

Type 2 Biomarkers and Prediction of Future Exacerbations and Lung Function Decline in Adult Asthma



Ruth Semprini, MBBS^{a,b}, Mathew Williams, DipExSci^a, Alex Semprini, MBBS^{a,b}, Alice McDouall, BSc^{a,c}, James Fingleton, PhD^{a,d}, Cecile Holweg, PhD^e, Mark Weatherall, MAppStats^c, Richard Beasley, MD^{a,b,d}, and Irene Braithwaite, PhD^{a,d} Wellington, New Zealand; and South San Francisco, Calif

What is already known about this topic? In studies primarily undertaken in patients with severe eosinophilic asthma, higher levels of blood eosinophils, fractional nitric oxide, and serum periostin predict an increased risk of severe exacerbations.

What does this article add to our knowledge? In a broader asthma population, higher levels of these predictive biomarkers are associated with fewer asthma exacerbations.

How does this study impact current management guidelines? This study highlights the importance of non-type 2 asthma as a phenotype associated with an increased risk of exacerbations and the inherent difficulty of applying highly specific biomarkers to this more diverse group.

BACKGROUND: Type 2 biomarkers that predict both likelihood of future severe exacerbations and response to mAb therapy in asthma would be useful clinically in identifying patients both at greater risk of hospitalization and most likely to benefit from mAb therapy.

OBJECTIVE: To describe the association between type 2 biomarkers, blood eosinophils, fractional exhaled nitric oxide (FENO), serum periostin, and serum IgE, and time to severe exacerbation in a broad asthma population.

METHODS: Participants from 2 adult asthma cohorts with baseline measurements of blood eosinophils, FENO, serum periostin, and serum IgE were reviewed after at least 12 months to obtain an exacerbation history, corroborated with general practitioner and hospital medical records. The association

between baseline type 2 biomarkers and time to exacerbation was described by Cox proportional hazard ratios (HRs) using multivariate models.

RESULTS: A total of 212 participants were followed for a median (range) 3.8 (1.1-5.3) years; 67 of 212 (32%) had at least 1 severe exacerbation. The HRs (95% CI) of baseline type 2 biomarkers and time to exacerbation were as follows: blood eosinophils per $0.1 \times 10^9/L$ increase, 0.89 (0.76-1.05), $P = .17$; log FENO per 0.693 increase, 0.65 (0.52-0.81), $P < .001$; log serum periostin per 0.693 increase, 0.62 (0.35-1.09), $P = .10$; log serum IgE per 0.693 increase, 0.89 (0.80-1.00), $P = .05$.

CONCLUSIONS: The positive association between type 2 biomarkers and risk of severe exacerbations in populations with severe refractory asthma does not extend to mild and moderate asthma. Non-type 2 asthma may represent a phenotype associated with an increased risk of severe exacerbations in a broad asthma population. © 2018 American Academy of Allergy, Asthma & Immunology (J Allergy Clin Immunol Pract 2018;6:1982-8)

Key words: Asthma; Exacerbation; Noneosinophilic asthma; Type 2 biomarkers

INTRODUCTION

Severe exacerbations of asthma are a significant public health burden, leading to around 500,000 hospitalizations per year in the United States, costing about one-third of the total of \$14.7 billion spent on asthma-related health care.¹ Severe asthma exacerbations are associated with increased morbidity and risk of mortality and excess lung function decline over time.^{2,3} Predictors of severe exacerbations are clinically useful to identify patients at risk of these associated outcomes. Clinical features reported to be associated with an increased risk of severe

^aMedical Research Institute of New Zealand, Wellington, New Zealand

^bVictoria University of Wellington, Wellington, New Zealand

^cUniversity of Otago, Wellington, New Zealand

^dCapital & Coast District Health Board, Wellington, New Zealand

^eGenentech Inc, South San Francisco, Calif

This study was funded by Genentech, Inc, member of the Roche Group. The funder was involved in the study design, and in critical review of the drafted manuscript.

The funder was not involved in the collection, analysis, and interpretation of data; nor in the decision to submit the manuscript for publication.

Conflicts of interest: C. Holweg is an employee of Genentech, Inc, a member of the Roche Group. The rest of the authors declare that they have no relevant conflicts of interest.

Received for publication January 8, 2018; revised March 12, 2018; accepted for publication March 20, 2018.

Available online March 30, 2018.

Corresponding author: Irene Braithwaite, PhD, Medical Research Institute of New Zealand, Private Bag 7902, Wellington 6242, New Zealand. E-mail: irene.braithwaite@mrnz.ac.nz.

2213-2198

© 2018 American Academy of Allergy, Asthma & Immunology

<https://doi.org/10.1016/j.jaip.2018.03.004>

Abbreviations used

FENO- Fractional exhaled nitric oxide

GP- General practitioner

ICS- Inhaled corticosteroid

LABA- Long-acting beta-agonist

exacerbations include poor symptom control, 1 or more severe exacerbations in the previous year, high short-acting beta-agonist use, exposure to tobacco smoke, high allergen exposures, poor lung function, comorbidities including obesity and rhinosinusitis, and major psychosocial problems.⁴

Other predictors of exacerbations in patients with severe asthma are type 2 biomarkers, including peripheral blood eosinophils, fractional exhaled nitric oxide (FENO), serum periostin, and serum IgE. Randomized controlled trials of mAb therapies directed against type 2 asthma report that higher levels of blood eosinophils,⁵⁻⁷ FENO,⁶ and serum periostin^{7,8} are associated with higher exacerbation rates in populations with severe asthma. While these type 2 biomarkers predict the likelihood of future exacerbations in adults with severe, uncontrolled type 2 asthma, it is unclear whether these associations extend to a more general group of adults with asthma with a range of asthma severity.

