a primary end-point of annual asthma exacerbation rate; enrolled patients with severe, uncontrolled asthma; used a large, pooled dataset from seven clinical trials of biologic therapies. Together these trials included more than 5000 patients, 2016 of whom were randomised to placebo. In addition to baseline factors, risk for exacerbations was also evaluated for patients who received placebo and had persistent versus fluctuating type 2 (T2) inflammation status during the studies, as defined by Global Initiative for Asthma (GINA) criteria [2]. Some of the results of these analyses have been previously reported in the form of an abstract [9].

All studies were conducted in accordance with the principles of Good Clinical Practice. Study protocols received independent ethics committee approval at each study site and all participants provided written informed consent.

**Statistical analyses**

Data were analysed for the full analysis set, as defined by each study. Demographics and baseline clinical characteristics for the placebo group of 2016 patients were compared with the 5701 total patients in the seven studies to determine how representative the placebo patients were of the entire pooled dataset. In addition, demographics and baseline characteristics were compared in four clinically relevant subgroups: patients with eosinophil counts ≥300 versus <300 cells·µL⁻¹, ≥3 versus ≤2 exacerbations in the past 12 months, medium- versus high-dosage ICS regimens and use versus nonuse of maintenance oral corticosteroids (OCS).

The primary efficacy variable was the annualised asthma exacerbation rate (AAER). The AAERs and corresponding 95% confidence intervals were estimated using a negative binomial model, with the number of exacerbations as the response variable. The AAER for placebo patients during the study treatment period was evaluated for subgroups, including race, geographic region, background ICS dosage (medium versus high), number of exacerbations in the past 12 months (≤2 versus ≥3), number of exacerbations resulting in hospitalisation or emergency department visit in the past 12 months (0 versus ≥1), age at asthma diagnosis (<18 versus ≥18 years), diagnosis of allergic rhinitis, presence of nasal polyposis, atopic status (positive versus negative) and maintenance OCS use (yes versus no).
The AAER in randomised placebo patients was also evaluated by baseline biomarker values, including total serum IgE concentrations, blood eosinophil counts and exhaled nitric oxide fraction (F\textsubscript{ENO}) concentrations, both continuously and using specific subgroup thresholds. F\textsubscript{ENO} concentration data were collected in the phase IIb benralizumab, STRATOS 1, STRATOS 2 and PATHWAY studies. T2 airway inflammation status was defined using the GINA criteria for blood eosinophil counts and/or F\textsubscript{ENO} concentration, with T2 inflammation thresholds of $\geq 150$ cells·$\mu$L$^{-1}$ and $\geq 20$ ppb, respectively [2].

Additional details regarding study design, patient enrolment criteria and statistical methods are provided in the supplementary material.

### Results

#### Demographics and baseline clinical characteristics

In total, 5701 patients from seven studies were included in the analysis population. The placebo group comprised 2016 patients who had been randomised to the placebo arm in their respective clinical trials. Baseline demographics and clinical characteristics were similar in the overall cohort and the placebo group (table 1). In the placebo group, the mean age was 49 years and two-thirds of patients were female. At baseline, mean Asthma Control Questionnaire-6 score was 2.6 and 29% of the patient population had experienced $\geq 3$ exacerbations in the year before study entry. Most patients (55%) were receiving a background high-dosage ICS regimen. Seven percent of patients were on maintenance OCS and 3% had a history of omalizumab use. There was a wide range of baseline blood eosinophil levels, F\textsubscript{ENO} concentrations and IgE values, consistent with the nondiscriminatory inclusion criteria.

With the exception of associations among spirometry and patient-reported outcome parameters, no strong correlations were detected between baseline demographics and clinical characteristics for the overall analysis cohort (supplementary tables S3 and S4). However, differences in baseline parameters were observed when patients were dichotomised according to four clinically relevant subgroups (supplementary table S5). Patients with a baseline eosinophil count $\geq 300$ cells·$\mu$L$^{-1}$ were more likely to have had

