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Biomarkers of Type 2 Airway Inflammation as Predictors of Loss of Asthma Control During Step-Down Therapy for Well-Controlled Disease: The Long-Acting Beta-Agonist Step-Down Study (LASST)

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Abstract

BACKGROUND: Biomarkers that can predict loss of asthma control among patients being considered for step-down therapy in well-controlled disease are lacking.

OBJECTIVE: To evaluate whether baseline biomarkers of type 2 airway inflammation and/or serial measurement of fractional exhaled nitric oxide (FENO) predict loss of asthma control as therapy is stepped down.

METHODS: In subanalyses of a multicenter randomized, double-blind, parallel 3-arm trial comparing strategies for step-down therapy in well-controlled asthma (Long-Acting Beta-Agonist

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Step-Down Study), we assessed whether baseline atopy as determined by serum aeroallergen allergy screening test (Phadiatop), baseline serum eosinophil peroxidase, or baseline or serial FENO measurements during follow-up predicted the time to loss of asthma control among participants. Loss of asthma control was defined in the study protocol. We analyzed these associations in adjusted models including all participants, after testing for interactions with assignment to each of the 3 treatment groups (continuation of stable dose of combination inhaled corticosteroid-long-acting beta-agonist, step-down of inhaled corticosteroid, or discontinuation of long-acting bronchodilator).

RESULTS: Four hundred forty-seven of the 553 Long-Acting Beta-Agonist Step-Down Study participants who were randomized to 1 of 3 treatment arms and had at least 1 biomarker measurement were included in this analysis. At baseline, higher levels of FENO were significantly associated with greater levels of multiallergen IgE levels ($P < .001$), but not with serum eosinophil peroxidase ($P = .742$). Among all participants as a group, elevations in baseline biomarkers were not predictive of a higher risk of treatment failure. In addition, FENO levels measured serially at 6-week intervals demonstrated that compared with participants with low levels (<25 parts per billion), those with intermediate ($25\text{--}50$ parts per billion) and high (>50 parts per billion) levels did not have significantly increased likelihood of subsequent treatment failure (hazard ratios, 1.03 [95% CI, 0.59–1.78] and 1.29 [95% CI, 0.65–2.54], respectively). There were no significant interactions of treatment group and baseline biomarkers.

CONCLUSIONS: In patients with well-controlled asthma, neither baseline levels of type 2 airway inflammatory biomarkers nor serial measures of FENO are strong predictors of treatment failure.

Keywords

Asthma; Biomarkers; Fractional exhaled nitric oxide; Eosinophil peroxidase; IgE; Step-down therapy

INTRODUCTION

Biomarkers of type 2 airway inflammation and allergic responses may be useful in characterizing patients with asthma, monitoring disease activity, and assessing response to treatment. Well-studied biomarkers in patients with asthma include fractional exhaled nitric oxide (FENO), serum IgE, and peripheral blood absolute eosinophil counts (AECs). Of these, FENO is particularly appealing as a clinical decision-making tool given the point of care availability, ease of use, and ability to obtain repeated measurements in the outpatient setting. FENO levels greater than 50 parts per billion (ppb) have been associated with responsiveness to inhaled corticosteroids (ICSs), more frequent exacerbations, and nonadherence with ICS treatment; however, its use as a tool to guide treatment has had variable results.^{1–5} In addition, determination of atopy by an allergen screening test for multiple aeroallergens has been found to correlate with the probability of wheezing and response to ICSs among children with asthma.⁶ Although AEC is the most well-validated blood measure of eosinophilic asthma, other eosinophil-derived proteins detectable in blood include serum eosinophil peroxidase (EPX)—an enzyme necessary for the development of eosinophilic inflammation. EPX is an eosinophil granule protein that is released from

activated eosinophils via an IgE-mediated mechanism and can be quantified in the blood.⁷ Serum EPX has been found to be positively correlated with peripheral and sputum eosinophilia, and positively associated with more severe asthma.^{7–11}