We aimed to assess whether the risk of severe exacerbation was greater in those with high levels of type 2 biomarkers in a relatively nonspecific group of adults with asthma with a range of severity. This was by following up patients from 2 previously recruited cohorts of research participants who had baseline measurements of blood eosinophils, FENO, serum periostin, and serum IgE. The first cohort was a sample of 235 adults with asthma, who had predominantly intermittent asthma of mild or moderate severity, recruited by a simple random sample from the electoral role in the greater Wellington area.⁹ The second smaller cohort consisted of 60 adults with stable moderate to severe asthma on a regular regimen of inhaled corticosteroids (ICSs) and a long-acting beta-agonist (LABA), who had participated in a study of the longitudinal variation of serum periostin levels.¹⁰ On the basis of reported data for type 2 biomarkers in severe asthma, we hypothesized that those with high baseline levels of peripheral blood eosinophils, FENO, serum periostin, or serum IgE would be more likely to have a severe exacerbation and would result in greater decreases in lung function over time.

METHODS

This study evaluated participants with asthma from 2 previous nonexperimental cross-sectional asthma biomarker studies conducted by the Medical Research Institute of New Zealand.^{9,10} For each of the original studies, participants had baseline measures of lung function, FENO levels, blood eosinophil count, serum periostin levels, and total serum IgE. A total of 15 participants were enrolled in both the original studies, and the baseline data used were from their first original study visit with complete data. Between May 2015 and June 2016, participants were invited to reattend the outpatient facility at the Medical Research Institute of New Zealand for a single further visit, which was at least 12 months after their original baseline measurements.

Ethical approval for this study was given by the New Zealand Ethics Committee (15/CEN/33). The study was prospectively registered with the Australia New Zealand Trial Registry

(ACTRN12615000503527). All participants provided written informed consent before participation.

Clinical data

A full description of study methodology can be found in this article's [Online Repository](http://www.jaci-inpractice.org) at www.jaci-inpractice.org. In brief, participants were asked about severe exacerbations since the baseline visit. A severe exacerbation was identified in accordance with the American Thoracic Society and European Respiratory Society criteria,¹¹ requiring the use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days or a hospitalization or emergency department visit because of asthma requiring systemic corticosteroids. Courses of corticosteroids separated by more than a week were treated as separate severe exacerbations. Patient-reported severe exacerbations, including date, treatment provided, and details of the health care provider, were recorded in an investigator-led questionnaire and then corroborated with general practitioner (GP) and hospital electronic medical records (see this article's [Online Repository](http://www.jaci-inpractice.org) at www.jaci-inpractice.org).

Study size

In total, 330 participants were invited to take part in the study where the assumed attrition rate would be of the order of 30%, anticipating a total of 230 participants would be enrolled. We estimated that this sample size would have 95% CIs for a proportion of 20% (the anticipated proportion of participants with a severe exacerbation) of $\pm 5\%$.

Statistical methods

Multivariate models, adjusting for the participant's original cohort and whether or not they were on ICSs at baseline, were used to estimate the relative differences in time to a severe exacerbation by estimating the hazard ratio between the type 2 biomarkers and time to severe exacerbation using the Cox proportional hazards model. In the analysis, lower hazard ratio corresponds with a longer time to a severe exacerbation.

Comparison of type 2 biomarkers was made between those who had a severe exacerbation and those who did not, using *t* tests. The predictor variables FENO, serum periostin, and serum IgE required logarithm transformation to better meet normal distribution. A ratio of geometric means was used to describe the exponent of these variables. A multiplier of 0.693 represents a doubling of the geometric mean.

Linear regression models were used to describe the relationship between baseline type 2 biomarkers and change in FEV₁, including multivariate analysis adjusting for age, sex, ICS use, and smoking status at baseline. A significant interaction term suggested that the change in FEV₁ with time was dependent on the type 2 biomarker. Statistical software used was SAS version 9.4 (SAS Institute Inc., Cary, NC).

RESULTS

A total of 212 out of the potential 330 participants returned for testing, of which 177 (84%) were from the New Zealand Health Respiratory Survey.⁹ The median (range) time between baseline and follow-up visits was 3.8 (1.1-5.3) years (mean, 2.8 $\pm$ 1.5 years, and median [interquartile range, IQR], 3.4 [1.3-3.9] years), with a total of 642 patient-years of follow-up. The flow of participants through the study is shown in [Figure 1](#). All (212 of 212; 100%) hospital electronic medical records were reviewed to