### Table 1: Demographics and baseline clinical characteristics

<table>
<thead>
<tr>
<th></th>
<th>All patients</th>
<th>All placebo patients</th>
<th>Placebo patients with $\geq 1$ exacerbations</th>
<th>Placebo patients with 0 exacerbations</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients</td>
<td>5701</td>
<td>2016</td>
<td>850</td>
<td>1166</td>
</tr>
<tr>
<td>Age years</td>
<td>49.5±14.0</td>
<td>49.6±14.3</td>
<td>50.1±13.7</td>
<td>49.2±14.8</td>
</tr>
<tr>
<td>Female</td>
<td>3756 (66)</td>
<td>1331 (66)</td>
<td>583 (69)</td>
<td>748 (64)</td>
</tr>
<tr>
<td>Ex-smoker</td>
<td>1085 (19)</td>
<td>378 (19)</td>
<td>183 (22)</td>
<td>195 (17)</td>
</tr>
<tr>
<td>BMI kg·m$^{-2}$</td>
<td>28.6±6.3</td>
<td>28.6±6.4</td>
<td>28.9±6.9</td>
<td>28.4±6.0</td>
</tr>
<tr>
<td>$\geq 3$ exacerbations in past 12 months</td>
<td>1642 (29)</td>
<td>586 (29)</td>
<td>324 (38)</td>
<td>262 (22)</td>
</tr>
<tr>
<td>$\geq 1$ exacerbations resulting in hospitalisation or ED visit in past 12 months</td>
<td>1877 (33)</td>
<td>673 (34)</td>
<td>330 (40)</td>
<td>343 (30)</td>
</tr>
<tr>
<td>Age at asthma diagnosis years</td>
<td>29.9±18.7</td>
<td>29.8±19.1</td>
<td>30.2±18.4</td>
<td>29.5±19.5</td>
</tr>
<tr>
<td>Asthma diagnosed as an adult</td>
<td>3962 (69)</td>
<td>1398 (69)</td>
<td>611 (72)</td>
<td>787 (68)</td>
</tr>
<tr>
<td>Pre-bronchodilator FEV$_1$ % pred</td>
<td>60.5±14.9</td>
<td>60.7±15.0</td>
<td>58.4±15.7</td>
<td>62.4±14.2</td>
</tr>
<tr>
<td>Reversibility %</td>
<td>22.9±26.3</td>
<td>23.3±28.7</td>
<td>23.8±34.4</td>
<td>22.9±23.7</td>
</tr>
<tr>
<td>Blood eosinophil count cells·$\mu$L$^{-1}$</td>
<td>260 (0–7510)</td>
<td>250 (0–5330)</td>
<td>280 (0–3000)</td>
<td>240 (0–5330)</td>
</tr>
<tr>
<td>$F_{\text{ENO}}$ ppb$^4$</td>
<td>21.5 (0–312.5)</td>
<td>21.2 (0–276.3)</td>
<td>22.7 (3.9–193.8)</td>
<td>20.0 (0–276.3)</td>
</tr>
<tr>
<td>Total serum IgE kU·L$^{-1}$</td>
<td>170.1 (0.3–46983.8)</td>
<td>171.3 (1.0–24 749.4)</td>
<td>182.7 (2.0–17317.0)</td>
<td>167.1 (1.0–24 749.4)</td>
</tr>
<tr>
<td>ACQ-6 score</td>
<td>2.6±0.9</td>
<td>2.6±0.9</td>
<td>2.7±0.9</td>
<td>2.6±0.9</td>
</tr>
<tr>
<td>Diagnosis of allergic rhinitis$^5$</td>
<td>1297 (38)</td>
<td>494 (39)</td>
<td>218 (47)</td>
<td>276 (35)</td>
</tr>
<tr>
<td>Nasal polyposis</td>
<td>746 (13)</td>
<td>260 (13)</td>
<td>139 (16)</td>
<td>121 (10)</td>
</tr>
<tr>
<td>Atopic-positive per Phadiatop/FEIA$^4$</td>
<td>3347 (61)</td>
<td>1194 (62)</td>
<td>507 (63)</td>
<td>687 (62)</td>
</tr>
<tr>
<td>Background high-dosage ICS</td>
<td>3205 (56)</td>
<td>1110 (55)</td>
<td>471 (55)</td>
<td>639 (55)</td>
</tr>
<tr>
<td>Maintenance OCS use</td>
<td>420 (7)</td>
<td>140 (7)</td>
<td>90 (11)</td>
<td>50 (4)</td>
</tr>
<tr>
<td>History of omalizumab$^5$</td>
<td>130 (3)</td>
<td>49 (3)</td>
<td>39 (6)</td>
<td>10 (1)</td>
</tr>
</tbody>
</table>