Guidelines recommend stepping down treatment when asthma is well controlled for at least 3 months. In a recent double-blind randomized controlled comparative effectiveness trial (Long-acting Beta-Agonist Step-Down Study [LASST]) investigating the optimal step-down therapy strategy in patients with asthma well controlled on combination medium-dose ICS and long-acting beta-agonists (ICS/LABAs), participants were randomized to 3 treatment arms: those who maintained the ICS/LABA dose, those who reduced the ICS dose and maintained LABA, and those who discontinued LABA and maintained ICS.¹² We observed a similarly high treatment failure rate (~30% over 48 weeks) across treatment arms. However, it is not clear whether available biomarkers can help to distinguish which patients are most susceptible to treatment failure among patients with well-controlled disease, particularly for those in whom the ICS dose is reduced.

Therefore, in the present study, we analyzed whether any of 3 biomarkers—FENO, aeroallergen multiallergen screening test (Phadiatop), or serum EPX—would predict treatment failure among patients with well-controlled asthma enrolled in a comparative trial of step-down strategies. Specifically, we hypothesized that (1) baseline elevated biomarker levels would predict treatment failure during ICS step down and (2) elevated serial FENO levels among participants randomized to ICS reduction would predict treatment failure.

METHODS

Study design

The LASST study was a multicenter, randomized, double-blind, parallel 3-arm, 56-week trial designed to compare strategies for managing patients with well-controlled asthma while receiving medium-dose ICS/LABA ([ClinicalTrials.gov NCT01437995](https://clinicaltrials.gov/ct2/show/study/NCT01437995)). Details of the study design are described elsewhere.¹² Briefly, participants with a history of at least 4 weeks of stable well-controlled asthma on medium-dose ICS/LABA at enrollment underwent an 8-week open-label run-in on medium-dose ICS/LABA. Participants aged 12 years or older with a total of 3 months of stable well-controlled asthma (as defined by a score of >19 on the Asthma Control Test [ACT])^{13,14} with no unscheduled health care encounters, no change in asthma medication, a prebronchodilator FEV₁ value of greater than or equal to 70% predicted, the use of fewer than 16 puffs of rescue beta-agonist per week, and no more than 10 puffs of rescue beta-agonist on 2 consecutive days were randomized (1:1:1) to receive blinded treatment with (1) continuation of fluticasone/salmeterol 250 µg/50 µg; (2) reduction in the ICS dose and maintenance of LABA with fluticasone-salmeterol combination 100 µg/50 µg; or (3) discontinuation of LABA and maintenance of the ICS dose with fluticasone 250 µg. Participants returned to the study center for reassessment every 6 weeks. A total of 553 patients were enrolled in the trial, with 459 patients successfully completing the 8-week run-in period and proceeding to randomization to 1 of the above 3 step-down strategies. Data from those 459 individuals were eligible for inclusion in this analysis.

Study procedures

Study questionnaires.—Participants completed the ACT,^{13,14} Asthma Symptom Utility Index,¹⁵ and Marks Asthma Quality of Life Questionnaire¹⁶ for adults 18 years and older, and Children's Health Survey for asthma—Child version¹⁷ for those 12 to 17 years old at randomization and during visits every 6 weeks postrandomization for the duration of the study. Participants completed daily asthma diaries to track morning peak flow, asthma medication use, and urgent care for asthma.

Spirometry.—At the time of randomization and during clinic visits every 6 weeks postrandomization, spirometry was performed (Koko Spirometry software, version 4.15, KoKo, LLC, Longmont, Colo) and following American Thoracic Society recommendations.¹⁸

