

Asthma and fixed airflow obstruction: Long-term trajectories suggest distinct endotypes

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Abstract

Background: Long-term trajectories of asthma with fixed airflow obstruction (FAO) may reveal links to inflammatory endotypes.

Objective: We investigated whether measures of asthma control and airway inflammation and remodelling differed by long-term FAO status in moderate-to-severe asthma.

Methods: Adults enrolled in the Difficult Asthma Study assessed initially using serial Asthma Control Questionnaire (ACQ), exacerbation history, spirometry and sputum cytology over 12 months, as well as endoscopic bronchial biopsy with airway smooth muscle (ASM) quantification, were reevaluated three or more years later with questionnaires and spirometry. FAO was defined as a persistent post-bronchodilator forced expired volume in one second (FEV₁)-to-forced vital capacity ratio below 0.70.

Results: Sixty-two participants (mean $\pm$ SD age 48 ± 11 years; 50% female; 75% atopic; asthma duration 24 ± 14 years) returned for follow-up assessment (median interval 7.9 years; IQR: 5.4–8.8 years). Compared to participants without FAO ($n = 28$), those with FAO at baseline and long-term follow-up ($n = 18$) had higher baseline sputum neutrophil content and ASM, and a higher exacerbation frequency that persisted at long-term follow-up. Sputum eosinophils, ACQ and long-term FEV₁ decline did not differ. Participants with incident FAO at long-term follow-up ($n = 16$) had higher baseline exacerbation frequency, sputum eosinophil content, higher ACQ scores and greater decline in FEV₁, whereas baseline ASM was similar to those without FAO.

Conclusion: In moderate-to-severe asthma, long-term FAO is characterized by neutrophilic sputum inflammation and airway remodelling, but FEV₁ decline is similar to those without FAO. Long-term incident FAO is preceded by higher exacerbation frequency, higher sputum eosinophil content and significant FEV₁ decline.

KEYWORDS

asthma, epidemiology, remodelling

1 | INTRODUCTION

Approximately 5%–10% of patients with asthma have severe disease.¹ These patients experience the greatest health status impairment

and consume the greatest proportion of healthcare resources.^{2–5} Despite advances in therapies over the last two decades, the disproportionate burden of severe asthma persists today.^{1,6} Several phenotypes of severe asthma have been proposed based on various

patient characteristics such as lung function, age of onset of asthma, atopic status, sputum eosinophil, fraction of exhaled nitric oxide and other characteristics.⁷⁻¹⁰ One such phenotype is that associated with apparent permanent loss of lung function. Identifying features that predispose to fixed airflow obstruction (FAO) in asthma may identify mechanisms to prevent or treat this burdened subgroup of severe asthma.

Fixed airflow obstruction (FAO) is a common feature of severe asthma.^{7,11,12} Asthma with FAO is also associated with lower quality of life compared to asthma without FAO or chronic obstructive pulmonary disease (COPD) with a similar degree of obstruction.¹³ Asthma with FAO is also associated with increased mortality.^{14,15} Asthma with FAO exhibits greater airway inflammation as indicated by higher sputum leucocytes and fraction of exhaled nitric oxide (FeNO).¹⁶⁻¹⁹ Endobronchial airway biopsies demonstrate increased airway smooth muscle (ASM) area that correlates negatively with the forced expiratory volume in 1 second (FEV₁).^{17,20,21} The timing of airway remodelling is uncertain but increase in ASM mass has been documented also in children between the ages of 8 and 12 years and is associated with lower lung function.²²

This study sought to characterize asthma control and airway inflammation and assessed repeatedly over time, as well as airway remodelling, according to long-term FAO status in a well-characterized cohort of moderate-to-severe asthma.²⁰ Furthermore, we sought to compare the proportion of previously described asthma phenotype clusters,¹² defined by readily available clinical variables, according to long-term FAO status. We hypothesized that those with FAO, whether persistent or incident, would have poorer disease control in terms of higher exacerbation rates, lower quality-of-life scores and higher proportion of severe phenotype clusters, compared to those without FAO. However, the incident FAO group would not have evidence of airway remodelling as defined by increased ASM at study entry, in contrast to the persistent FAO subjects, but are expected to have loss of lung function compared to those without FAO. We postulate that they would demonstrate evidence of elevated Th2-mediated inflammation such as elevated sputum eosinophils and FeNO at studying entry which would suggest that Th2-mediated inflammation plays an important role in the development of airway remodelling.

