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

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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_{ENO}) concentration, with the greatest AAER occurring for patients with eosinophils $\geqslant$300 cells·μL^{-1} and F_{ENO} $\geqslant$50 ppb. No relationship was observed between baseline serum IgE concentration and AAER. Combining type 2 inflammation criteria for eosinophils and F_{ENO} had greater prognostic value than either biomarker alone. Persistent eosinophil and F_{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_{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 IIb study (NCT01238861) of benralizumab, two phase III studies (STRATOS 1 (NCT02161757) and STRATOS 2 (NCT02194699)) and one phase IIb 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; and had a common sponsor. All studies enrolled adults or adults and adolescents who had physician-diagnosed asthma requiring a medium- or high-dosage inhaled corticosteroid (ICS) regimen plus a long-acting β-agonist. No baseline criteria were imposed for inflammatory biomarker concentrations. Formal heterogeneity testing among the studies was not performed; however, the methods (supplementary table S1) and demographics and baseline characteristics (supplementary table S2) were considered sufficiently similar for pooling.

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_{ENO}) concentrations, both continuously and using specific subgroup thresholds. F_{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_{ENO} concentration, with T2 inflammation thresholds of ⩾150 cells·μL^{-1} and ⩾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 ⩾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_{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 ⩾300 cells·μL^{-1} were more likely to have had

TABLE 1 Demographics and baseline clinical characteristics

	All patients	All placebo patients	Placebo patients with ⩾1 exacerbations	Placebo patients with 0 exacerbations
Patients	5701	2016	850	1166
Age years	49.5±14.0	49.6±14.3	50.1±13.7	49.2±14.8
Female	3756 (66)	1331 (66)	583 (69)	748 (64)
Ex-smoker	1085 (19)	378 (19)	183 (22)	195 (17)
BMI kg·m^{-2}	28.6±6.3	28.6±6.4	28.9±6.9	28.4±6.0
⩾3 exacerbations in past 12 months	1642 (29)	586 (29)	324 (38)	262 (22)
⩾1 exacerbations resulting in hospitalisation or ED visit in past 12 months	1877 (33)	673 (34)	330 (40)	343 (30)
Age at asthma diagnosis years	29.9±18.7	29.8±19.1	30.2±18.4	29.5±19.5
Asthma diagnosed as an adult	3962 (69)	1398 (69)	611 (72)	787 (68)
Pre-bronchodilator FEV_1 % pred	60.5±14.9	60.7±15.0	58.4±15.7	62.4±14.2
Reversibility %	22.9±26.3	23.3±28.7	23.8±34.4	22.9±23.7
Blood eosinophil count cells·μL^{-1}[#]	260 (0–7510)	250 (0–5330)	280 (0–3000)	240 (0–5330)
F_{ENO} ppb[¶]	21.5 (0–312.5)	21.2 (0–276.3)	22.7 (3.9–193.8)	20.0 (0–276.3)
Total serum IgE kU·L^{-1}[+]	170.1 (0.3–46 983.8)	171.3 (1.0–24 749.4)	182.7 (2.0–17 317.0)	167.1 (1.0–24 749.4)
ACQ-6 score	2.6±0.9	2.6±0.9	2.7±0.9	2.6±0.9
Diagnosis of allergic rhinitis[§]	1297 (38)	494 (39)	218 (47)	276 (35)
Nasal polyposis	746 (13)	260 (13)	139 (16)	121 (10)
Atopic-positive per Phadiatop/FEIA[ƒ]	3347 (61)	1194 (62)	507 (63)	687 (62)
Background high-dosage ICS	3205 (56)	1110 (55)	471 (55)	639 (55)
Maintenance OCS use	420 (7)	140 (7)	90 (11)	50 (4)
History of omalizumab[##]	130 (3)	49 (3)	39 (6)	10 (1)

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_{ENO}: exhaled nitric oxide fraction; ACQ-6: Asthma Control Questionnaire-6; FEIA: fluorescence enzyme immunoassay; ICS: inhaled corticosteroid; OCS: oral corticosteroid. [#]: data available for 5621 patients overall and for 1993 patients in the placebo group; [¶]: data available for 3014 patients overall and for 1111 patients in the placebo group; [+]: data available for 5528 patients overall and for 1957 patients in the placebo group; [§]: data available for 3453 patients overall and for 1255 patients in the placebo group; [ƒ]: data available for 5482 patients overall and for 1926 patients in the placebo group; [##]: data available for 4188 patients overall and for 1536 patients in the placebo group.