Data are presented as n, mean±SD, n (%) or median (range). BMI: body mass index; ED: emergency department; FEV$_1$: forced expiratory volume in 1 s; $F_{\text{ENO}}$: exhaled nitric oxide fraction; ACQ-6: Asthma Control Questionnaire-6; FEIA: fluorescence enzyme immunoassay; ICS: inhaled corticosteroid; OCS: oral corticosteroid. $^4$: data available for 5621 patients overall and for 1993 patients in the placebo group; $^5$: data available for 3014 patients overall and for 1111 patients in the placebo group; $^6$: data available for 5528 patients overall and for 1957 patients in the placebo group; $^7$: data available for 3453 patients overall and for 1255 patients in the placebo group; $^8$: data available for 5482 patients overall and for 1926 patients in the placebo group.

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≥3 exacerbations in the previous 12 months (33% versus 25%), had higher median baseline $F_{\text{ENO}}$ (31.0 versus 18.3 ppb) and total serum IgE (236.0 versus 125.7 kU·L$^{-1}$) concentrations, and were more likely to have a history of nasal polyposis (20% versus 7%) than patients with lower eosinophil counts. Patients with a history of ≥3 exacerbations in the past 12 months were more likely to have experienced ≥1 exacerbations resulting in hospitalisation or emergency department visit in the previous 12 months (45% versus 29%), use a high-dosage ICS regimen (63% versus 54%) and receive maintenance OCS (14% versus 5%) compared with patients with fewer recent exacerbations. High- versus medium-dosage ICS use was associated with a greater prevalence of ≥3 exacerbations in the previous year (32% versus 24%). Receiving versus not receiving maintenance OCS was associated with a greater prevalence of ≥3 exacerbations in the prior 12 months (54% versus 27%), asthma diagnosed as an adult (78% versus 69%) and high-dosage ICS use (74% versus 55%). Fewer patients receiving maintenance OCS had a diagnosis of allergic rhinitis (24% versus 38%) or were atopic (50% versus 62%), but a greater percentage had a history of nasal polyposis (31% versus 12%).

**Baseline parameters and exacerbation occurrence**

During the study treatment period, 850 (42%) patients in the placebo group experienced ≥1 exacerbations (table 1). A greater percentage of patients who experienced an exacerbation had a history of ≥3 exacerbations in the past 12 months (38% versus 22%), ≥1 exacerbations resulting in hospitalisation or emergency department visit in the past 12 months (40% versus 30%), diagnosed allergic rhinitis (47% versus 35%) and nasal polyposis (16% versus 10%) compared with patients who did not experience an exacerbation. Baseline biomarker concentrations, including eosinophil counts (280 ± 356 versus 240 ± 300 cells·µL$^{-1}$), $F_{\text{ENO}}$ concentration (22.7 ± 24.0 versus 20.0 ppb) and serum IgE concentration (182.7 ± 200.0 versus 167.1 kU·L$^{-1}$), were greater for patients with versus without an exacerbation during the study. Maintenance OCS use and history of omalizumab treatment were also more prevalent for patients with versus without an exacerbation (11% versus 4% and 6% versus 1%, respectively).

**Clinical predictors of exacerbation risk**

For patients in the placebo group, the overall AAER was 0.91 (95% CI 0.84–0.98) (figure 1). Greater AAERs were associated with a history of ≥3 exacerbations in the 12 months prior to study entry, ≥1 exacerbations resulting in hospitalisation or emergency department visit in the 12 months prior to study entry, presence of nasal polyposis, maintenance OCS use, Asian race and study sites in Asia or Western Europe. AAERs below the overall placebo group 95% confidence intervals were associated with a history of ≥1 exacerbations resulting in hospitalisation or emergency department visit in the 12 months prior to study entry and study sites in Eastern Europe.