2 | METHODS

We conducted a long-term follow-up study of adults three or more years after completing the Difficult Asthma Study at the Montreal Chest Institute of the McGill University Health Centre and at Sacré-Coeur Hospital, Montreal, Canada, which has been described in detail elsewhere.²⁰ A subset of these patients also underwent endobronchial biopsy for airway smooth muscle analysis as reported in previous studies of this cohort.^{17,20} The protocols were reviewed and approved by institutional review boards of both institutions, and informed consent was obtained.

2.1 | Study sample

Patients carrying a diagnosis of moderate or severe asthma made by a respirologist and supported by either an improvement in FEV₁ of 12% or greater with an absolute increase of at least 200 mL following administration of a β -adrenergic agonist, or variability over time, and having demonstrated at least 70% compliance with four inhalations of fluticasone (1000 μ g) and salmeterol (200 μ g) per day during a 1-month run-in period were eligible for enrolment into the original study. All subjects were non-smokers or ex-smokers of at least 2 years with a smoking history of <15 pack-years.

Severe asthma was based on the American Thoracic Society (ATS) Workshop for Refractory Asthma²³ and required at least one major criterion: (a) treatment with daily oral steroids for more than 50% of the previous 12 months or (b) treatment with high-dose inhaled steroid (≥ 1000 μ g of fluticasone or equivalent per day) and at least one other add-on therapy (long-acting β -agonist, leukotriene receptor antagonist or theophylline) continuously over the previous 12 months. In addition, at least 2 or more minor criteria had to be present: (a) daily use of short-acting β -agonist; (b) persistent airflow obstruction, as documented with a pre-bronchodilator forced expiratory volume in one second (FEV₁) of <70% and an FEV₁/forced vital capacity ratio of <80% of predicted value; (c) 1 or more urgent care visits in the previous 12 months; (d) 3 or more steroid bursts in the previous 12 months; (e) prompt deterioration with <25% dose reduction of steroids; or (f) a near-fatal asthma event in the last 3 years.

Moderate asthma required (a) well-controlled asthma on at least 200 μ g/d of fluticasone or equivalent but not exceeding 1000 μ g/d, with or without the use of a long-acting β -agonist; (b) 2 or less steroid bursts in the previous 12 months and none in the past 3 months (the total number of days taking oral steroids could not exceed 30 days in the previous 12 months); (c) FEV₁ of >70% of predicted value and >90% of personal best from the previous 2 years; and (4) no more than 1 non-scheduled urgent care or office visit in the previous 12 months.

2.2 | Baseline measurements

The Difficult Asthma Study included clinic assessments every 1 month for severe asthma and every 3 months for moderate asthma over a 1-year period. Each visit included the Asthma Control Questionnaire (ACQ) and the Asthma Quality of Life Questionnaire (AQLQ),^{24,25} along with spirometry and fraction of exhaled nitric oxide (FeNO), both performed according to ATS standards. Sputum leucocyte differential was assessed at every visit using hypertonic saline-induced expectorated sputum and processed within 2 hours.

The baseline measures of ACQ, spirometry, FeNO and sputum neutrophil percent were normally distributed. The mean and variability of these repeated baseline measures were therefore calculated as the arithmetic mean and standard deviation (SD), respectively. The distribution of sputum eosinophils (percent) was skewed and

therefore stratified as <3%, 3%-10% and >10%, and variability was quantified as the interquartile range. FEV₁ bronchodilator responsiveness was defined as a 200 mL and 12% increments in FEV₁ or FVC 10-15 minutes after two metre-dose inhalations of salbutamol via a spacer device.

Allergy skin testing was performed at one visit to 13 aeroallergens (*Dermatophagoides pteronyssinus*, *Dermatophagoides farinae*, cat, dog, horse, ragweed, grass mix, tree mix, weed mix, feather mix, *Alternaria*, *Aspergillus* and *Cladosporium*), as well as a positive histamine, and negative diluent control. Atopy was defined by a positive skin test to any of the aeroallergens.

Participants were classified according to asthma phenotype by a clustering algorithm described by Moore and colleagues for the Severe Asthma Research Program (SARP) using baseline FEV₁ (percent predicted) maximum FEV₁ (percent predicted) during the initial study's 12-month follow-up and age at asthma onset.¹²

Bronchoscopy with endobronchial airway biopsies from the right lung at various segmental and subsegmental carinae was performed on a subset of subjects at a single visit.²⁰ Tissues were fixed in formaldehyde and embedded in paraffin before cutting 5-µm sections, and staining was performed using a peroxidase-based method. ASM was identified using human smooth muscle-specific α -actin antibody (Novocastra Laboratories Ltd), and area was quantified using the Image Pro-Plus 4.0 system (Media Cybernetics) from images recorded by CCD colour video camera (Sony) mounted on a conventional light microscope (Olympus Optical Co.) with a 4× objective. ASM area was quantified on 2 sections from 2 biopsy specimens for each subject.