⩾3 exacerbations in the previous 12 months (33% *versus* 25%), had higher median baseline F_{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 *versus* 240 cells·μL^{-1}), F_{ENO} concentration (22.7 *versus* 20.0 ppb) and serum IgE concentration (182.7 *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 ⩽2 exacerbations 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_{ENO} and IgE concentrations was unimodal with a rightward skew (supplementary figure S1). The AAER increased with greater baseline blood eosinophil counts and F_{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_{ENO} concentration from 0 to ∼60 ppb, with flattening of the relationships above these thresholds.

Both baseline eosinophil counts and F_{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_{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_{ENO} ⩾50 ppb and eosinophils <300 cells·μL^{-1} or F_{ENO} <50 ppb and eosinophils ⩾300 cells·μL^{-1}); the AAER in this group was 0.83. Intermediate T2 inflammation (F_{ENO} ⩾20–<50 ppb and eosinophils <300 cells·μL^{-1} or F_{ENO} <20 ppb and eosinophils ⩾150–<300 cells·μL^{-1}) was the largest subgroup, encompassing 467 (42.5%) patients. Low T2 inflammation (F_{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_{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_{ENO} ⩾20 *versus* <20 ppb and blood eosinophils ⩾150 *versus* <150 cells·μL^{-1}. Patients who had both F_{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_{ENO} and eosinophil criteria) (figure 4a).