Biomarker measurements.—FENO was measured using a Food and Drug Administration—approved portable device, the NIOX MINO, in accordance with American Thoracic Society guidelines.¹⁹ The aeroallergen multiallergen IgE assay (Phadiatop, Pharmacia& Upjohn, Uppsala, Sweden) provided a single value based on total responses to a panel of specific inhalant aeroallergens and expressed as Phadia Arbitrary Units/L, which was used to quantitatively assess the degree of allergic sensitization.^{20,21} Higher levels of Phadiatop values reflect higher levels of specific IgE antibodies. A level below the limit of quantification indicates that IgE antibodies to allergens in the panel are undetectable and the subject is nonatopic. Complete blood cell count with differential was not collected as part of this study initiated in 2011 and thus AECs were not available for analysis. In lieu of AECs, we retrospectively assessed serum EPX as a surrogate for blood and airway eosinophilia using the sandwich EPX ELISA protocol.^{9,22,23} Baseline values for FENO, aeroallergen multiallergen assay, and serum EPX were measured at the time of randomization. FENO was also measured every 6 weeks during the postrandomization 48-week study period.

Outcome measures.—For the current analysis, the main outcome measure was time to treatment failure, defined in the trial as time from randomization to the occurrence of any 1 of the following events: (1) hospitalization or an urgent medical visit for asthma initiated by the patient or physician; (2) use of systemic corticosteroids for asthma or increase in controller therapy as reported in diaries and verified by the study physician or asthma care provider; (3) decrease in prebronchodilator FEV₁ to more than 20% below the baseline value measured at randomization; (4) a decrease in the morning peak expiratory flow rate to more than 35% below the baseline value (the mean over the final 2 weeks of the stable treatment phase) on 2 consecutive days; (5) use of 10 puffs or more per day of rescue beta-agonist for 2 consecutive days (excluding premedication for exercise); (6) patient refusal to continue due to medication intolerance or failure of the treatment to provide symptomatic relief; or (7) judgment by a physician that the patient should stop treatment for reasons of safety.

Data analysis.—The baseline distributions for 3 biomarkers (FENO, multiallergen IgE assay, and serum EPX) were summarized using medians and quartiles, as well as by the number and percent within each level of clinically relevant categorizations. Based on American Thoracic Society recommendations,²⁴ FENO was divided into 3 categories related

to the likelihood of type 2 airway inflammation and responsiveness to corticosteroids: less than 25 ppb (less likely), 25 to 50 ppb (intermediate likelihood), and more than 50 ppb (more likely). Multiallergen IgE measurements were classified as less than 0.35 kUA/L (normal, nonallergic) versus greater than or equal to 0.35 kUA/L (elevated, allergic). Because there is no well-defined threshold level for EPX, we categorized levels above and below an *a priori*—designated threshold of 400 ng/mL of the cohort to differentiate between individuals with low (<400 ng/mL) and elevated ($\geq$ 400 ng/mL) serum EPX levels.²² Box plots and the Kruskal-Wallis test were used to assess the association between the 3 biomarkers. Demographic characteristics were compared across categories of FENO levels using χ^2 tests (for categorical variables) and Kruskal-Wallis tests (for continuous variables).

Associations between the baseline values for each of the biomarkers and treatment failure were analyzed separately using Cox proportional hazards models. The extended Cox proportional hazards model was used to assess the association between treatment failure and FENO variables (<25, 25–50, >50 ppb) measured every 6 weeks. We created unadjusted models and models adjusting for hospitalization or systemic corticosteroid prescription in the year before the trial, sex (female vs male), and age category (adult [$\geq$ 18 years] vs child [$<$ 18 years]). We performed sensitivity analyses adding treatment group as a covariate to our adjusted models. Race, body mass index, and percent predicted FEV₁ were also assessed for inclusion in the adjusted models but were not statistically significant and were removed from the model for parsimony. To specifically test the hypothesis that baseline biomarkers of type 2 airway inflammation or time-variant FENO would predict participants vulnerable to treatment failure, after reduction of inhaled steroid compared with the other groups, we evaluated the interaction of treatment group by biomarker category using type III tests. Analyses were performed using SAS (SAS, Version 9.4, SAS, Inc, Cary, NC). In these exploratory analyses, a *P* value of less than .05 was considered statistically significant.