2.3 | Long-term follow-up measurements

Participants enrolled into the Difficult Asthma Study three or more years earlier were contacted by phone or mail and invited to return for a single long-term follow-up study assessment. The assessment occurred, while the participant was not in exacerbation and included ACQ, and spirometry according to ATS standards.²⁶

At baseline and long-term follow-up assessments, age, gender, smoking status, medication inventory and exacerbation history were assessed by interviewer-administered questionnaire. An exacerbation was defined as a worsening of asthma symptoms requiring initiation or increase in corticosteroid. Body height and weight were measured using a standardized protocol. Body mass index (BMI) was computed as the weight in kilograms divided by the squared height in metres.

2.4 | Trajectories of fixed airflow obstruction (FAO)

Participants with a post-bronchodilator FEV₁/FVC ratio below 0.70 at each assessment during the 12-month baseline Difficult Asthma Study and at the long-term follow-up assessment were classified as having *long-term persistent FAO* (n = 18). Participants who did

not exhibit FAO during the baseline study period but had a post-bronchodilator FEV₁/FVC < 0.70 at the long-term follow-up assessment were classified as having *long-term incident FAO* (n = 16). The remaining participants were classified as not having FAO (n = 28). No participant with FAO during the baseline study period exhibited an FEV₁/FVC ratio above 0.70 at the long-term follow-up assessment.

2.5 | Statistical analysis

Dichotomous variables are presented as proportions and continuous variables as means with SD unless otherwise indicated. Primary analysis used linear regression to estimate differences in baseline ACQ score, spirometry measures, FeNO, sputum differential, as well as variability of these measures among participants with long-term persistent FAO, long-term incident FAO, using those without FAO as the reference group. Long-term follow-up ACQ score, spirometry measures and long-term changes from baseline were similarly compared using linear regression to adjust for variable follow-up intervals. Secondary analyses compared baseline SARP phenotype cluster between groups using the Fisher exact test. Differences in baseline ASM were estimated using linear regression between fixed airflow obstruction groups in the subset of participants who underwent bronchoscopy. Gender interactions were assessed on a multiplicative scale. A *post hoc* sensitivity analyses stratified participants free of FAO at baseline according to an FEV₁ threshold of 80% predicted. Statistical computations were performed using SAS 9.3 (Cary, NC). A two-tailed P-value < .05 was considered to indicate statistical significance.

3 | RESULTS

Of 90 participants completing the baseline 12-month Difficult Asthma Study and eligible for the long-term follow-up, 62 returned for assessment (median interval 7.9 years; IQR: 5.4-8.8 years) and were included in the present analysis. The mean baseline age of returning participants was 48 ± 11 years, 50% were female, the mean BMI was 28.5 ± 6.7 kg/m², 63% had severe asthma, 87% were atopic, and the mean asthma duration was 24 ± 14 years. Participants who were eligible but did not return for long-term follow-up assessment had similar clinical characteristics (Table A1). All patients who returned for follow-up remained non-smokers. Thirteen subjects were systemic corticosteroid-dependent, as defined as requiring oral prednisone for 4 or more consecutive months.

The characteristics of participants according to FAO status are summarized in Table 1. Twenty-eight participants did not have FAO at baseline or long-term follow-up; 18 had FAO at baseline and long-term follow-up (long-term persistent FAO); and 16 had long-term incident FAO, with median long-term follow-up intervals of 8.0, 7.4 and 8.0 years, respectively (P = .49). Age, gender, height, BMI, atopic status, age of asthma onset, childhood asthma onset and asthma duration were similar among the three groups