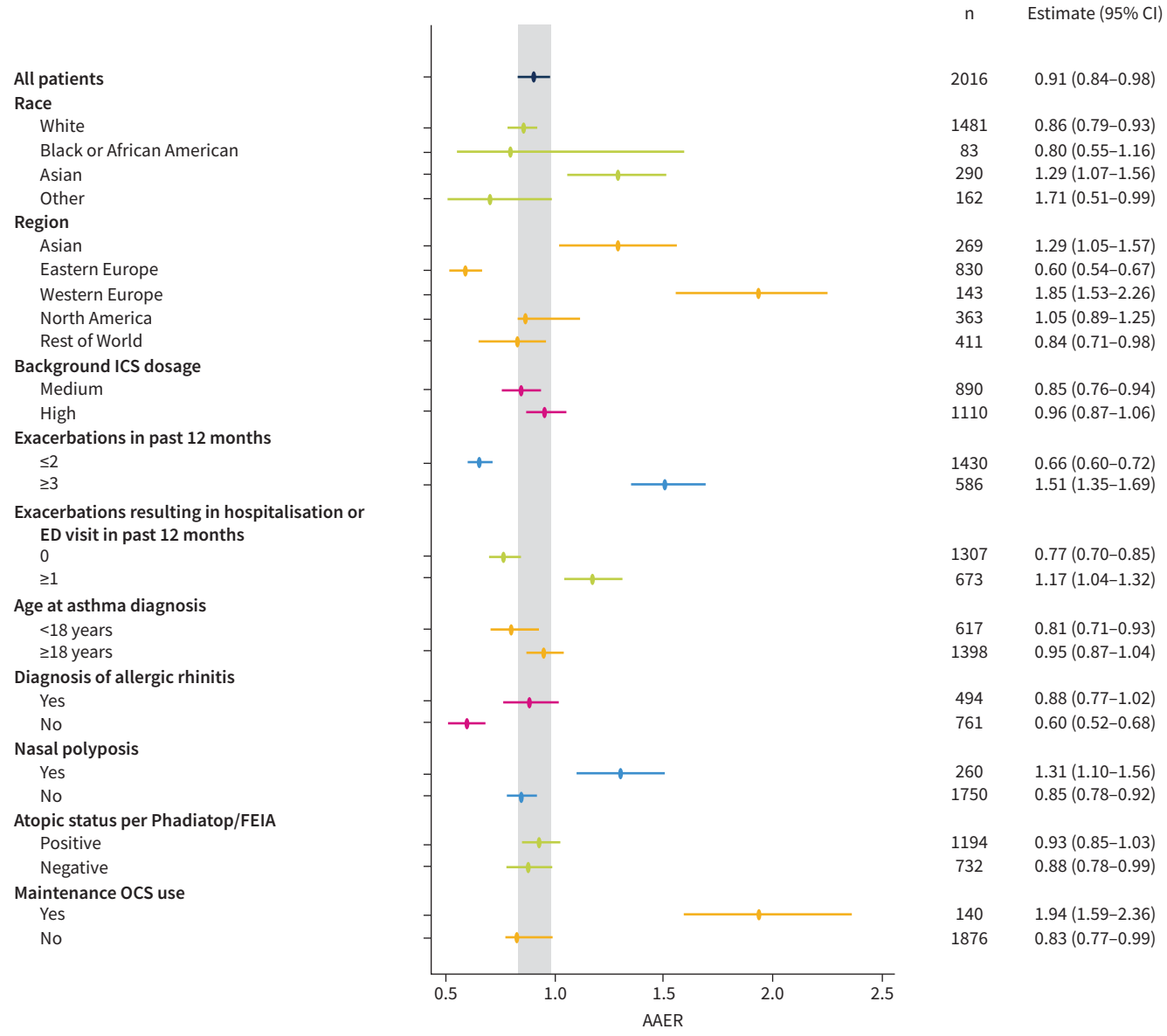

FIGURE 1 Annualised asthma exacerbation rates (AAERs) in the placebo group by demographics and baseline clinical characteristics. ICS: inhaled corticosteroid; ED: emergency department; FEIA: fluorescence enzyme immunoassay; OCS: oral corticosteroid. Shading indicates the 95% confidence interval range for the AAER overall for patients in the placebo group.

Patients who had F_{ENO} ⩾20 ppb or blood eosinophils ⩾150 cells·μL^{-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_{ENO} concentrations or eosinophil counts (*i.e.* inconsistently meeting thresholds during post-baseline measurements) behaved similarly to the <20 ppb group for F_{ENO} concentration and the ⩾150 cells·μL^{-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_{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).