RESULTS

Participant characteristics at baseline by FENO category

A total of 447 of the 459 eligible participants (97%) enrolled in the LASST and randomized to 1 of 3 strategies had data available at randomization, including biomarkers, FENO (*n* = 435), multiallergen IgE (*n* = 439), and serum EPX (*n* = 431), and were included in the analysis. One hundred twenty-two participants (27%) met the definition of treatment failure, the primary outcome of the study, with a median time to failure (95% CI) of 23.1 (19.1–28.0) weeks. Ninety-nine randomized participants (22%) were younger than 18 years, 281 (63%) were female, and 202 participants (45%) were black or Hispanic (Table I). High baseline ACT and Asthma Symptom Utility Index scores and low Mark's Asthma Quality of Life Questionnaire scores reflected a cohort of participants with well-controlled asthma. A total of 1658 measurements were taken on 379 participants who had FENO at least twice between randomization and failure date, with an intraperson correlation of 0.73 (95% CI, 0.70–0.77). Participants with a baseline FENO level of less than 50 ppb were more likely to be younger, male, or black, compared with participants with low (<25 ppb) or intermediate (25–50 ppb) FENO levels. Participants with a FENO level of less than 25 ppb were associated with a higher body mass index (*P* = .009). At the time of randomization, there were no

differences in FENO category according to smoking status, age of onset of asthma, likelihood of acute care for asthma, baseline asthma treatment, history of oral corticosteroid use or beta-agonist use, comorbid allergic conditions, Asthma Quality of Life Questionnaire scores, or Asthma Symptom Utility Index scores.

Distribution and relationships among biomarkers

The distribution of results for each evaluated biomarker at the time of randomization is illustrated in Figure 1. In this study cohort of participants with well-controlled asthma with ACT score more than 19 and 12 weeks of stable asthma on medium-dose fluticasone-salmeterol combination therapy, most (73%) had FENO values of less than 25 ppb, 20% had FENO values between 25 and 50 ppb, and a small proportion (7%) had a FENO value of more than 50 ppb at randomization. Most participants (87%) had evidence of type 2 inflammation by at least 1 of the measured biomarkers, with 79% with positive multiallergen IgE levels (79%) and 36% with a serum EPX level greater than or equal to 400 ng/mL. Although no associations were found between serum EPX and multiallergen IgE levels nor EPX and FENO, increased multiallergen IgE levels were significantly associated with higher FENO levels ($P < .001$) (Figure 2).

Baseline biomarker levels and time to trial definition of failure

The lack of associations between each baseline type 2 biomarker and the primary study end point of time to treatment failure is presented in Table II. Results were similar in both unadjusted and multivariate analyses (Table II). In sensitivity analyses, including treatment group as a covariate in the models did not meaningfully change our results (data not shown). Furthermore, type 2 status—as defined by the presence of elevated levels of any 1 of the 3 biomarkers at randomization—was not associated with a significantly higher rate of treatment failure. Although baseline FENO more than 50 ppb had a point estimate of elevated risk, it failed to reach statistical significance, along with other high T2 biomarkers (Table II). We tested for interactions of treatment groups with baseline biomarkers and found no evidence for interaction with any of the assays: FENO ($P = .668$), multiallergen IgE ($P = .413$), or serum EPX ($P = .757$). Furthermore, there were no significant differential effects when examined by treatment group.

Changes in FENO postrandomization and risk of treatment failure

To be included in the analysis using multiple FENO measurements per participant, a participant had to have a FENO value at randomization and at least 1 follow-up visit ($N = 446$). FENO was evaluated every 6 weeks at routine study visits postrandomization over the 48-week period. Treatment failure events were analyzed for associations with FENO levels measured at the visit immediately before the event to assess the ability of the previous visit to predict loss of control. Follow-up was censored after a treatment failure. Of the 2514 nonmissing FENO levels used in these analyses, most (1738) had FENO measurements of less than 25 ppb. There were 541 visits with FENO in the intermediate range and 235 visits with FENO more than 50 ppb. There was a large amount of informative, nonmonotone missingness in FENO data throughout the trial, so a missing category was added to this analysis. Missing a FENO measurement was associated with a lower risk of treatment failure for the next 6 weeks (Table III).