TABLE 1 Participant characteristics according to FAO status

	No FAO at baseline or at long-term follow-up ^a n = 28	Long-term incident FAO n = 16	Long-term persistent FAO n = 18
Long-term follow-up interval—years	8.0 (6.3–9.0)	8.0 (5.8–9.3)	7.4 (4.9–8.6)
Baseline age—years	48 ± 12	48 ± 11	49 ± 12
Female—%	57	56	39
Height—cm	169 ± 9	172 ± 12	166 ± 9
Body mass index—kg/m ²	29 ± 7	29 ± 8	28 ± 5
Smoking status—%			
Never	50	56	83
Former (<15 pack-years)	50	44	17
Atopy ^b —%	96	75	83
Age of asthma onset—years	24 ± 18	25 ± 18	23 ± 16
Childhood onset asthma—%	39.3	37.5	38.9
Asthma duration—years	24 ± 11	23 ± 20	26 ± 10
Asthma severity—%			
Moderate	64	25	6
Severe	36	75	94
Asthma severity—n			
Moderate	17	5	1
Severe	11	11	17
Asthma pharmacotherapy at baseline/follow-up—% ^c			
Systemic corticosteroid dependence ^d	0/4	38/25	39/22
High-dose inhaled corticosteroid	43/39	75/69	94/72
Medium-dose inhaled corticosteroid	39/39	25/25	6/22
Long-acting beta-agonist	85/71	87/88	94/94
Long-acting anti-muscarinic	7/11	13/19	17/28
Leukotriene receptor antagonist	19/21	31/25	33/22
Theophylline	4/0	0/6	11/11
Biologic	4/4	6/13	6/6

Note: Mean ± SD or median (IQR) unless otherwise indicated.

Abbreviations: ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FAO, denotes fixed airflow obstruction; FEV₁, forced expired volume in the first second; SD, standard deviation.

^aBaseline FAO defined as post-bronchodilator FEV₁-to-FVC ratio <0.70 at all study visits during the 12-mo baseline study period. Long-term incident FAO was defined as FAO at long-term follow-up but not at baseline. Long-term persistent FAO was defined as FAO at baseline and long-term follow-up.

^bPresence of atopy was defined as a positive skin test to any of 13 aeroallergens.⁴⁸

^cAll participants had a prescription for short-acting beta-agonist.

^dSystemic corticosteroid dependence defined as utilization of the equivalent of 5 mg or more of prednisone for 4 or more consecutive months.

(Table 1). The proportion of former smokers among participants with long-term persistent FAO (17%) was lower than those with long-term incident FAO (44%), or those without FAO (50%). The prevalence of baseline severe asthma status among participants without FAO was 36% and was significantly higher among those with long-term persistent FAO (94%; odds ratio 30.6 95% CI: 3.5–265; *P* = .002) and those with long-term incident FAO (75%; odds ratio 5.4 95% CI: 1.4–21; *P* = .016).

3.1 | Asthma control and quality of life

At baseline, participants without FAO had a mean ACQ score of 1.2 ± 0.7 , which was lower when compared to participants with long-term persistent FAO (1.7 ± 0.7 ; *P* = .030), and also tended to be lower than those with long-term incident FAO (1.7 ± 1.1 ; *P* = .168). Long-term changes in ACQ scores were small (Table 2) and were similar by FAO status (*P* ≥ .14). Baseline and long-term

TABLE 2 Participant characteristics according to FAO status at baseline and long-term follow-up

	No FAO at baseline or long-term follow-up ^a n = 28	Long-term incident FAO n = 16	Long-term persistent FAO n = 18
ACQ score			
Baseline	1.2 ± 0.7	1.7 ± 1.1	1.7 ± 0.7 [*]
Long-term follow-up	1.5 ± 1.2	1.7 ± 1.2	1.7 ± 1.0
Absolute change	0.3 ± 1.0	0.0 ± 0.8	0.0 ± 0.6
AQLQ score			
Baseline	5.3 ± 1.1	5.1 ± 1.2	5.3 ± 1.0
Long-term follow-up	5.3 ± 1.3	5.3 ± 1.4	5.7 ± 1.1
Absolute change	0.0 ± 1.1	0.1 ± 1.2	0.5 ± 0.9
Exacerbations in the preceding year—no. (range)			
Baseline	0 (0-5)	1 (0-7) [*]	1 (0-6) [*]
Long-term follow-up	0 (0-4)	1.5 (0-4) ^{***}	1 (0-4) ^{**}
FEV ₁ percent predicted			
Baseline	85 ± 10	76 ± 22	57 ± 17 ^{***}
Long-term follow-up	84 ± 13	63 ± 10 ^{**}	56 ± 18 ^{***}
Absolute difference	-1 ± 14	-13 ± 22 ^{**}	-1 ± 9
Annualized difference	-0.1 ± 1.9	-1.8 ± 5.2 ^{**}	-0.1 ± 1.5
FEV ₁ —L			
Baseline	3.1 ± 1.0	2.7 ± 1.1	2.2 ± 1.0 ^{***}
Long-term follow-up	2.8 ± 0.8	2.0 ± 0.5 [*]	1.8 ± 0.7 ^{***}
Absolute difference	-0.3 ± 0.8	-0.7 ± 1.0 [*]	-0.4 ± 0.7
Annualized difference	-0.05 ± 0.15	-0.09 ± 0.20 [*]	-0.05 ± 0.14
FEV ₁ /FVC			
Baseline	77 ± 6	71 ± 6 ^{**}	56 ± 7 ^{***}
Long-term follow-up	75 ± 6	62 ± 6 ^{**}	57 ± 9 ^{***}
Absolute difference	-2 ± 5	-9 ± 9 ^{**}	1 ± 7
Annualized difference	-0.2 ± 0.7	-1.2 ± 1.1 ^{**}	0.2 ± 1.2
Bronchodilator responsive at baseline—%	29	25	17
Sputum neutrophils at baseline—%	46 ± 17	43 ± 20	57 ± 19 [*]
Sputum eosinophils at baseline—%		*	
Proportion < 3%	31	13	22
Proportion 3%-10%	54	47	39
Proportion > 10%	15	40	39
% Sputum eosinophils at baseline	7.40 ± 5.90	9.33 ± 9.97	10.38 ± 8.09
FeNO at baseline—ppb	30 ± 14	31 ± 14	31 ± 14
Airway smooth muscle area at baseline—mm ²	0.12 ± 0.14 [n = 8]	0.14 ± 0.10 [n = 8]	0.32 ± 0.03 [n = 6] [*]
Asthma phenotype ^b at baseline—%		***	***
SARP cluster 1	7	19	0
SARP cluster 2	61	25	11
SARP cluster 3	25	6	6
SARP cluster 4	4	44	28
SARP cluster 5	4	6	56