**Biomarker predictors of exacerbation risk**

The baseline distribution of blood eosinophil counts and $F_{\text{ENO}}$ and IgE concentrations was unimodal with a rightward skew (supplementary figure S1). The AAER increased with greater baseline blood eosinophil counts and $F_{\text{ENO}}$ concentrations, but did not change with increasing serum IgE concentration (figure 2). The addition of atopic status to baseline serum IgE concentration provided no additional predictive information. Approximately linear increases in AAER were observed with increasing baseline blood eosinophil count from 0 to ~650 cells·µL$^{-1}$ and with increasing baseline $F_{\text{ENO}}$ concentration from 0 to ~60 ppb, with flattening of the relationships above these thresholds.

Both baseline eosinophil counts and $F_{\text{ENO}}$ concentrations were available for 1098 of the 2016 placebo patients, and these values were used to categorise the degree of airway inflammation observed in the study populations (figure 3). A small number of patients (108 (9.8%)) met the very stringent criteria for very high degrees of T2 inflammation ($F_{\text{ENO}}$ ≥50 ppb and eosinophils ≥300 cells·µL$^{-1}$) at baseline. These patients had the highest AAER of any subgroup (1.00). There were 312 (28.4%) patients categorised as having medium-to-high T2 inflammation ($F_{\text{ENO}}$ ≥50 ppb and eosinophils <300 cells·µL$^{-1}$ or $F_{\text{ENO}}$ <50 ppb and eosinophils ≥300 cells·µL$^{-1}$); the AAER in this group was 0.83. Intermediate T2 inflammation ($F_{\text{ENO}}$ ≥20–<50 ppb and eosinophils <300 cells·µL$^{-1}$ or $F_{\text{ENO}}$ <20 ppb and eosinophils ≥150–<300 cells·µL$^{-1}$) was the largest subgroup, encompassing 467 (42.5%) patients. Low T2 inflammation ($F_{\text{ENO}}$ <20 ppb and eosinophils <150 cells·µL$^{-1}$) was detected in 211 (19.2%) patients. Both the intermediate and low T2 inflammation groups had AAERs of 0.58. A sensitivity analysis using a low $F_{\text{ENO}}$ threshold of <25 ppb yielded similar results (supplementary figure S2).

When patients in the placebo group were dichotomised by baseline biomarker values, AAERs were greater for patients with $F_{\text{ENO}}$ ≥20 versus <20 ppb and blood eosinophils ≥150 versus <150 cells·µL$^{-1}$. Patients who had both $F_{\text{ENO}}$ ≥20 ppb and eosinophils ≥150 cells·µL$^{-1}$ at baseline had greater AAERs compared with those below these thresholds and patients with GINA-defined T2 inflammation (by $F_{\text{ENO}}$ and eosinophil criteria) (figure 4a).
Patients who had $F_{\text{ENO}} \geq 20$ ppb or blood eosinophils $\geq 150$ cells·$\mu$L$^{-1}$ throughout the entire treatment period (i.e. persistent elevation) had greater AAERs compared with those with persistently low values for either biomarker (figure 4b). Patients with fluctuating $F_{\text{ENO}}$ concentrations or eosinophil counts (i.e. inconsistently meeting thresholds during post-baseline measurements) behaved similarly to the $<20$ ppb group for $F_{\text{ENO}}$ concentration and the $\geq 150$ cells·$\mu$L$^{-1}$ group for eosinophil count. The greatest AAER (0.85, 95% CI 0.63–1.14) was observed for patients with persistent elevations in both $F_{\text{ENO}}$ and eosinophils, which exceeded the AAER for patients with persistent GINA-defined T2 inflammation (0.71, 95% CI 0.60–0.85). The percentage of patients without evidence of GINA-defined T2 inflammation throughout the entire treatment period was very small (33 (3.0%)).

