



Plasma interleukin-6 concentrations, metabolic dysfunction, and asthma severity: a cross-sectional analysis of two cohorts

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Summary

Background Severe asthma is a complex heterogeneous disease associated with older age and obesity. The presence of eosinophilic (type 2) inflammation in some but not all patients with severe asthma predicts responsiveness to current treatments, but new treatment approaches will require a better understanding of non-type 2 mechanisms of severe asthma. We considered the possibility that systemic inflammation, which arises in subgroups of obese and older patients, increases the severity of asthma. Interleukin-6 (IL-6) is a biomarker of systemic inflammation and metabolic dysfunction, and we aimed to explore the association between IL-6 concentrations, metabolic dysfunction, and asthma severity.

Methods In this cross-sectional analysis, patients were recruited from two cohorts: mainly non-severe asthmatics from the University of California San Francisco (UCSF) and mainly severe asthmatics from the Severe Asthma Research Program (SARP). We generated a reference range for plasma IL-6 in a cohort of healthy control patients. We compared the clinical characteristics of asthmatics with plasma IL-6 concentrations above (IL-6 high) and below (IL-6 low) the upper 95% centile value for plasma IL-6 concentration in the healthy cohort. We also compared how pulmonary function, frequency of asthma exacerbations, and frequency of severe asthma differed between IL-6 low and IL-6 high asthma populations in the two asthma cohorts.

Findings Between Jan 1, 2005, and Dec 31, 2014, we recruited 249 patients from UCSF and between Nov 1, 2012, and Oct 1, 2014, we recruited 387 patients from SARP. The upper 95th centile value for plasma IL-6 concentration in the healthy cohort ($n=93$) was 3.1 pg/mL , and 14% (36/249) of UCSF cohort and 26% (102/387) of the SARP cohort had plasma IL-6 concentrations above this upper limit. The IL-6 high patients in both asthma cohorts had a significantly higher average BMI ($p<0.0001$) and a higher prevalence of hypertension ($p<0.0001$) and diabetes ($p=0.04$) than the IL-6 low patients. IL-6 high patients also had significantly worse lung function and more frequent asthma exacerbations than IL-6 low patients (all p values <0.0001). Although 80% (111/138) of IL-6 high asthmatic patients were obese, 62% (178/289) of obese asthmatic patients were IL-6 low. Among obese patients, the forced expiratory volume in 1 s (FEV_1) was significantly lower in IL-6 high than in IL-6 low patients (mean percent predicted FEV_1 68.6% [SD 18.7] vs 76.0% [19.4]; $p=0.001$), and the percentage of patients reporting an asthma exacerbation in the past 1–2 years was higher in IL-6 high than in IL-6 low patients (66% [73/111] vs 48% [85/178]; $p=0.003$). Among non-obese asthmatics, FEV_1 values and the frequency of asthma exacerbations within the past 1–2 years were also significantly worse in IL-6 high than in IL-6 low patients (mean FEV_1 64.2% [SD 22.7] vs 81.1% [20.1] predicted; $p<0.0001$; 59% [16/27] vs 34% [108/320]; $p=0.01$).

Interpretation Systemic IL-6 inflammation and clinical features of metabolic dysfunction, which occur most commonly in a subset of obese asthma patients but also in a small subset of non-obese patients, are associated with more severe asthma. These data provide strong rationale to undertake clinical trials of IL-6 inhibitors or treatments that reduce metabolic dysfunction in a subset of patients with severe asthma. Plasma IL-6 is a biomarker that could guide patient stratification in these trials.

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Introduction

Asthma is a disease with variable clinical features and underlying cellular and molecular mechanisms. Type 2 inflammation is important in asthma, but a significant subgroup of asthma patients do not have type 2 inflammation in their airways and do not respond to treatments targeting this pathway.¹ Many of these type-2 low asthma patients have severe disease and considerable unmet treatment needs.

Obesity is common in patients with severe asthma,² but the mechanism of the association between obesity

and severe forms of asthma is not fully understood. One possibility is that obesity-related systemic inflammation contributes to development of severe asthma. Low-grade systemic inflammation occurs in a subset of obese patients because adipocytes and inflammatory macrophages in adipose tissue secrete a variety of pro-inflammatory cytokines (including interleukin-6 [IL-6]).³ Although this low-grade systemic inflammation is known to be associated with development of insulin resistance, dyslipidaemia, atherosclerosis, type 2 diabetes, and hypertension,³ little attention has been paid

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Research in context

Evidence before this study

A subset of patients with asthma have severe disease, characterized by high medication use, frequent exacerbations, and abnormal lung function. Obesity is common in patients with severe asthma, but the mechanism of the association between obesity and severe forms of asthma is not fully understood. One possibility is that obesity related systemic inflammation contributes to the development of severe asthma. Although this low-grade systemic inflammation is known to be associated with the metabolic diseases of hypertension, atherosclerosis, and type 2 diabetes, the possibility that systemic inflammation could lead to more severe forms of asthma has not been studied in detail.