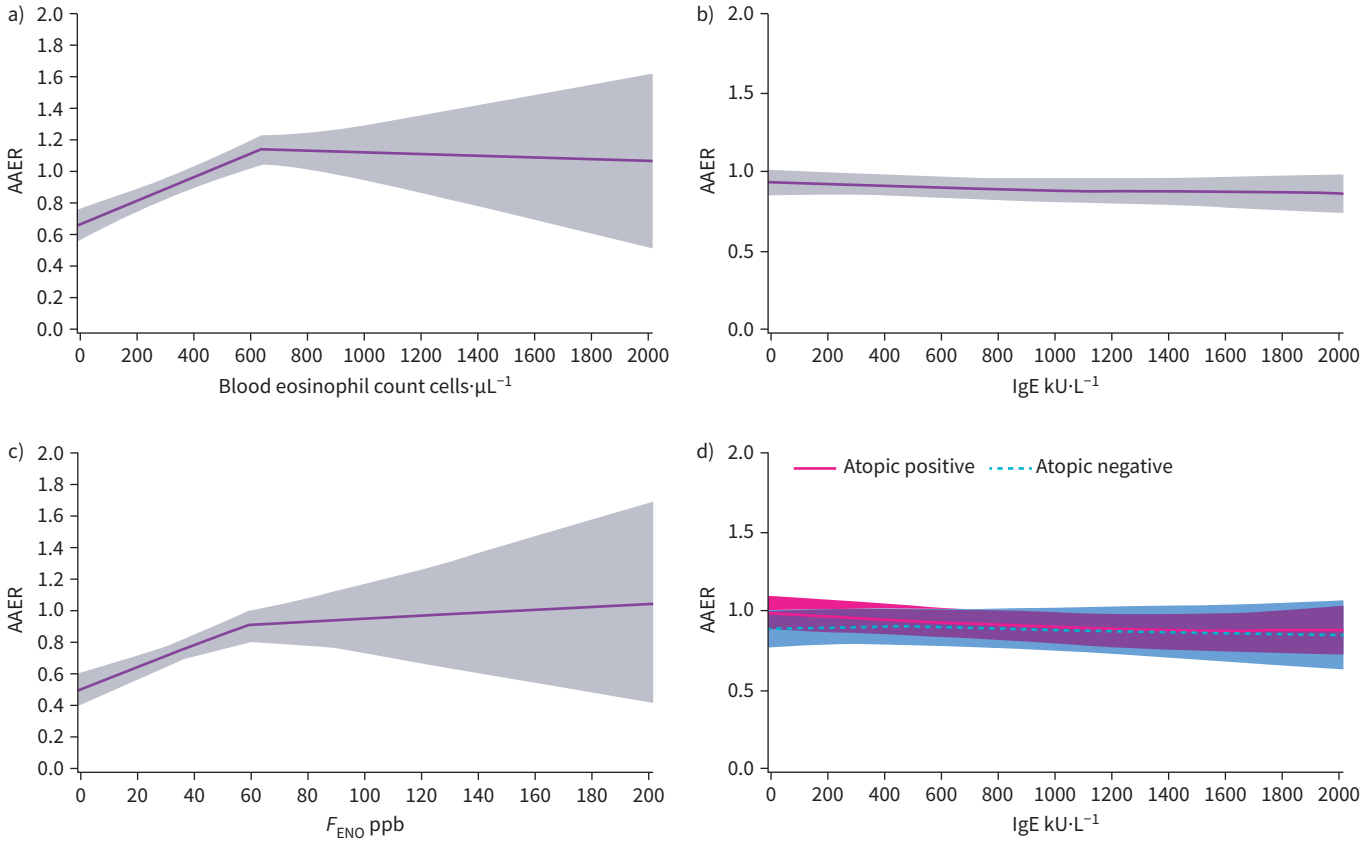

FIGURE 2 Modelling the effect of baseline biomarker concentrations on annualised asthma exacerbation rate (AAER) in the placebo group: a) blood eosinophil count, b) serum IgE concentration, c) exhaled nitric oxide fraction (F_{ENO}) and d) serum IgE concentration with atopic status. Lines indicate LOESS (locally estimated scatterplot smoothing) regression plots and shading indicates the 95% confidence interval. Baseline blood eosinophil counts and baseline serum IgE concentrations include data from all seven studies: benralizumab (SIROCCO, CALIMA and phase IIb), tralokinumab (STRATOS 1, STRATOS 2 and phase IIb) and tezepelumab (PATHWAY). Baseline F_{ENO} includes data from four studies: benralizumab (phase IIb), tralokinumab (STRATOS 1 and 2) and tezepelumab (PATHWAY).

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

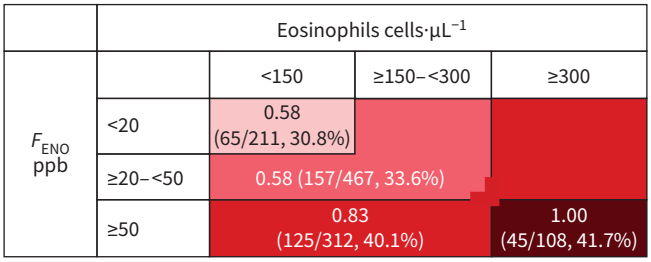

FIGURE 3 Annualised asthma exacerbation rates (AAERs) for blood eosinophil counts and exhaled nitric oxide fraction (F_{ENO}) concentration subgroups. Data are presented as AAER (number of patients who experienced an exacerbation/number of patients per subgroup (percentage of patients with an exacerbation)) in the pooled placebo group. These subgroups are based on noninvasive measures of inflammation. Data are derived from four studies in which F_{ENO} concentration was measured: benralizumab (phase IIb), tralokinumab (STRATOS 1 and 2) and tezepelumab (PATHWAY).

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 *versus* <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 IIb DREAM study, which enrolled patients with severe eosinophilic asthma (n=606), exacerbation risk was greatest for patients in the