Combining baseline biomarkers sequentially by categorical subgroups, with the inclusion of baseline IgE concentration, did not yield additional prognostic information regarding asthma exacerbations risk (supplementary figure S3).
Discussion

Despite the availability and widespread use of OCS and other standard-of-care therapies, many patients with asthma, particularly those with severe, uncontrolled disease, remain at elevated risk for recurrent exacerbations [16, 17]. In this study, we used data from a large, multinational dataset to assess relationships between patient characteristics and asthma exacerbation risk for patients with severe, uncontrolled asthma enrolled in clinical trials of biologic therapies. There are three notable findings from this analysis. First, for patients randomised to placebo, patient-related and clinical factors, including exacerbation history, presence of nasal polyposis, maintenance OCS use, Asian race and enrolment at a study site in Asia or Western Europe, were associated with increased exacerbation frequency. Second, baseline blood eosinophil count and \( F_{ENO} \) concentration, but not IgE concentration, were associated with exacerbation risk. Third, in a novel longitudinal assessment of T2 inflammation criteria and exacerbation risk, a combination of elevations in blood eosinophils and \( F_{ENO} \), both at baseline and persistent over time, identified patients at the highest risk of exacerbations.

Individual characteristics such as exacerbation history and OCS use have previously been linked to asthma exacerbation risk [3, 18–22]. The large population evaluated in our study lends further credence to these data and provides additional insights for smaller patient subpopulations, such as those with nasal polyposis. Particularly notable in our analysis was the regional variation in AAER. Increased AAER was observed for patients enrolled at study sites in Asia; however, equivalent elevations were associated with Asian racial designation, thus confounding the distinction between inherent and environmental factors contributing to exacerbation risk. Also noted was an increased AAER for Western Europe but a decreased...
AAER in Eastern Europe. Further work is needed to more fully understand these regional differences in exacerbation risk.

Of the biomarkers tested, baseline blood eosinophil count and baseline $F_{ENO}$ concentration were predictors of exacerbation risk, with a graded association between baseline elevations and AAER. In contrast, no prognostic relationship was observed between baseline IgE concentration, even when combined with allergic status, and exacerbations. The eosinophil and IgE findings are consistent with a pooled analysis from the SIROCCO and CALIMA studies, in which asthma exacerbation risk increased in conjunction with baseline blood eosinophil count for patients with severe, uncontrolled asthma randomised to placebo [23]. No association with exacerbation risk was observed for baseline IgE concentration and the addition of atopic status did not improve the predictive value of IgE. A separate pooled analysis of SIROCCO and CALIMA reported greater reductions in AAER with active treatment (benralizumab) versus placebo for patients with greater versus lesser blood eosinophil counts and in those with a more versus less pronounced exacerbation history [24]. These results support the elevated exacerbation risk associated with greater baseline blood eosinophil count and exacerbation history, as well as the effectiveness of therapies targeted at decreasing eosinophilia in these patients.

The influence of elevated eosinophil counts on exacerbation risk has been well documented [22, 25–31]. Data from the longitudinal COBRA study demonstrated that patients with asthma ($n=1080$) who had the greatest degree of eosinophilia (>300 cells·μL$^{-1}$) were more likely to experience severe exacerbations and have poor asthma control compared with patients with lower eosinophil counts [28]. Moreover, increases in eosinophil counts over time were associated with subsequent exacerbations. Using a greater threshold for eosinophil elevations (>400 cells·μL$^{-1}$) in an historical, observational, primary care cohort of 130,248 adults and adolescents with asthma of any severity, Price et al. [25] reported a 42% increase in the occurrence of severe exacerbations for patients with blood eosinophil counts >400 versus ≤400 cells·μL$^{-1}$. Moreover, patients with greater blood eosinophil counts were 26% less likely to achieve asthma control. Compared with a reference value of ≤200 cells·μL$^{-1}$, asthma exacerbation rates increased with each successively greater blood eosinophil count category. Baseline blood eosinophil count as a predictor of exacerbation risk has also been reported in the context of mild asthma [31] and chronic obstructive pulmonary disease [32, 33], suggesting that its prognostic value is not limited to moderate or severe asthma. Emerging data support a correlation between $F_{ENO}$ concentration and exacerbation risk [34–36]. In a study of patients with late-onset asthma and sputum eosinophilia ($n=110$) despite standard-of-care therapy, risk for ≥2 exacerbations per year was markedly increased for patients with $F_{ENO}$ concentrations ≥50 ppb (OR 5.4, 95% CI 1.9–11.6) [34]. For an unselected population of real-world patients with severe asthma ($n=115$), $F_{ENO}$ concentration more strongly correlated with the frequency of exacerbations requiring OCS use than either peripheral blood eosinophil count or serum periostin concentration [35]. In another study, baseline $F_{ENO}$ concentration correlated with time to first severe exacerbation, demonstrating an even stronger correlation than blood eosinophil count, serum periostin concentration or serum IgE concentration [36].