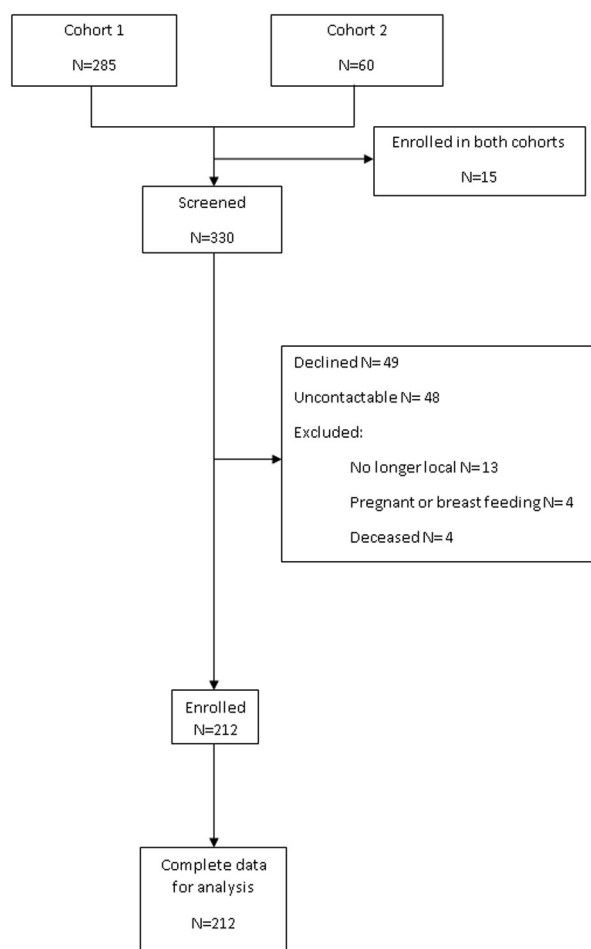


FIGURE 1. Flow of participants through the study.

corroborate hospital attendances and prescriptions of systemic corticosteroids. A total of 22 of 212 (10%) participants did not have GP electronic records reviewed by an investigator because permission was not granted by the GP and 4 of 212 (2%) participants had severe exacerbations outside of New Zealand, which could not be corroborated locally. There were 67 of 212 (32%) participants with at least 1 severe exacerbation of asthma. There were a total of 189 severe exacerbations, representing a rate of 0.29 severe exacerbations per patient-year. The participants had well-controlled asthma of mild to moderate severity (Table I). At baseline/follow-up, there were 41/38 participants prescribed no inhalers, 16/37 participants prescribed a short-acting beta-agonist only, 63/36 prescribed an ICS with short-acting beta-agonist inhaler, and 92/101 participants prescribed an ICS with a LABA. No participants were prescribed maintenance systemic corticosteroids for their asthma, although at follow-up 3 people reported the chronic use of oral corticosteroids for non-asthma-related conditions. The mean scores at baseline for the 5-item Asthma Control Questionnaire and Asthma Quality of Life Questionnaire for Standardized activities were 0.91 and 6.13, respectively. The mean FEV₁ percent predicted was 88.9% and 87.3% at baseline and follow-up visits, respectively.

Association between type 2 variables and time to severe exacerbation

The associations between type 2 biomarkers and time to severe exacerbation are presented in Table II. The multivariate associations are adjusted for which recruitment cohort the participants were from and for use of ICSs or not. There was no statistically significant evidence of an association between the cohort the participants were from and time to severe exacerbation in any of the multivariate analyses. In the univariate analysis, the hazard ratio (95% CI) for time to exacerbation was consistent with the larger original cohort being less at risk: 0.40 (0.21-0.85). However, in all the multivariate models, this estimate of association, once adjusted for whether on ICSs or not and the individual type 2-related measurement, had increased to 0.55 with wide CIs (0.28-1.17).

Peripheral blood eosinophils

Mean blood eosinophil counts were $(0.25 \pm 0.19) \times 10^9/L$ and $(0.24 \pm 0.18) \times 10^9/L$, at baseline and follow-up visits, respectively. There was no significant association between baseline blood eosinophil count and time to severe exacerbation. The adjusted hazard ratio (95% CI) for association between higher eosinophil count and time to severe exacerbation was 0.89 (0.76-1.05) per $0.1 \times 10^9/L$ eosinophil count increase ($P = .17$; Figure 2, A).

FENO

Median (IQR) FENO levels were 22.5 (13.6-44.4) ppb and 22 (15-33) ppb, at baseline and follow-up visits, respectively. The analysis of FENO was on the natural logarithm-transformed scale. The adjusted hazard ratio (95% CI) for association between baseline log FENO and time to severe exacerbation was 0.65 (0.52-0.81), per 0.693 log FENO increase ($P < .001$; Figure 2, B). This 0.693 unit log FENO difference represents a 2-fold change in geometric mean FENO.

Serum periostin

Median (IQR) serum periostin levels were 52.8 (45-65.5) ng/mL and 52.6 (43.7-61.7) ng/mL, at baseline and follow-up visits, respectively. The analysis of serum periostin was on the natural logarithm-transformed scale. The adjusted hazard ratio (95% CI) for association between baseline log serum periostin and time to severe exacerbation was 0.62 (0.35-1.09) per 0.693 log periostin increase ($P = .1$; Figure 2, C).

Serum IgE

Median (IQR) serum IgE levels were 123 (32-262.5) IU/L and 103 (28-243) IU/L, at baseline and follow-up visits, respectively. The analysis of serum IgE was on the natural logarithm-transformed scale. The adjusted hazard ratio (95% CI) for association between baseline log serum IgE and time to severe exacerbation was 0.89 (0.80-1.00) per 0.693 log IgE increase ($P = .05$; Figure 2, D).

ICS use at baseline

There was strong evidence of an association between ICS use at baseline and time to severe exacerbation. In all the models, ICS use was associated with an increased risk of exacerbation. The univariate hazard ratio (95% CI) was 3.86 (1.76-8.48) and the adjusted hazard ratio (95% CI) was 3.66 (1.65-8.08).