Interleukin-6 (IL-6) is a biomarker of obesity-related systemic inflammation and metabolic dysfunction. It is also a pleiotropic cytokine and a plausible mediator of airway dysfunction in asthma. Previous studies in small numbers of patients have shown an increase in plasma and serum levels of IL-6 in asthma patients compared with healthy controls, and genetic studies have found associations between single nucleotide polymorphisms at the IL-6 receptor and risk of asthma diagnosis. To date, there has been an absence of research into the relationships between systemic IL-6 inflammation, obesity, and outcomes of asthma severity.

Added value of this study

Our analysis of two cohorts of asthma patients shows that systemic IL-6 inflammation occurs in a subset of asthmatics with obesity and metabolic dysfunction. These IL-6 high asthmatics have much lower lung function and more frequent exacerbations than do IL-6 low asthmatics. Although the average BMI in patients with IL-6 high asthma is higher than in those with IL-6 low asthma, most obese asthma patients are IL-6 low, and metabolic diseases such as hypertension and diabetes occur much more commonly in the IL-6 high subgroups. Notably, asthma is much more severe in obese asthma patients who are IL-6 high than those who are IL-6 low, and this association remains in non-obese asthmatics.

Implications of all the available evidence

Increased systemic IL-6 concentrations, occurring independently of type 2 inflammation, are associated with a severe asthma phenotype. Variability in systemic IL-6 concentrations in obese patients helps to explain the heterogeneity of asthma severity in these patients. Systemic IL-6 inflammation represents an extrapulmonary mechanism of severe asthma, and treatment of low-grade systemic inflammation (including with IL-6 inhibitors) or treatment of metabolic dysfunction represent rational clinical trials to pursue in a subset of patients with severe asthma. Plasma IL-6 is a biomarker that could guide patient stratification in the trials.

to the role of systemic inflammation and metabolic dysfunction as a risk for development of severe asthma. Previous studies with small sample sizes have described increased plasma IL-6 concentrations in patients with asthma,⁴ and findings of genetic studies have shown that genes relevant to IL-6 signalling are associated with asthma,⁵ but these studies have not assessed the relation between airway and systemic levels of IL-6 in asthma, the role of systemic IL-6 inflammation in explaining the variable effects of obesity on asthma severity, and the association between IL-6 inflammation and type 2 inflammation in asthma.

Materials and methods

Study design

Participants studied included a reference (healthy) cohort and two asthma cohorts—mainly non-severe asthma patients recruited at University of California, San Francisco (UCSF; n=249) and mainly severe asthma patients recruited by the Severe Asthma Research Program (SARP; n=387). These cohorts were well characterised with data available for outcomes related to lung function, asthma control, and asthma exacerbations, and data for metabolic health. Data for history of diabetes was only available in the SARP cohort. Both cohorts also had blood and airway biospecimens available for analysis. The appendix (pp 2, 3) provides details about recruitment methods, subject enrolment, study measurements, and study procedures.

Participants

Healthy control participants and patients with asthma had been recruited to research studies in the UCSF Airway Clinical Research Center between Jan 1, 2005, and Dec 31, 2014. All studies included one or two baseline visits, which used standardised protocols for clinical characterisation and collection, processing, and storage of blood and induced sputum. Healthy participants had no history of pulmonary disease, no history of atopic disease or allergic rhinitis, and had normal airway responses to inhaled methacholine. Participants in the asthma cohorts had a previous physician diagnosis of asthma and either bronchial hyper-responsiveness to methacholine or reversible airflow obstruction. These participants had no lifetime history of any other pulmonary disease. All participants provided informed consent and they also provided consent for their biospecimens to be placed in a tissue bank for studies in addition to the original protocol.

Adult patients with asthma were recruited to the SARP between Nov 1, 2012, and Oct 1, 2014, by seven clinical research centres (including UCSF) in the USA. The SARP protocol is an ongoing, six-visit, 3-year, longitudinal cohort study in which 60% of participants have severe asthma as defined by the European Respiratory Society/American Thoracic Society (ERS/ATS) criteria.⁶ The SARP protocol included two baseline visits in which patients underwent detailed characterisation studies and provided samples of blood

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See Online for appendix

and induced sputum. The data reported here are from these two baseline visits.