Table III illustrates the association between FENO measured at the previous visit and the likelihood of treatment failure for the cohort as a whole. The point estimates for risk of treatment failure increased with greater FENO levels measured at the visit before exacerbation, but the association did not reach statistical significance (Table III).

DISCUSSION

This retrospective subgroup analysis of the LASST cohort of patients with well-controlled asthma examined the utility of serum biomarkers to predict disease stability among a multicenter cohort of adult patients with well-controlled asthma undergoing step-down therapy. In the parent study, all 3 treatment strategies, including stable-ICS/LABA, reduced ICS/LABA, and LABA-step-off arms, had significant rates of treatment failure (26%, 28%, and 31%, respectively), with similar outcomes across all groups.¹² However, despite these rates of treatment failure, we found that baseline levels of biomarkers of type 2 airway inflammation, including multiallergen IgE and EPX assays, did not significantly predict time to treatment failure over the following 48 weeks. Furthermore, FENO levels assessed within the previous 6 weeks were also not clearly predictive of having a subsequent treatment failure. These results raise the question of the utility of using these specific biomarkers for monitoring changes in asthma control specifically among clinically well-controlled patients.

Biomarkers may have predictive, prognostic, and in some situations, pharmacodynamic properties in the management of asthma. Thus far, only a few biomarkers have been identified and used in clinical management.²⁵ Available biomarkers reflect only the underlying T2 airway inflammation. Although previous studies have largely focused on the role of these biomarkers to guide clinical decisions regarding escalation of treatments in uncontrolled asthma, their utility in guiding *de-escalation* of asthma therapy has not been established. Given the critical importance of maintaining stability among patients with asthma, including those undergoing step-down therapy for well-controlled disease, further prospective studies are warranted to identify alternate biomarker profiles to better predict future treatment failure and improve real-time clinical decision making in this well-controlled patient population.

Our data indicated a nonsignificant point estimate of increased risk of treatment failure for FENO more than 50 ppb both at baseline and longitudinally. Although larger studies are needed to confirm these findings, we hypothesize that a FENO value greater than 50 ppb may be reflective of airway inflammation severe enough to predict treatment failure, despite a clinical assessment of good control. A clinical assessment of “well-controlled” may be insensitive to underlying immunologic derangement and biomarkers could add information about patient status. If these associations are confirmed in future studies, real-time FENO measurements may have adjunctive value to improve the characterization of disease status for individual patients.

Thus far, the use of FENO as a surrogate for eosinophilic airway inflammation in the management of asthma has been somewhat controversial.^{1,4,5,26–29} Recently published data among patients with severe asthma in the placebo arm of a phase 2b study of mepolizumab in patients with severe eosinophilic asthma (DREAM [Dose Ranging Efficacy And safety

with Mepolizumab in severe asthma study]) demonstrated that those patients with severe asthma who had elevated FENO at baseline (in combination with high blood eosinophil counts) had a 2-fold rate of severe exacerbations requiring oral corticosteroids compared with those patients on placebo who had low or discordant biomarker levels.³⁰ Other data suggest that FENO reflects IL-4— and IL-13—mediated airway inflammation, rather than eosinophilic airway inflammation *per se* among patients with severe asthma.³¹ These results highlight the potential utility of biomarker profiling in predicting patients who are at risk of loss of asthma control within a population with severe asthma. However, little is known about the predictive value of biomarkers in a well-controlled population. Patients with well-controlled asthma may be a pathobiologically distinct phenotype, different from patients with severe asthma, such that serial FENO assessments are not reflective of the risk of disease relapse. Future studies are needed to evaluate the mechanistic underpinnings of FENO as a predictive tool for respiratory morbidity across the spectrum of asthma phenotypes.