TABLE I. Characteristics of participants enrolled in the study

Continuous variable	Mean $\pm$ SD		Difference* (SD)
	Baseline	Follow-up	
Age (y)	50.5 $\pm$ 13.2		
BMI (kg/m ²)	29 $\pm$ 6.1		
ACQ-5 score	0.91 $\pm$ 0.74	0.73 $\pm$ 0.76	−0.17 (0.71)
AQLQ-S score	6.13 $\pm$ 0.92	6.23 $\pm$ 0.81	0.09 (0.75)
FEV ₁ (L)	2.90 $\pm$ 0.91	2.77 $\pm$ 0.87	−0.14 (0.34)
FEV ₁ % predicted (%)	88.9 $\pm$ 19.8	87.3 $\pm$ 18.7	−1.6 (10.2)
Blood eosinophils ($\times 10^9$ /L)	0.25 $\pm$ 0.19	0.24 $\pm$ 0.18	−0.01 (0.15)
FENO (ppb) [†]	22.5 (13.6 to 44.4)	22 (15 to 33)	−0.4 (−9.5 to 5)
Serum periostin (ng/mL) [†]	52.8 (45 to 65.5)	52.6 (43.7 to 61.7)	−2.24 (−8.1 to 4.4)
Serum IgE (IU/L) [†]	123 (32 to 262.5)	103 (23 to 243)	−8 (−74.8 to 8)

Categorical variables	N/212 (%)		Difference (N)
	Baseline	Follow-up	
Ethnicity			
Asian	8 (3.8)	8 (3.8)	
European	189 (89.2)	189 (89.2)	
Maori	8 (3.8)	8 (3.8)	
Pacific	5 (2.4)	5 (2.4)	
Other	2 (0.9)	2 (0.9)	
Female	109 (51.4)	109 (51.4)	
Current smoker	13 (6.1)	13 (6.1)	0
No inhalers	41 (19.3)	38 (17.9)	−3
SABA inhaler only	16 (7.5)	37 (17.5)	21
ICS use	155 (74.1)	137 (64.1)	−18
ICS/LABA	92 (43.4)	101 (47.6)	9

AQLQ-S, Asthma Quality of Life Questionnaire for Standardized activities; BMI, body mass index; SABA, short-acting beta-agonist.

*Calculated as follow-up visit value minus baseline visit value.

[†]Medians (IQR) given here as variables skewed.

TABLE II. The associations between type 2 biomarkers and time to exacerbation

Type 2 biomarkers	Univariate		Multivariate	
	Hazard ratio (95% CI)	P value	Hazard ratio (95% CI)	P value
Per 0.1 unit increase in blood eosinophils	0.92 (0.79-1.07)	.28	0.89 (0.76-1.05)	.17
Per 0.693 unit increase in log FENO	0.64 (0.52-0.79)	<.001	0.65 (0.52-0.81)	<.001
Per 0.693 unit increase in log serum periostin	0.59 (0.33-1.07)	.08	0.62 (0.35-1.09)	.10
Per 0.693 unit increase in log serum IgE	0.93 (0.83-1.04)	0.20	0.89 (0.80-1.00)	.05

The multivariate associations are adjusted for which recruitment cohort the participants were from and for use of ICSs or not.

Change in FEV₁ over time

There was no evidence of an association between the change in FEV₁ over time and baseline FENO, serum periostin, or serum IgE: 0.02 (−0.02 to 0.06), −0.01 (−0.12 to 0.09), and 0.01 (−0.01 to 0.04) difference in FEV₁ per 0.693 increase in log FENO, log periostin, and log IgE, respectively. In a multivariate model, with adjustments made for age, sex, ICS use, and smoking status at baseline, there was strong evidence of an interaction between change in FEV₁ between the follow-up and baseline visits and blood eosinophil count at baseline ($P < .001$). This was best illustrated by plotting predicted change against time, where the blood eosinophil counts of 0.1 and 0.3×10^9 /L represented the first and third quartiles, respectively (Figure 3). Where the baseline blood eosinophil count was “low,” the decrease in FEV₁ was more rapid with time; however, “high”

baseline blood eosinophil counts were associated with an increase in FEV₁ with time. The multivariate model estimated the cutoff point between high and low to be 0.21×10^9 /L.

DISCUSSION

This study found a statistically significant association between high baseline FENO and serum IgE levels and a lower risk of a severe exacerbation in adults with predominantly intermittent asthma of mild to moderate severity. Those who had at least 1 severe exacerbation had lower baseline FENO levels than those who did not exacerbate. The association between high baseline peripheral blood eosinophils and serum periostin levels and time to a severe exacerbation was not statistically significant. A greater decline in FEV₁ was associated with a baseline peripheral blood eosinophil count of less than 0.21×10^9 /L.