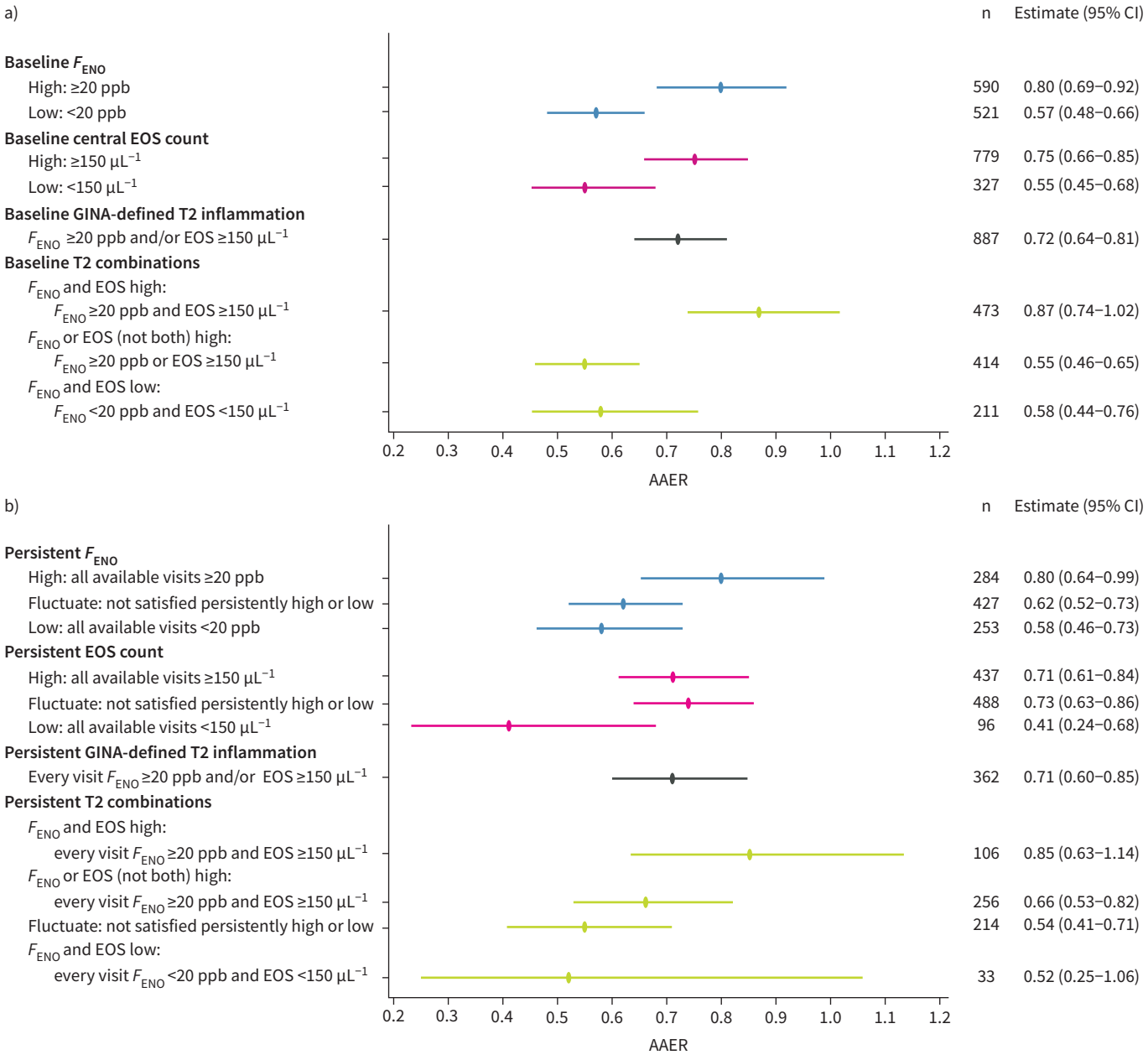

FIGURE 4 Annualised asthma exacerbation rates (AAERs) in the placebo group by Global Initiative for Asthma (GINA)-defined type 2 (T2) airway inflammation endotype criteria at baseline and over the course of the treatment period: eosinophil (EOS) count and exhaled nitric oxide fraction (F_{ENO}) concentration 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).

placebo group who had both high baseline peripheral blood eosinophil counts (⩾150 cells·μL^{-1}) and high F_{ENO} concentrations (⩾25 ppb) [30]. 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

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_{ENO} ⩾50 ppb and eosinophils ⩾300 cells·μL^{-1} than patients with F_{ENO} <25 ppb and eosinophils <150 cells·μL^{-1}. Taken together with previous findings, these results suggest that F_{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 ⩾150 cells·μL^{-1} and/or F_{ENO} ⩾20 ppb (among other factors) [2]. Our analysis detected a greater AAER for patients with elevations in both baseline eosinophil count and F_{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_{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_{ENO} and fluctuating eosinophil categories warrant further exploration. Notably, the greatest AAER occurred for patients with persistent elevations in both eosinophil count and F_{ENO} concentration, and the lowest AAER was observed for patients with persistently low eosinophil counts and F_{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_{ENO} concentrations. The wide ranges of baseline blood eosinophil counts and F_{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_{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_{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_{ENO}. Moreover, persistence in eosinophil and F_{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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References