Plasma IL-6 and C-reactive protein measurements

Assays were done at UCSF using aliquots of ethylenediaminetetraacetic acid-treated plasma and high sensitivity human IL-6 Quantikine ELISA Kits (R&D Systems, Minneapolis, MN, USA; lower and upper limit of detection, 0.16 and 10.5 pg/mL, respectively).

To calculate a reference interval for plasma IL-6 in healthy participants, we followed the guidelines of the national committee for clinical laboratory standards.⁷ First, we log transformed plasma IL-6 values in healthy participants to normalise the distribution and to work out the upper 95th centile value as the upper limit of normal (figure 1A). This approach yielded 3.1 pg/mL as the cutoff value.

To explore whether the plasma IL-6 levels detected were associated with downstream markers of IL-6

activity, we measured plasma C-reactive protein (CRP) levels⁸ in a subset (n=123) of the UCSF asthma cohort and a subset (n=38) of the healthy participants.

CRP was measured in plasma from healthy participants from UCSF and UCSF patients with asthma using the Quantikine ELISA kit (R&D Systems, Minneapolis, MN, USA; lower and upper limit of detection, 0.78 and 20 ng/L, respectively).

Induced sputum measures

Total and differential cell counts were quantified in both UCSF and SARP patients using methods previously described.^{9,10} IL-6 protein was measured in induced sputum supernatants from UCSF asthma patients using the human IL-6 DuoSet Kit (R&D Systems Minneapolis, MN, USA; lower limit of detection, 9.38 pg/mL). The reliability of sputum IL-6 protein measures was confirmed by spike and recovery experiments (appendix pp 3,4). IL-6 gene expression was measured from RNA isolated from induced

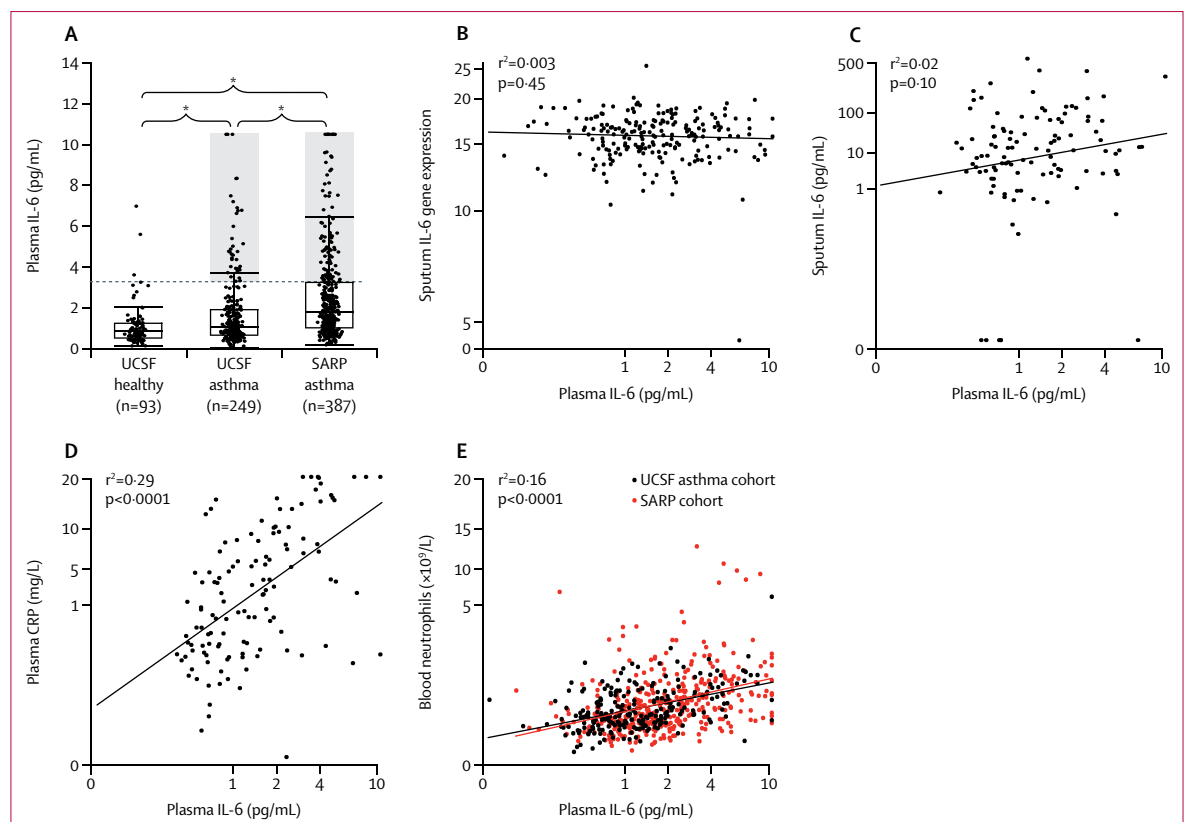


Figure 1: Plasma IL-6 measurements in healthy controls and asthmatics, and the association between blood and airway measures of IL-6