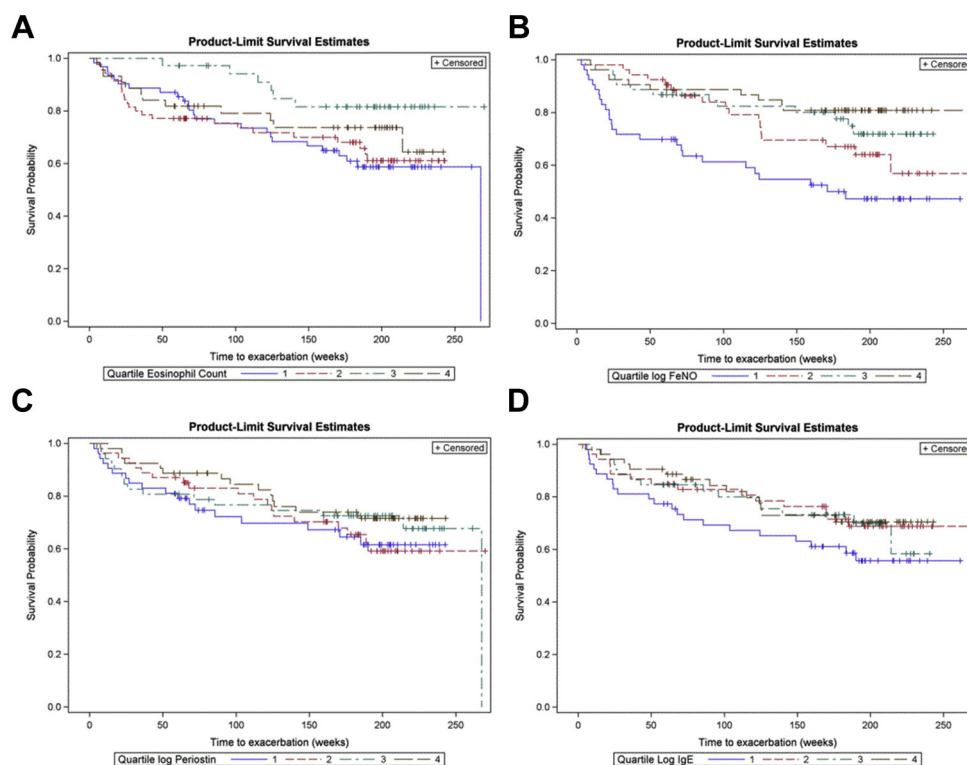


FIGURE 2. Kaplan-Meier survival curves for quartiles of blood eosinophil count (A), log FeNO (B), log serum periostin (C), and log serum IgE (D).

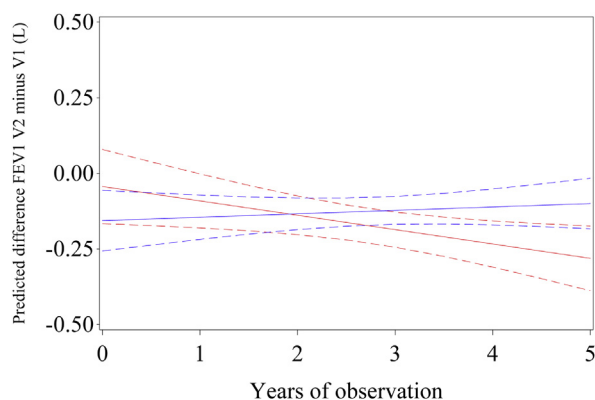


FIGURE 3. Change in FEV₁ with time associated with baseline eosinophil count. The solid red line is the predicted mean rate of change with time for an eosinophil count of 0.1 at baseline. The dotted red lines are the upper and lower confidence limits for the predicted mean. The solid blue line is the predicted mean rate of change with time for an eosinophil count of 0.3 at baseline. The dotted blue lines are the upper and lower confidence limits for the predicted mean. Both prediction models are without the other covariates.

These results do not confirm findings from previous randomized controlled trials of the efficacy of mAb therapies targeting components of the type 2 inflammatory pathways, in populations with severe asthma⁵⁻⁸ or observational cohorts with more severe persistent asthma than those in our study.¹²⁻¹⁴ This

may be due to differences in the population studied, as outlined below. Although we could not identify a statistically significant association between time to severe exacerbation and blood eosinophil or serum periostin levels, the width of the CIs means that we cannot rule out substantial and possibly meaningful associations between time to severe exacerbation and the type 2 measurements. For example, for blood eosinophils the lower 95% confidence limit was 0.76, which could be consistent with a strong association.

The most important consideration in this study is confounding by ICS therapy, which is more likely to be prescribed to patients with more severe persistent disease,⁴ reduces peripheral blood eosinophil, FeNO, and serum periostin levels,^{9,15,16} and reduces the risk of severe exacerbation,^{17,18} with clinical efficacy greater in those with raised blood or sputum eosinophils or FeNO levels.^{19,20} Our finding that ICS use was associated with an increased risk of severe exacerbations suggests that it was likely to be a marker of more severe disease. Furthermore, the observation that low FeNO was associated with both high inhaled fluticasone dose and shorter time to severe exacerbation may suggest that the FeNO was reduced by high-dose inhaled fluticasone therapy, but that other factors associated with severe asthma may confound the association.