Note: Mean ± 1 SD unless otherwise indicated.

Abbreviations: ACQ, Asthma Control Questionnaire; AQLQ, Asthma Quality of Life Questionnaire; FAO, denotes fixed airflow obstruction; FEV₁, forced expired volume in the first second; SARP, Severe Asthma Research Program; SD, standard deviation.

^aBaseline FAO defined as post-bronchodilator FEV₁-to-FVC ratio <0.70 at all study visits during the 12-mo baseline study period. Long-term incident FAO was defined as FAO at long-term follow-up but not at baseline. Long-term persistent FAO was defined as FAO at baseline and long-term follow-up. Sputum eosinophil content is presented as proportions due to skewed distribution.

^bAsthma phenotype was defined by the Moore clustering algorithm.¹²

*P < .05, **P < .01 and ***P < .001 for comparison with the no FAO group.

changes in AQLQ scores did not differ significantly by FAO status (Table 2; $P \geq .12$).

3.2 | Exacerbation frequency

Twelve-month exacerbation frequency at baseline and at long-term follow-up is summarized according to FAO status in Table 2. Compared to participants without FAO, exacerbation frequency during the baseline study period was higher among participants with long-term persistent FAO (Poisson regression coefficient: +0.7 95% CI +0.1 to +1.2; $P = .022$) and among participants with long-term incident FAO (Poisson regression coefficient: +0.7 95% CI +0.1 to +1.3; $P = .019$). Exacerbation frequencies remained higher at long-term follow-up for those with long-term persistent FAO (Poisson regression coefficient: +1.5 95% CI +0.8 to +2.1; $P < .001$) and for those with long-term incident FAO (Poisson regression coefficient: +1.1 95% CI +0.4 to +1.7; $P = .003$).

3.3 | Airflow obstruction

Baseline FEV₁ (percent predicted) by FAO status is summarized in Table 2. Compared to participants without FAO, baseline FEV₁ percent predicted was lower among participants with long-term persistent FAO (mean difference: -28% 95% CI: -38 to -18%; $P < .001$) and among participants with long-term incident FAO (mean difference: -10% 95% CI: -20 to +0.2%; $P = .06$). Compared to participants without FAO, those with long-term incident FAO had more variability in FEV₁ over the baseline study period (SD: 9.3; $P = .002$), whereas long-term persistent FAO exhibited a similar FEV₁ variability to those without FAO (SD: 5.3; $P = .982$).

The long-term change in FEV₁ (Figure 1) among participants without FAO was small ($-1 \pm 14\%$) and was similar among participants with long-term persistent FAO ($-1 \pm 9\%$). In contrast, participants with long-term incident FAO experienced a decline in FEV₁

($-13 \pm 22\%$), which differed significantly from those without FAO (mean difference: -13% 95% CI: -23 to -3%; $P = .011$).

Similar results were observed for FEV₁ in L and FEV₁/FVC.