1 Global Asthma Network. The Global Asthma Report 2018. Auckland, Global Asthma Network, 2018.
2 Global Initiative for Asthma (GINA). Global Strategy for Asthma Management and Prevention. 2019. Available from: http://ginasthma.org/.
3 Suruki RY, Daugherty JB, Boudiaf N, *et al.* The frequency of asthma exacerbations and healthcare utilisation in patients with asthma from the UK and USA. *BMC Pulm Med* 2017; 17: 74.
4 Jain N, Satish K, Abhyankar N, *et al.* Repeated exacerbation of asthma: an intrinsic phenotype of uncontrolled asthma. *Lung India* 2019; 36: 131–138.
5 Hekking PW, Wener RR, Amelink M, *et al.* The prevalence of severe refractory asthma. *J Allergy Clin Immunol* 2015; 135: 896–902.
6 Bourdin A, Bjermer L, Brightling C, *et al.* ERS/EAACI statement on severe exacerbations in asthma in adults: facts, priorities and key research questions. *Eur Respir J* 2019; 54: 1900900.
7 Castillo JR, Peters SP, Busse WW. Asthma exacerbations: pathogenesis, prevention, and treatment. *J Allergy Clin Immunol Pract* 2017; 5: 918–927.
8 Sundh J, Wireklint P, Hasselgren M, *et al.* Health-related quality of life in asthma patients – a comparison of two cohorts from 2005 and 2015. *Respir Med* 2017; 132: 154–160.
9 Kraft M, Brusselle G, FitzGerald JM, *et al.* Patient clinical characteristics and biomarkers associated with underlying exacerbation risk in asthma. *Am J Respir Crit Care Med* 2020; 201: A4523.
10 Bleecker ER, FitzGerald JM, Chanez P, *et al.* Efficacy and safety of benralizumab for patients with severe asthma uncontrolled with high-dosage inhaled corticosteroids and long-acting beta$_2$-agonists (SIROCCO): a randomised, multicentre, placebo-controlled phase 3 trial. *Lancet* 2016; 388: 2115–2127.
11 FitzGerald JM, Bleecker ER, Nair P, *et al.* Benralizumab, an anti-interleukin-5 receptor alpha monoclonal antibody, as add-on treatment for patients with severe, uncontrolled, eosinophilic asthma (CALIMA): a randomised, double-blind, placebo-controlled phase 3 trial. *Lancet* 2016; 388: 2128–2141.
12 Castro M, Wenzel SE, Bleecker ER, *et al.* Benralizumab, an anti-interleukin 5 receptor alpha monoclonal antibody, versus placebo for uncontrolled eosinophilic asthma: a phase 2b randomised dose-ranging study. *Lancet Respir Med* 2014; 2: 879–890.
13 Panettieri RA Jr, Sjöbring U, Péterffy A, *et al.* Tralokinumab for severe, uncontrolled asthma (STRATOS 1 and STRATOS 2): two randomised, double-blind, placebo-controlled, phase 3 clinical trials. *Lancet Respir Med* 2018; 6: 511–525.
14 Brightling CE, Chanez P, Leigh R, *et al.* Efficacy and safety of tralokinumab in patients with severe uncontrolled asthma: a randomised, double-blind, placebo-controlled, phase 2b trial. *Lancet Respir Med* 2015; 3: 692–701.
15 Corren J, Parnes JR, Wang L, *et al.* Tezepelumab in adults with uncontrolled asthma. *N Engl J Med* 2017; 377: 936–946.

16 Zeiger RS, Schatz M, Dalal AA, *et al.* Utilisation and costs of severe uncontrolled asthma in a managed-care setting. *J Allergy Clin Immunol Pract* 2016; 4: 120–129.

17 Maio S, Baldacci S, Bresciani M, *et al.* RItA: the Italian severe/uncontrolled asthma registry. *Allergy* 2018; 73: 683–695.

18 Quezada W, Kwak ES, Reibman J, *et al.* Predictors of asthma exacerbation among patients with poorly controlled asthma despite inhaled corticosteroid treatment. *Ann Allergy Asthma Immunol* 2016; 116: 112–117.

19 Papaioannou AI, Kostikas K, Bakakos P, *et al.* Predictors of future exacerbation risk in patients with asthma. *Postgrad Med* 2016; 128: 687–692.

20 Boer S, Sont JK, Loijmans RJB, *et al.* Development and validation of personalised prediction to estimate future risk of severe exacerbations and uncontrolled asthma in patients with asthma, using clinical parameters and early treatment response. *J Allergy Clin Immunol Pract* 2019; 7: 175–182.