The other main difference is that we studied an adult population with predominantly intermittent asthma of mild to moderate severity, with only two-thirds taking ICS therapy, and just less than half on concomitant LABA therapy. In contrast, the randomized controlled studies of mAb therapy that demonstrated that the type 2 biomarkers predicted severe exacerbation risk selected patients with severe refractory asthma,

despite high-dose ICS therapy, with or without maintenance oral corticosteroids, who also required additional controller medications.⁵⁻⁸

Contrasting results have been reported in observational studies such as the National Heart, Lung, and Blood Institute Severe Asthma Program, in which there was a positive association between severe exacerbations of asthma and blood eosinophils, but not FENO, and low serum IgE was associated with severe exacerbation risk.²¹

Our population may also explain the difference from others in our findings of an association between low blood eosinophils levels and a greater decline in lung function, and the lack of an association for either FENO or periostin. In contrast, studies in populations with more severe asthma have shown no consistent association between accelerated decline in lung function and high blood eosinophils,²² FENO,²³ or periostin.²⁴

Limitations of our study include multiplicity of analyses, which may have resulted in type 1 error, but also for some of the associations assessed the CIs were quite wide. There may also have been other factors affecting an individual's risk of severe exacerbation not adjusted for in the analyses, such as baseline ICS dose, exacerbation history, particulate or environmental exposures, treatment adherence, or socioeconomic factors, because these data were not collected at baseline. We did not investigate whether the severe exacerbations were of viral and/or allergic etiology and so differential associations based on exacerbation type could not be undertaken. In addition, this study was not designed to investigate potential mechanisms relating to the pathways associated with the type 2 biomarkers studied; however, the positive results for FENO and IgE and negative results for eosinophils might indicate that the exacerbations in our population could be driven by IL-13 rather than IL-5. With respect to lung function, we did not standardize the bronchodilator withdrawal criteria before lung function testing in either the initial study or the follow-up study, which would have been preferable for an analysis of lung function decline. The follow-up rate of 64% was lower than anticipated and may have resulted in selection bias in our relatively small population.

The patients' reports of severe exacerbations of asthma were corroborated with medical records where possible. All electronic records of severe exacerbations were confirmed by the investigator, regardless of whether the event was reported by the patient. Episodes where there was concordance between the patient-reported date of severe exacerbation and medical records were confirmed by the investigator. Where the dates of severe exacerbations were not concordant with medical records, the patient-reported severe exacerbation was not counted by the investigator, unless the medical records could not be accessed such as severe exacerbations that occurred outside of New Zealand ($n = 4$) or where permission to access medical records was declined by the primary health care provider ($n = 22$). Given these small numbers, we do not anticipate this resulted in significant recall bias with the data.

This study comprised 2 cohorts with asthma from previous studies, which were recruited using different methodologies. The first cohort consisted of randomly selected participants from the electoral role, resulting in the inclusion of patients with mild to moderate asthma. The second cohort consisted of patients with asthma on ICS and LABA therapy, to include those with more severe disease. Despite differences in recruitment methodology

between the cohorts, baseline measurements for both studies were undertaken using the same guidelines and laboratory assays. In addition, all participants underwent baseline testing having been free from upper or lower respiratory tract infections for a minimum of 3 weeks, ensuring stability of disease immediately before measurements were taken.

Whereas type 2 high asthma has been well characterized and described, type 2 low disease is relatively understudied. It is likely to align with the broad group of noneosinophilic asthma, which is an important clinical entity comprising multiple phenotypes with differing inflammatory profiles.²⁵ Patients with type 2 low asthma may have predominantly neutrophilic airway inflammation, which is the most common inflammatory phenotype in severe exacerbations.²⁶ Significantly, type 2 low asthma is relatively resistant to treatment with corticosteroid therapy,^{19,20,27,28} and may be the predominant mechanism in sudden-onset fatal asthma.²⁹ Taken together, it may explain why those with low baseline blood eosinophils were more likely to have a greater decline in lung function at their follow-up visit. Our findings suggest that type 2 low asthma may be highly prevalent among those with mild to moderate asthma in the general population, which has important clinical implications, given its relative steroid insensitivity and lack of alternative treatment strategies available.

We conclude that the positive association between type 2 biomarkers and risk of severe exacerbations in populations with severe refractory asthma may not extend to mild and moderate asthma. This study highlights the importance of type 2 low asthma as a phenotype associated with an increased risk of severe exacerbations in those with mild to moderate asthma and which warrants further study.

Acknowledgments

We thank the study participants for their involvement in this study.

REFERENCES

1. Dougherty RH, Fahy JV. Acute exacerbations of asthma: epidemiology, biology and the exacerbation-prone phenotype. *Clin Exp Allergy* 2009;39:193-202.
2. Crane J, Pearce N, Burgess C, Woodman K, Robson B, Beasley R. Markers of risk of asthma death or readmission in the 12 months following a hospital admission for asthma. *Int J Epidemiol* 1992;21:737-44.
3. Bai TR, Vonk JM, Postma DS, Boezen HM. Severe exacerbations predict excess lung function decline in asthma. *Eur Respir J* 2007;30:452-6.
4. Global Initiative for Asthma — GINA. 2017 GINA Report: Global Strategy for Asthma Management and Prevention; 2017. Available from: <http://ginasthma.org/2017-gina-report-global-strategy-for-asthma-management-and-prevention/>. Accessed January 7, 2018.
5. Pavord ID, Korn S, Howarth P, Bleecker ER, Buhl R, Keene ON, et al. Mepolizumab for severe eosinophilic asthma (DREAM): a multicentre, double-blind, placebo-controlled trial. *Lancet* 2012;380:651-9.
6. Ortega HG, Yancey SW, Mayer B, Gunsoy NB, Keene ON, Bleecker ER, et al. Severe eosinophilic asthma treated with mepolizumab stratified by baseline eosinophil thresholds: a secondary analysis of the DREAM and MENSA studies. *Lancet Respir Med* 2016;4:549-56.
7. Hanania NA, Wenzel S, Rosén K, Hsieh H-J, Mosesova S, Choy DF, et al. Exploring the effects of omalizumab in allergic asthma: an analysis of biomarkers in the EXTRA study. *Am J Respir Crit Care Med* 2013;187:804-11.
8. Wenzel S, Swanson B, Teper A, Hamilton J, Izuhara K, Ohta S, et al. Dupilumab reduces severe exacerbations in periostin-high and periostin-low asthma patients. *Eur Respir J* 2016;48:OA1798.
9. Fingleton J, Travers J, Williams M, Charles T, Bowles D, Strik R, et al. Treatment responsiveness of phenotypes of symptomatic airways obstruction in adults. *J Allergy Clin Immunol* 2015;136:601-9.