There are several unique strengths of our study. To begin with, we designed our study to address a highly clinically relevant—but understudied—question of the ability of peripheral biomarkers to predict treatment failure in a treatment step-down study among patients with well-controlled asthma. Most studies to understand the utility of biomarkers have been done in uncontrolled or refractory disease and evaluated biomarkers as potential guides for escalation of asthma therapy. Given guideline mandates to consider stepping down therapy among those patients who have demonstrated clinical stability, our results inform the potential role of biomarker data to identify patients who will be harmed by following those guidelines. Second, the LASST provided data over a follow-up period of 48 weeks, enabling capture of time-varying effects of a biomarker upon a robustly defined asthma outcome. Furthermore, in our serial analysis, FENO testing was performed at high frequency, every 6 weeks, and therefore able to detect emerging clinical instability in patients potentially undergoing step-down therapy. In sum, our study helps to fill the wide evidence gap required to practically guide the clinical management of well-controlled patients with asthma.

There were also limitations to our work. Although we chose to focus on 3 individual biomarkers commonly linked to asthma control, other biomarkers not tested here may have stronger predictive capability. For example, peripheral blood eosinophilia has been associated with high asthma exacerbation rates.^{32–34} However, in a previous study of tapering patients well controlled on low-dose ICS alone, we found that eosinophil biomarkers (blood eosinophil counts and serum eotaxin) were not predictive of subsequent exacerbation.³ Similarly in this study, using EPX in lieu of AECs, was not predictive of treatment failure. Furthermore, EPX assays were not repeated in our study and the predictive value of serial EPX testing remains to be explored. Future efforts to identify optimal, potentially composite, biomarkers that can most reliably predict the risk of future asthma events in well-controlled patients are warranted.

CONCLUSIONS

Baseline type 2 inflammatory biomarkers or serial measurements of FENO are not clearly an informative approach to predict subsequent treatment failure in clinically well-controlled

patients with asthma. Large, prospective studies are needed to identify optimal biomarkers that can help to guide asthma management among those with well-controlled disease.

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Abbreviations used

ACT	Asthma Control Test
AEC	Absolute eosinophil count
EPX	Eosinophil peroxidase
FENO	Fractional exhaled nitric oxide
ICS	Inhaled corticosteroid
LABA	Long-acting beta-agonist
LASST	Long-Acting Beta-Agonist Step-Down Study
ppb	Parts per billion

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What is already known about this topic?

The use of biomarkers of type 2 inflammation has been suggested to guide escalation of asthma therapy in uncontrolled disease. However, whether these biomarkers can also predict loss of control in those patients being stepped down for well-controlled asthma remains unclear.

What does this article add to our knowledge?

Neither baseline type 2 inflammatory biomarkers such as serum aeroallergen multiallergy screening tests and eosinophil peroxidase assays nor baseline or serial measures of fractional exhaled nitric oxide were clear predictors of treatment failure in those with well-controlled asthma, regardless of maintenance or reduction of controller therapy.

How does this study impact current management guidelines:

Type 2 inflammatory biomarkers may not be a robust predictor of loss of asthma control among well-controlled patients. Our results highlight the need to identify alternative means of predicting treatment failure in well-controlled patients undergoing step-down of their treatment.

FIGURE 1.
Distribution of each serum biomarker at trial randomization.

FIGURE 2.

Boxplots of inflammatory markers by cutoff values. *P* values are from the Kruskal-Wallis test.

TABLE I.