Three recent analyses evaluated the combination of blood eosinophil count and $F_{ENO}$ concentration on asthma exacerbation risk [30, 31, 37]. In a post hoc analysis of the phase Ib DREAM study, which enrolled patients with severe eosinophilic asthma ($n=606$), exacerbation risk was greatest for patients in the
The effect of active treatment (mepolizumab) was greater for patients with baseline elevations in both biomarkers compared with patients who had baseline elevations in only one biomarker or in neither biomarker. In a prespecified subgroup analysis of Novel START, a 52-week, open-label, randomised controlled trial that enrolled patients with mild asthma (n=675), greater reductions in exacerbations and severe exacerbations with maintenance inhaled budesonide were observed for patients with high (≥300 cells·μL⁻¹) versus low (<150 cells·μL⁻¹) blood eosinophil counts [31]. No consistent interaction between treatment response and \( F_{ENO} \) concentration was observed in this study cohort; however, maintenance budesonide plus as-needed salbutamol had a greater effect on severe exacerbations compared with as-needed salbutamol alone for patients with \( F_{ENO} <20 \) versus >50 ppb. The third analysis

**Baseline F\(_{ENO}\)**
- High: ≥20 ppb
- Low: <20 ppb

**Baseline T2 combinations**
- \( F_{ENO} \) and EOS high: \( F_{ENO} \geq 20 \text{ ppb and EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) or EOS (not both) high: \( F_{ENO} \geq 20 \text{ ppb or EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) and EOS low: \( F_{ENO} <20 \text{ ppb and EOS} <150 \text{ μL}^{-1} \)

**Baseline central EOS count**
- High: ≥150 μL⁻¹
- Low: <150 μL⁻¹

**Baseline GINA-defined T2 inflammation**
- \( F_{ENO} \) ≥20 ppb and/or EOS ≥150 μL⁻¹

**Baseline T2 combinations**
- \( F_{ENO} \) and EOS high: \( F_{ENO} \geq 20 \text{ ppb and EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) or EOS (not both) high: \( F_{ENO} \geq 20 \text{ ppb or EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) and EOS low: \( F_{ENO} <20 \text{ ppb and EOS} <150 \text{ μL}^{-1} \)

**Persistent F\(_{ENO}\)**
- High: all available visits ≥20 ppb
- Low: all available visits <20 ppb

**Persistent EOS count**
- High: all available visits ≥150 μL⁻¹
- Low: all available visits <150 μL⁻¹

**Persistent T2 combinations**
- \( F_{ENO} \) and EOS high: every visit \( F_{ENO} \geq 20 \text{ ppb and EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) or EOS (not both) high: every visit \( F_{ENO} \geq 20 \text{ ppb and EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) and EOS low: every visit \( F_{ENO} <20 \text{ ppb and EOS} <150 \text{ μL}^{-1} \)

**Persistent GINA-defined T2 inflammation**
- Every visit \( F_{ENO} \geq 20 \text{ ppb and/or EOS} \geq 150 \text{ μL}^{-1} \)

**Persistent T2 combinations**
- \( F_{ENO} \) and EOS high: every visit \( F_{ENO} \geq 20 \text{ ppb and EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) or EOS (not both) high: every visit \( F_{ENO} \geq 20 \text{ ppb and EOS} \geq 150 \text{ μL}^{-1} \)
- \( F_{ENO} \) and EOS low: every visit \( F_{ENO} <20 \text{ ppb and EOS} <150 \text{ μL}^{-1} \)