10. Semprini R, Caswell-Smith R, Fingleton J, Holweg C, Matthews J, Weatherall M, et al, Periostin Study Team. Longitudinal variation of serum periostin levels in adults with stable asthma. *J Allergy Clin Immunol* 2017;139:1687-1688.e9.
11. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;180:59-99.
12. Malinovschi A, Janson C, Borres M, Alvig K. Simultaneously increased fraction of exhaled nitric oxide levels and blood eosinophil counts relate to increased asthma morbidity. *J Allergy Clin Immunol* 2016;138:1301-8.
13. Schleich FN, Chevremont A, Paulus V, Henket M, Manise M, Seidel L, et al. Importance of concomitant local and systemic eosinophilia in uncontrolled asthma. *Eur Respir J* 2014;44:97-108.
14. Zeiger RS, Schatz M, Dalal AA, Chen W, Sadikova E, Suruki RY, et al. Blood eosinophil count and outcomes in severe uncontrolled asthma: a prospective study. *J Allergy Clin Immunol Pract* 2017;5:144-53.
15. Silkoff PE, McClean P, Spino M, Erlich L, Slutsky AS, Zamel N. Dose-response relationship and reproducibility of the fall in exhaled nitric oxide after inhaled beclomethasone dipropionate therapy in asthma patients. *Chest* 2001;119:1322-8.
16. Anderson WJ, Short PM, Williamson PA, Manoharan A, Lipworth BJ. The inverse agonist propranolol confers no corticosteroid-sparing activity in mild-to-moderate persistent asthma. *Clin Sci* 2014;127:635-43.
17. Suissa S, Ernst P, Benayoun S, Baltzan M, Cai B. Low-dose inhaled corticosteroids and the prevention of death from asthma. *N Engl J Med* 2000;343:332-6.
18. Donahue JG, Weiss ST, Livingston JM, Goetsch MA, Greineder DK, Platt R. Inhaled steroids and the risk of hospitalization for asthma. *JAMA* 1997;277:887-91.
19. Berry M, Morgan A, Shaw DE, Parker D, Green R, Brightling C, et al. Pathological features and inhaled corticosteroid response of eosinophilic and non-eosinophilic asthma. *Thorax* 2007;62:1043-9.
20. Martin MJ, Wilson E, Gerrard-Tarpey W, Meakin G, Hearson G, McKeever TM, et al. The utility of exhaled nitric oxide in patients with suspected asthma. *Thorax* 2016;71:562-4.
21. Denlinger LC, Phillips BR, Ramratnam S, Ross K, Bhakta NR, Cardet JC, et al. Inflammatory and comorbid features of patients with severe asthma and frequent exacerbations. *Am J Respir Crit Care Med* 2017;195:302-13.
22. Brightling CE, George L. Is the eosinophil a leading villain in lung function decline? *Chest* 2015;148:844-6.
23. van Veen IH, ten Brinke A, Sterk PJ, Sont JK, Gauw SA, Rabe KF, et al. Exhaled nitric oxide predicts lung function decline in difficult-to-treat asthma. *Eur Respir J* 2008;32:344-9.
24. Kanemitsu Y, Matsumoto H, Izuhara K, Tohda Y, Kita H, Horiguchi T, et al. Increased periostin associates with greater airflow limitation in patients receiving inhaled corticosteroids. *J Allergy Clin Immunol* 2013;32:305-12.
25. Wenzel SE. Asthma phenotypes: the evolution from clinical to molecular approaches. *Nat Med* 2012;18:716-25.
26. Fahy JV, Kim KW, Liu J, Boushey HA. Prominent neutrophilic inflammation in sputum from subjects with asthma exacerbation. *J Allergy Clin Immunol* 1995;95:843-52.
27. Cowan DC, Taylor DR, Peterson LE, Cowan JO, Palmay R, Williamson A, et al. Biomarker-based asthma phenotypes of corticosteroid response. *J Allergy Clin Immunol* 2015;135:877-83.
28. Green RH, Brightling CE, Woltmann G, Parker D, Wardlaw AJ, Pavord ID. Analysis of induced sputum in adults with asthma: identification of subgroup with isolated sputum neutrophilia and poor response to inhaled corticosteroids. *Thorax* 2002;57:875-9.
29. Sur S, Crotty TB, Kephart GM, Hyma BA, Colby TV, Reed CE, et al. Sudden-onset fatal asthma: a distinct entity with few eosinophils and relatively more neutrophils in the airway submucosa? *Am Rev Respir Dis* 1993;148:713-9.