21 Ueno H, Koya T, Hasegawa T, *et al.* A study of factors related to asthma exacerbation using a questionnaire survey in Niigata Prefecture, Japan. *Asian Pac J Allergy Immunol* 2020; 38: 108–113.

22 Peters MC, Mauger D, Ross KR, *et al.* Evidence for exacerbation-prone asthma and predictive biomarkers of exacerbation frequency. *Am J Respir Crit Care Med* 2020; 202: 973–982.

23 Jackson DJ, Humbert M, Hirsch I, *et al.* Ability of serum IgE concentration to predict exacerbation risk and benralizumab efficacy for patients with severe eosinophilic asthma. *Adv Ther* 2020; 37: 718–729.

24 FitzGerald JM, Bleecker ER, Menzies-Gow A, *et al.* Predictors of enhanced response with benralizumab for patients with severe asthma: pooled analysis of the SIROCCO and CALIMA studies. *Lancet Respir Med* 2018; 6: 51–64.

25 Price DB, Rigazio A, Campbell JD, *et al.* Blood eosinophil count and prospective annual asthma disease burden: a UK cohort study. *Lancet Respir Med* 2015; 3: 849–858.

26 Zeiger RS, Schatz M, Dalal AA, *et al.* Blood eosinophil count and outcomes in severe uncontrolled asthma: a prospective study. *J Allergy Clin Immunol Pract* 2017; 5: 144–153.

27 Hoch HE, Calatroni A, West JB, *et al.* Can we predict fall asthma exacerbations? Validation of the seasonal asthma exacerbation index. *J Allergy Clin Immunol* 2017; 140: 1130–1137.

28 Pretolani M, Soussan D, Poirier I, *et al.* Clinical and biological characteristics of the French COBRA cohort of adult subjects with asthma. *Eur Respir J* 2017; 50: 1700019.

29 Vedel-Krogh S, Fallgaard Nielsen S, Lange P, *et al.* Association of blood eosinophil and blood neutrophil counts with asthma exacerbations in the Copenhagen General Population Study. *Clin Chem* 2017; 63: 823–832.

30 Shrimanker R, Keene O, Hynes G, *et al.* Prognostic and predictive value of blood eosinophil count, fractional exhaled nitric oxide, and their combination in severe asthma: a post hoc analysis. *Am J Respir Crit Care Med* 2019; 200: 1308–1312.

31 Pavord ID, Holliday M, Reddel HK, *et al.* Predictive value of blood eosinophils and exhaled nitric oxide in adults with mild asthma: a prespecified subgroup analysis of an open-label, parallel-group, randomised controlled trial. *Lancet Respir Med* 2020; 8: 671–680.

32 Pascoe S, Locantore N, Dransfield MT, *et al.* Blood eosinophil counts, exacerbations, and response to the addition of inhaled fluticasone furoate to vilanterol in patients with chronic obstructive pulmonary disease: a secondary analysis of data from two parallel randomised controlled trials. *Lancet Respir Med* 2015; 3: 435–442.

33 Bafadhel M, Peterson S, De Blas MA, *et al.* Predictors of exacerbation risk and response to budesonide in patients with chronic obstructive pulmonary disease: a post-hoc analysis of three randomised trials. *Lancet Respir Med* 2018; 6: 117–126.

34 de Groot JC, Amelink M, de Nijs SB, *et al.* Risk factors for frequent severe exacerbations in late-onset eosinophilic asthma. *Am J Respir Crit Care Med* 2015; 192: 899–902.

35 Mansur AH, Srivastava S, Sahal A. Disconnect of type 2 biomarkers in severe asthma; dominated by FeNO as a predictor of exacerbations and periostin as predictor of reduced lung function. *Respir Med* 2018; 143: 31–38.

36 Semprini R, Williams M, Semprini A, *et al.* Type 2 biomarkers and prediction of future exacerbations and lung function decline in adult asthma. *J Allergy Clin Immunol Pract* 2018; 6: 1982–1988.

37 Busse W, Pavord I, Wenzel S, *et al.* Baseline FeNO as a prognostic biomarker for subsequent severe asthma exacerbations in patients with uncontrolled, moderate-to-severe asthma receiving placebo in the LIBERTY ASTHMA QUEST study. *J Allergy Clin Immunol* 2020; 145: AB21.

38 Luc F, Prieur E, Whitmore GA, *et al.* Placebo effects in clinical trials evaluating patients with uncontrolled persistent asthma. *Ann Am Thorac Soc* 2019; 16: 1124–1130.