**FIGURE 4** Annualised asthma exacerbation rates (AAERs) in the placebo group by Global Initiative for Asthma (GINA)-defined type 2 (T2) airway inflammation endotype criteria at baseline and over the course of the treatment period: eosinophil (EOS) count and exhaled nitric oxide fraction (\( F_{ENO} \)) concentration as predictors of AAER a) at baseline, both individually and jointly, and b) for patients with persistent (at each study visit) or fluctuating concentrations. Persistency was evaluated only for patients who had baseline and three or more post-baseline visit values available. Baseline \( F_{ENO} \) includes data from four studies: benralizumab (phase IIb), tralokinumab (STRATOS 1 and 2) and tezepelumab (PATHWAY).
used data from the placebo group of the LIBERTY ASTHMA QUEST study, which enrolled patients with uncontrolled, moderate-to-severe asthma (n=620) [37]. In this cohort, severe exacerbation rates were 3 times greater in patients with baseline $F_{\text{ENO}} \geq 50$ ppb and eosinophils $\geq 300$ cells·μL$^{-1}$ than patients with $F_{\text{ENO}} < 25$ ppb and eosinophils $< 150$ cells·μL$^{-1}$. Taken together with previous findings, these results suggest that $F_{\text{ENO}}$ concentration adds further prognostic value to eosinophil count for asthma exacerbation risk prediction.

Current GINA guidelines for identifying T2 inflammation for patients with severe asthma use cut-offs of blood eosinophils $\geq 150$ cells·μL$^{-1}$ and/or $F_{\text{ENO}} \geq 20$ ppb (among other factors) [2]. Our analysis detected a greater AAER for patients with elevations in both baseline eosinophil count and $F_{\text{ENO}}$ concentration compared with the less stringent GINA-defined biomarker elevation requirement. A novel observation in our analysis was the difference in AAER for patients with persistent versus fluctuating biomarker elevations. Exacerbation rates in the group of patients with fluctuating $F_{\text{ENO}}$ concentrations during the observation period aligned more closely with patients in the persistently low group. In contrast, patients categorised in the fluctuating eosinophil count group had exacerbation rates consistent with the persistently high group and notably elevated relative to the persistently low group. The observed differences in behaviour between the fluctuating $F_{\text{ENO}}$ and fluctuating eosinophil categories warrant further exploration. Notably, the greatest AAER occurred for patients with persistent elevations in both eosinophil count and $F_{\text{ENO}}$ concentration, and the lowest AAER was observed for patients with persistently low eosinophil counts and $F_{\text{ENO}}$ concentrations, a group that comprised only 3% of the analysis population.

There are strengths and weaknesses to this analysis. Strengths are the large sample size and recruitment of patients irrespective of baseline blood eosinophil counts and $F_{\text{ENO}}$ concentrations. The wide ranges of baseline blood eosinophil counts and $F_{\text{ENO}}$ and IgE concentrations provide confidence in our analysis of AAER by these continuous variables. All the studies met consistent, rigorous quality control standards. A further strength of this pooled analysis is that similar methods were used and comparable patient populations were included in the seven studies. Limitations of this study included the lack of $F_{\text{ENO}}$ measurement in several studies, which reduced the pool of patients available for T2 status evaluation. Asthma exacerbation rates may be greater in comparable real-world populations than reported in this study, in part because a strong placebo effect has been observed in clinical trials involving patients with uncontrolled persistent asthma [38]. Although adherence to background therapy was monitored and strongly encouraged during these studies, maintenance therapy was not provided universally in all studies by the study sponsor. Hence, adherence to background asthma therapy through these studies cannot be fully assured. Finally, as these subgroup analyses were not specified a priori, these results should be considered hypothesis generating.

In this cohort of patients with severe, uncontrolled asthma, exacerbation history, maintenance OCS use, patient demographics/clinical characteristics, geographic region, baseline blood eosinophil count and baseline $F_{\text{ENO}}$ concentration were relevant predictors of exacerbation risk. Risk elevation was particularly marked for patients who met the combination of GINA-defined T2 inflammation criteria for blood eosinophils and $F_{\text{ENO}}$. Moreover, persistence in eosinophil and $F_{\text{ENO}}$ elevations was associated with greater asthma exacerbation risk than values that fluctuated over the 1-year observation period. Further interrogation of datasets such as this will provide prognostic information that informs the development of individualised treatment strategies for the prevention of asthma exacerbations.

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Data sharing: Data underlying the findings described in this article may be requested in accordance with AstraZeneca’s data-sharing policy described at https://astrazenecagroup-dt.pharmcm.com/DT/Home.

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