Baseline characteristics at enrollment by baseline FENO category

Characteristic	Total (N = 447)	FENO < 25 ppb (N = 317)	Feno 25–50 ppb (N = 87)	FENO > 50 ppb (N = 31)
Baseline FENO (ppb), median (Q1, Q3)	17.5 (12.5, 26.0)	14.5 (11.0, 18.5)	32.5 (28.0, 39.0)	67.5 (62.5, 87.5)
Age (y)				
Median (Q1, Q3)	35 (20, 50)	36 (21, 51)	38 (23, 51)	18 (14, 39)
Child, n (%)	99 (22)	68 (21)	15 (17)	14 (45)
Sex: female, n (%)	281 (63)	217 (68)	45 (52)	11 (35)
Former smoker, n (%)	58 (13)	47 (15)	9 (10)	2 (6)
Secondhand smoke home/work, n (%)	54 (12)	40 (13)	12 (14)	2 (6)
Obese, n (%)	187 (42)	140 (44)	38 (44)	6 (19)
Body mass index, median (Q1, Q3)	28 (24, 34)	29 (24, 35)	28 (24, 33)	24 (21, 30)
Doctor/ER/hospital for asthma in past year, n (%)	132 (30)	88 (28)	34 (39)	7 (23)
Race or ethnic group, n (%)				
White	230 (51)	177 (56)	38 (44)	8 (26)
Black	151 (34)	101 (32)	32 (37)	16 (52)
Hispanic	51 (11)	30 (9)	14 (16)	5 (16)
Other	15 (3)	9 (3)	3 (3)	2 (6)
Asthma characteristics				
Age of asthma onset, median (Q1, Q3)	7 (2, 18)	8 (2, 20)	7 (3, 18)	4 (1, 11)
Hospitalized for asthma in past year, n (%)	132 (30)	88 (28)	34 (39)	7 (23)
Oral steroids for asthma in past year, n (%)	119 (27)	78 (25)	28 (32)	7 (23)
Daily short-acting beta-agonist use, n (%)	28 (6)	19 (6)	6 (7)	2 (6)
Daily antileukotriene use, n (%)	90 (20)	62 (20)	19 (22)	5 (16)
Self-reported atopic conditions, n (%)				
Rhinitis	243 (54)	171 (54)	47 (54)	20 (65)
Eczema	88 (20)	66 (21)	13 (15)	6 (19)
Food allergies	210 (47)	151 (48)	43 (49)	9 (29)
Allergies worsen asthma	353 (79)	251 (79)	66 (76)	27 (87)
Asthma questionnaires scores				
ACT score ($\bar{x}$) (range, 5–25)	22 (21–24)	22 (21–24)	22 (20–23)	21 (20–23)

Characteristic	Total (N = 447)	FENO < 25 ppb (N = 317)	FENO 25–50 ppb (N = 87)	FENO > 50 ppb (N = 31)
Asthma Symptom Utility Index ($\uparrow$) (range, 0–1)	0.94 (0.88–1.00)	0.94 (0.88–1.00)	0.94 (0.85–1.00)	0.95 (0.92–1.00)
Mark' s Asthma Quality of Life Questionnaire ($\downarrow$) (range, 0–80)	6 (3–13)	6 (3–13)	7 (4–12)	9 (3–15)
Spirometry, median (Q1, Q3)				
Prebronchodilator FEV ₁ (L)	2.7 (2.3, 3.3)	2.7 (2.3, 3.3)	2.7 (2.3, 3.3)	3.1 (2.1, 3.7)
Prebronchodilator FVC (L)	3.6 (3.0, 4.4)	3.5 (3.0, 4.4)	3.8 (3.1, 4.4)	3.9 (3.1, 4.9)
Postbronchodilator FVC (L)	3.6 (3.1, 4.4)	3.5 (3.0, 4.4)	3.8 (3.2, 4.5)	4.0 (3.2, 5.0)
Postbronchodilator FEV ₁ (L)	2.9 (2.4, 3.5)	2.9 (2.3, 3.4)	2.9 (2.4, 3.5)	3.3 (2.4, 3.7)
FEV ₁ /FVC ratio	0.8 (0.7, 0.8)	0.8 (0.7, 0.8)	0.7 (0.7, 0.8)	0.7 (0.7, 0.8)
Percent predicted spirometry, median (Q1, Q3)				
Prebronchodilator FEV ₁ (% predicted)	88 (80, 99)	88 (80, 99)	87 (79, 96)	89 (81, 99)
Prebronchodilator FVC (% predicted)	98 (89, 106)	97 (88, 106)	98 (89, 108)	103 (91, 117)
Prebronchodilator peak flow (% predicted)	94 (83, 106)	94 (83, 106)	94 (85, 106)	92 (70, 105)
Biomarkers				
Average serum EPX (ng/mL), median (Q1, Q3)	349.65 (272.79, 442.08)	349.90 (272.79, 436.83)	343.42 (255.72, 442.08)	353.86 (306.96, 473.05)
<400 ng/mL, n (%)	108 (25)	77 (25)	23 (28)	6 (20)
400 ng/mL, n (%)	323 (75)	229 (75)	60 (72)	24 (80)
Phadiatop (k UA/L), median (Q1, Q3)	8.08 (1.00, 31.50)	5.21 (0.32, 22.75)	15.80 (3.15, 45.30)	31.20 (2.62, 50.60)
<0.35 k UA/L, n (%)	90 (21)	80 (26)	6 (7)	2 (7)
0.35–34.9 k UA/L, n (%)	247 (56)	173 (55)	52 (61)	15 (50)
35 k UA/L, n (%)	102 (23)	59 (19)	27 (32)	13 (43)
Treatment, n (%)				
Stable therapy	150 (34)	104 (33)	32 (37)	9 (29)
Reduce ICS	146 (33)	105 (33)	25 (29)	11 (35)
LABA step-off	151 (34)	108 (34)	30 (34)	11 (35)