3.4 | Sputum cytology

The mean and standard deviation of sputum neutrophils measured repeatedly during the baseline study period are summarized according to FAO status in Table 2. Compared to participants without FAO, sputum neutrophil content was higher among participants with long-term persistent FAO (mean difference: +11% 95% CI: +0.1 to +22%; $P = .048$), but not different among participants with long-term incident FAO (mean difference: -3.2% 95% CI: -15 to 8%; $P = .59$).

Baseline sputum eosinophil proportions are presented in Table 2. Compared to participants without FAO, those with long-term incident FAO exhibited higher proportions of sputum eosinophils (p -trend = 0.03). Participants with long-term persistent FAO tended to have higher sputum eosinophil content compared to those without FAO, but these differences were not statistically significant ($P = .10$).

3.5 | Fraction of exhaled nitric oxide

Mean values of FeNO measured repeatedly during the baseline study period are summarized in Table 2 and did not differ significantly among participants with long-term persistent or long-term incident FAO when compared to participants without FAO ($P \geq .86$).

3.6 | Airway smooth muscle

A subset of participants ($n = 22$) underwent endobronchial biopsy with ASM quantification during the baseline study (Table 2 and Figure 1). Compared to participants without FAO ($n = 8$), ASM

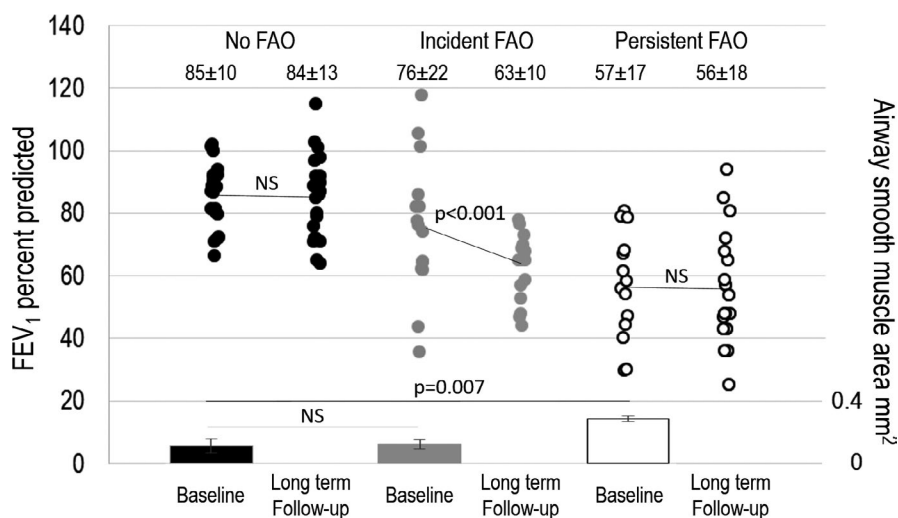


FIGURE 1 FEV₁ and airway smooth muscle by FAO status. Each dot represents the FEV₁ of a participant, and the line indicates the mean value at baseline and at long-term follow-up. Endobronchial biopsy and airway smooth muscle quantification were available in 22 participants (no FAO: 8; incident FAO: 8; persistent FAO: 6). The error bars indicate standard error of the airway smooth muscle area. FAO fixed airflow obstruction, and NS non-significant; FEV₁ denotes forced expired volume in the first second

content was higher among participants with long-term persistent FAO ($n = 6$; mean difference: $+0.2 \text{ mm}^2$ 95% CI: $0.1\text{--}0.3 \text{ mm}^2$; $P = .002$), but similar to those with long-term incident FAO ($n = 8$; mean difference: 0.0 mm^2 95% CI: -0.1 to 0.1 ; $P = .76$). While there was no evidence of interaction by gender, we did observe that, among the entire sample, higher baseline ASM area among women was associated with long-term increase in FEV_1 (10.3% increase per 1-SD increment in ASM area; $P = .007$), but not among men (2.1% increase per 1-SD increment in ASM area; $P = .81$).

3.7 | SARP asthma phenotype clusters

Prevalence of SARP phenotypes at baseline differed significantly by FAO group (Table 2; $P < .001$). Participants without FAO consisted mainly of cluster 2 (61%) and cluster 3 (25%) phenotypes. Those with long-term persistent FAO included predominantly phenotype clusters 2 (19%), 4 (27%) and 5 (50%). Participants with long-term incident FAO consisted mainly of phenotype clusters 2 (28%) and 4 (56%).