ONLINE REPOSITORY METHODS

Study population

This study followed up participants from 2 previous observational studies conducted by the Medical Research Institute of New Zealand. The New Zealand Respiratory Health Survey was a 3-phase cross-sectional study that enrolled 451 adults randomly selected from the electoral roll between November 2010 and August 2012, of whom 285 had a doctor's diagnosis of asthma and complete data.^{E1} The second study enrolled 60 adults with stable asthma, on ICS and LABA therapy, who had participated in a study of the longitudinal variation of serum periostin levels, and were recruited between March 2014 and February 2015.^{E2} A total of 15 participants were enrolled in both studies, and the baseline data used were from their first visit with complete data. For each study, participants had baseline measures of lung function, FENO levels, blood eosinophil count, serum periostin levels, and total serum IgE. Participants were invited to reattend the outpatient facility at the Medical Research Institute of New Zealand for a single follow-up visit, at least 12 months after their baseline measurements, which took place from May 2015 to June 2016.

Inclusion and exclusion criteria

Follow-up visits were rescheduled if participants reported sustaining a bone fracture, undergoing major general or dental surgery, or taking systemic corticosteroids within 3 months of the study visit. In addition, visits were rescheduled if participants reported an upper or lower respiratory tract infection in the 3 weeks before the study visit. Participants were excluded from follow-up if they were pregnant or breast-feeding.

Clinical data

Respiratory history. All participants were asked about severe exacerbations since the baseline visit. A severe exacerbation was identified in accordance with the American Thoracic Society (ATS) and European Respiratory Society criteria,^{E3} requiring the use of systemic corticosteroids (tablets, suspension, or injection), or an increase from a stable maintenance dose, for at least 3 days or a hospitalization or emergency department visit because of asthma requiring systemic corticosteroids. Courses of corticosteroids separated by more than a week were treated as separate severe exacerbations. Patient-reported exacerbations, including date, treatment provided, and health care provider details, were recorded in an investigator-led questionnaire and then corroborated with GP and hospital electronic medical records.

A patient-reported severe exacerbation was counted if there was a prescription of systemic corticosteroids for 3 or more days for the treatment of asthma in the patient's medical records, in concordance with the date provided by the patient. In the case of patient-reported self-medication, where patients followed an

existing acute management plan of taking systemic corticosteroids from a home rescue pack, GP medical records were checked for appointments or prescriptions where the rescue pack medication would have been replaced. In both cases, if there was no evidence of prescriptions for systemic corticosteroid, the patient-reported event was discounted. Regarding occasions where the severe exacerbation occurred abroad and no supporting documentation was accessible, the patient-reported exacerbations were counted. In the event that GP or hospital records were not available, all patient-reported exacerbations were counted.

The remaining questionnaires used were the Asthma Control Questionnaire and the Asthma Quality of Life Questionnaire for Standardized activities to establish respiratory health status and disease control.

Lung function. Spirometry was performed using a MasterScreen Pneumo (Masterscreen Version 2.0, Carefusion, Hoeberg, Germany), in accordance ATS guidelines.^{E4} Global Lung Initiative reference ranges were used.^{E5}

Biomarkers. Blood was drawn for complete blood cell count and differential (Sysmex platform, Mundelein, Ill), and creatinine, electrolytes, and serum IgE (Roche, Cobas 501, NZ) analyses, which were performed immediately in the local laboratory. Blood for serum periostin measurement was coagulated and centrifuged and serum aliquots stored at -80°C for analysis using the Elecysis periostin immunoassay (Roche Diagnostics, Penzberg, Germany) described previously.^{E6} FENO was measured using a NIOX MINO monitor (Aerocrine AB, Solna, Sweden) in accordance with ATS guidelines.^{E7}

REFERENCES

- E1. Fingleton J, Travers J, Williams M, Charles T, Bowles D, Strik R, et al. Treatment responsiveness of phenotypes of symptomatic airways obstruction in adults. *J Allergy Clin Immunol* 2015;136:601-9.
- E2. Semprini R, Caswell-Smith R, Fingleton J, Holweg C, Matthews J, Weatherall M, et al, Periostin Study Team. Longitudinal variation of serum periostin levels in adults with stable asthma. *J Allergy Clin Immunol* 2017;139:1687-1688.e9.
- E3. Reddel HK, Taylor DR, Bateman ED, Boulet L-P, Boushey HA, Busse WW, et al. An official American Thoracic Society/European Respiratory Society statement: asthma control and exacerbations: standardizing endpoints for clinical asthma trials and clinical practice. *Am J Respir Crit Care Med* 2009;180:59-99.
- E4. Miller MR, Hankinson J, Brusasco V, Burgos F, Casaburi R, Coates A, et al. Standardisation of spirometry. *Eur Respir J* 2005;26:319-38.
- E5. Quanjer PH, Stanojevic S, Cole TJ, Baur X, Hall GL, Culver BH, et al, ERS Global Lung Function Initiative. Multi-ethnic reference values for spirometry for the 3-95-yr age range: the global lung function 2012 equations. *Eur Respir J* 2012;40:1324-43.
- E6. Palme S, Christenson RH, Jortani SA, Ostlund RE, Kolm R, Kopal G, et al. Multicenter evaluation of analytical characteristics of the Elecysis® Periostin immunoassay. *Clin Biochem* 2017;50:139-44.
- E7. ATS/ERS recommendations for standardized procedures for the online and offline measurement of exhaled lower respiratory nitric oxide and nasal nitric oxide, 2005. *Am J Respir Crit Care Med* 2005;171:912-30.