ER, Emergency room; FVC, forced vital capacity; Q1, first quartile; Q3, third quartile.

$\uparrow$ High scores indicate better health.

$\downarrow$ Low scores indicate better health.

TABLE II.

Association between baseline inflammatory markers and time to trial definition of failure

Biomarker	Unadjusted			Adjusted*		
	No. of events/no. at risk	HR (95% CI)	P	HR (95% CI)	P	P
FENO (reference, <25 ppb)	87/317	1.00		1.00		
25–50 ppb	20/87	0.86 (0.53–1.4)	.538	0.86 (0.52–1.42)		.554
>50 ppb	12/31	1.56 (0.85–2.85)	.150	1.7 (0.9–3.2)		.100
Multiallergen IgE (reference, <0.35 k UA/L)	23/90	1.00		1.00		
0.35 k UA/L	96/349	1.12 (0.71–1.76)	.634	1.1 (0.69–1.75)		.684
Serum EPX (reference, <400 ng/mL)	75/274	1.00		1.00		
400 ng/mL	43/157	0.97 (0.67–1.41)	.882	0.99 (0.68–1.45)		.973
High T2 [†] (reference, no biomarkers elevated)	13/54	1.00		1.00		
Any biomarker elevated	102/365	1.23 (0.69–2.19)	.485	1.22 (0.68–2.18)		.506

HR, Hazard ratio; kUA/L, kilo units of antibody/liter.

* Adjusted models include whether or not a patient had an emergency doctor visit/systemic corticosteroid prescription for asthma in the year before the study, sex (female vs male), age category (adult vs child (<18 y)).

[†] Any of the biomarkers elevated (FENO > 50, multiallergen IgE 0.35, EPX 400 ng/mL).

TABLE III.

Association between FENO at previous visit and time to trial definition of failure

Biomarker and characteristic	Failures/intervals	Unadjusted			Adjusted*		
		HR	95% CI	P	HR	95% CI	P
FENO at previous visit (reference, FENO <25 ppb)	75/1738	Reference			Reference		
[25, 50] ppb	24/541	1.02	(0.64–1.62)	.927	1.03	(0.65–1.64)	.897
>50 ppb	14/235	1.38	(0.78–2.45)	.268	1.43	(0.79–2.57)	.235
Missing	8/318	0.40	(0.18–0.88)	.024	0.41	(0.18–0.91)	.029

* Adjusted models include whether or not a patient had a hospitalization/systemic corticosteroid prescription for asthma in the year before the study, sex (female vs male), and age category (adult vs child [<18 y]).