3.8 | Sensitivity analyses

Among 44 participants free of FAO at baseline, 11 (25%) had an $\text{FEV}_1 < 80\%$ predicted. Compared to participants free of FAO at baseline with FEV_1 of 80% or greater, those with an $\text{FEV}_1 < 80\%$ predicted did not differ significantly with respect to age, gender, BMI, atopy status or smoking status ($P \geq .32$), but were significantly more likely to have severe asthma (OR 9.14 95% CI: 1.95, 42.89; $P = .005$), of later onset (mean difference: 13 years 95% CI: 2, 23; $P = .019$), and were more likely to develop long-term incident FAO (OR 5.04; 95% CI: 1.14, 22.30; $P = .033$).

Restricting the comparison between participants without FAO and participants with incident FAO to those with an FEV_1 of 80% or greater yielded similar results as the main analysis (baseline ACQ score [$P = .18$], AQLQ score [$P = .16$], percent predicted FEV_1 [$P = .16$], FeNO [$P = .66$], sputum neutrophils [$P = .71$], sputum eosinophils [$P = .010$], long-term follow-up ACQ score [$P = .64$] and percent predicted FEV_1 [$P < .0001$] and baseline to long-term change in percent predicted FEV_1 [$P < .0001$]). The baseline and long-term exacerbation frequency Poisson regression coefficients were similar in magnitude to those in the main analysis ($+0.6$ and $+1.1$, respectively), but not statistically significant (0.094 and 0.055, respectively).

4 | DISCUSSION

Among middle-aged adults with moderate-to-severe asthma, long-term persistent and long-term incident FAO were common and had distinct clinical and biologic features when compared to participants without FAO. The long-term persistent FAO group exhibited poorer asthma control, higher exacerbation frequency and sputum neutrophil content, evidence of airway remodelling and lower baseline

FEV_1 , whereas the long-term change in FEV_1 was small, and similar to the group without FAO. The long-term incident FAO group, while also experiencing poorer asthma control and higher exacerbation frequency, exhibited higher sputum eosinophil content, baseline FEV_1 variability and longitudinal FEV_1 decrement, but lacked evidence of baseline airway remodelling. These findings suggest the existence of distinct or sequential asthma FAO endotypes, which are not easily discernible by asthma control or exacerbation history.

Few studies have examined long-term outcomes and FAO among adults with moderate-to-severe asthma beyond 12 months.^{16,27-30} Consistent with our findings, these studies found FAO to be associated with increased exacerbation rate,^{29,30} and that incident FAO was preceded by lung function decline.^{16,27,28} A population-based cohort study of asthma reported an FAO prevalence of 11.4% and an FAO incidence of 17.6% over 28 years.²⁷ Baseline characteristics predicting incident FAO were male gender, older age and smoking status, whereas obesity was protective. In contrast to these findings, we observed a much higher FAO prevalence (29.0%) and incidence (36.4% over 8 years) and did not observe associations with gender, age, smoking status or BMI. These differences are likely due, in part, to differences in study design: we studied a more severe spectrum of asthma severity, where FAO is more common,^{7,12,31} and where female- and obesity-associated asthma phenotypes are more common.¹² We also excluded heavy smokers to avoid misclassification of asthma with COPD.

To our knowledge, this is the first study where long-term FAO status was correlated with baseline airway inflammation and remodelling in moderate-to-severe asthma. We observed distinct inflammatory profiles for long-term persistent and incident FAO suggesting at least two different or sequential FAO endotypes. While both groups experienced poorer control and frequent exacerbations, the persistent FAO group exhibited neutrophilic airway inflammation and remodelling, but relatively stable lung function. In contrast, the incident FAO group exhibited eosinophilic airway inflammation without (or prior to) airway remodelling, followed by significant loss of lung function. Eosinophilic asthma has been linked previously to recurrent exacerbations associated with a decline in lung function.³² However, in this latter study the decline was attenuated by inhaled budesonide, whereas in the current sample high-dose inhaled steroids did not prevent incident FAO. More recent research implicates refractory airway type 2 inflammation and sputum eosinophilia in steroid-resistant asthma,³³ which may be mediated by innate lymphoid cells (eg ILC2s).^{34,35} The association between an asthma subgroup with steroid resistance, sputum eosinophilia and incident FAO underscores the need to identify the molecular mechanisms of steroid-resistant sputum eosinophilia and to determine whether this subgroup transitions to neutrophilic inflammation with remodelled airways. Alternatively, these may represent distinct non-overlapping endotypes.

The potential causative role of eosinophils in airway remodelling has biologic plausibility. Several important aspects of remodelling such as airway smooth muscle growth and fibrosis may be mediated by the eosinophil. Eosinophils synthesize cysteinyl leukotrienes, molecules upstream of the epidermal growth receptor,

known to cause allergen driven airway remodelling in animal models.^{36,37} Eosinophils are also a source of fibrogenic cytokines that may also be involved in the airway remodelling process.^{38,39} The finding that FAO at baseline evaluation was associated with sputum neutrophilia raises the question of the role that neutrophils might play in FAO. The failure of this subset to evolve further argues that the neutrophil is unlikely causative of the permanent loss of function. Additional study is needed to determine whether these groups are indeed two separate manifestations of asthma or if they exist on a continuum where the eosinophilic group will eventually become neutrophilic and stabilize following a period of lung function decline and remodelling.

FeNO did not differ significantly among participants with incident FAO when compared to those without FAO, in contrast to sputum eosinophils. Poor correlation between FeNO and sputum eosinophils has been demonstrated among participants with oral corticosteroid-dependent asthma.⁴⁰ The 38% prevalence of oral corticosteroid use in the incident FAO group, combined with a modest sample size, may explain the discordant associations between FeNO and sputum eosinophils in the present study.

SARP asthma phenotype clusters, derived from clinical variables using an unbiased statistical approach,¹² differed by FAO group. Forty-four percent of participants with long-term incident FAO belonged to milder phenotype clusters (1 or 2), suggesting important within-cluster heterogeneity, or potential misclassification when relying on clinical variables alone.⁴¹ The most prevalent phenotype among participants with incident FAO, however, was cluster 4, suggesting that this is readily identifiable asthma subgroup is at greater risk of losing lung function. Cluster 5, associated with the longest duration of asthma,¹² represented the majority of participants with long-term persistent FAO. Longer observation, ideally from early childhood, is required to determine whether long-term FAO observed in adulthood is acquired or congenital.⁴²

Strengths of the present study include baseline compliance assessment to minimize misclassification of disease severity; repeated, non-invasive baseline measures of airway inflammation severity and its variability over 12 months; and a median long-term follow-up of 8 years. Our study has limitations. First, incomplete follow-up of some participants in the baseline study, which had they returned, may have altered our findings. We think this is unlikely, however, since baseline characteristics were similar among participants returning for long-term assessment and those not. Second, our well-characterized sample was modest in size, and thus, some clinically relevant differences may not have achieved statistical significance. Third, self-reported exacerbation frequency is susceptible to recall error. We think substantial error is unlikely since medical records among the subgroup of participants followed clinically at our institution were consistent with self-reported events. Fourth, endotypes defined by distinct molecular mechanisms or targeted pharmacotherapy responsiveness were not assessed in this study,⁴³ and therefore, the findings suggest but do not confirm novel endotypes. Finally, our long-term follow-up evaluation consisted of a single visit despite the known temporal variability of asthma symptoms

and airflow obstruction, which may have resulted in misclassification of FAO. To minimize this bias, we evaluated subjects while not in exacerbation.

In summary, long-term follow-up of adults with moderate-to-severe asthma revealed that nearly one-third had persistent FAO characterized by neutrophilic sputum inflammation, remodelled airways and low but stable FEV₁. Another one-quarter, who developed incident FAO, exhibited higher baseline sputum eosinophil content and greater FEV₁ decline, but did not exhibit airway remodelling at baseline. These findings suggest at least two distinct FAO endotypes with potential therapeutic implications: first, therapies targeting neutrophilic inflammation are needed for a substantial proportion of patients with moderate-to-severe asthma. Second, therapies targeting eosinophilic inflammation⁴⁴⁻⁴⁷ in appropriate patients may help to prevent incident FAO before airway remodelling occurs. Endotype-guided therapeutic trials are needed to test these hypotheses.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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APPENDIX 1

TABLE A1 Baseline Difficult Asthma Study participant characteristics according to the long-term follow-up status

	Attended long-term follow-up assessment N = 62	Attempted but did not attend long-term follow-up assessment ^b N = 28
Age—years	48 ± 11	52 ± 14
Female—%	50	52
Height—cm	169 ± 10	168 ± 8
Body mass index—kg/m ²	28.5 ± 6.7	27.7 ± 4.9
Smoking status—%		
Never	57	61
Former	43	39
Atopy ^a —%	75	87
Age of asthma onset—years	24 ± 17	25 ± 18
Asthma duration—years	24 ± 14	27 ± 20
Asthma severity—%		
Moderate	37	29
Severe	63	71

Note: Mean ± 1 SD (standard deviation) unless otherwise indicated.

^aPresence of atopy was defined as a positive skin test to any of the 13 aeroallergens.⁴⁸

^bOne participant had died, two had withdrawn from the study, 14 refused (2 too busy, 2 too ill, 10 no reason given), and 11 were unreachable.