(A) Plasma IL-6 values in UCSF healthy participants, UCSF asthma patients, and SARP asthma patients. The horizontal dashed line indicates the upper 95th centile value for plasma IL-6 in the healthy participants. The shaded areas show the patients who had plasma IL-6 concentrations above the upper reference limit. Horizontal line within the box represents median value, ends of box plots display 1st and 3rd quartiles, and whiskers show values that fall between 1st quartile -1.5 (IQR) and 3rd quartile $+1.5$ (IQR). (B) Correlation between plasma IL-6 protein concentrations and log, normalised gene expression levels for IL-6 in sputum cells (n=210 in the SARP asthma cohort). Black line is line of best fit. (C) Correlation between plasma IL-6 protein concentration and sputum IL-6 protein (n=109, UCSF asthma cohort). Black line is line of best fit. (D) Association between plasma IL-6 concentration and plasma CRP (n=123, UCSF asthma cohort). Black line is line of best fit. (E) Association between plasma IL-6 concentration and blood neutrophils in the UCSF asthma cohort (black symbols and black best-fit line) and the SARP cohort (red symbols and red best-fit line). Best-fit lines were created in each cohort separately and the Pearson coefficient r^2 was calculated with the cohorts combined. CRP=C-reactive protein. IL-6=interleukin-6. UCSF=University of California San Francisco. SARP=Severe Asthma Research Program. * $p<0.05$ from ANOVA comparison.

sputum cell pellets from 210 SARP patients and 55 UCSF patients with asthma using previously described methods.¹¹

Statistical methods

Analyses were done with JMP 10 software package (SAS Institute, Cary, NC, USA) and Stata 12.0 (StataCorp College Station, TX, USA), and p values less than 0.05 were deemed significant. Three-group comparisons between participants in the UCSF healthy, UCSF asthma, and SARP asthma cohorts were made using ANOVA followed by a Bonferroni correction for continuous variables and a Fisher's exact test for categorical variables. Two-group comparisons between IL-6 high and IL-6 low asthma were made with a Student *t* test for numeric variables with approximately normal distributions, a Wilcoxon rank-sum for numeric variables with non-normal distributions, and a Fisher's exact test for dichotomous variables. Correlation was assessed using Pearson correlation for numeric variables with approximately normal distributions. Numeric variables with non-normal distributions were log transformed to achieve normality before Pearson correlation analysis. Best-fit lines were constructed using least-squares regression. Linear and logistic regression modelling was used to assess the relation between natural log transformed plasma IL-6 levels and measures of asthma control, including measures of pulmonary function, asthma exacerbation frequency, and prevalence of severe asthma.

Older age, increased body mass, and increased type 2 inflammation have all been associated with increased plasma IL-6 levels and increased asthma severity. To establish whether these factors confounded the association between plasma IL-6 measures and asthma severity, we did linear and logistic regression models using continuous measures of plasma IL-6 as the predictor of interest. Asthma severity was determined using data from pulmonary function testing and questionnaires that recorded asthma symptoms and asthma exacerbation history. Race, sex, and medication use were controlled for as a sensitivity analysis and inclusion of these variables into linear and logistic regression models did not significantly alter the relationship between IL-6 and asthma severity.

The dependent variables (outcome) for the linear regression models were forced expiratory volume in 1 s (FEV₁), and predicted forced vital capacity (FVC). The dependent variables for the logistic regression modelling were asthma exacerbation (defined as having been prescribed a course of systemic corticosteroids) in the past year (SARP cohort) or 2 years (UCSF asthma cohort), admittance to hospital for asthma in the past year, asthma-related emergency department visit in the past year, and meeting ERS/ATS criteria for severe asthma. Interaction terms were constructed for the interaction between the predictor variables of plasma IL-6 and BMI on the dependent variables of asthma severity. Including these interaction terms did not significantly change the models and therefore were not included. Although all

	Healthy UCSF (N=93)	Asthma UCSF (N=249)	SARP (N=387)
Age (years)	35.9 (12.1)	37.3 (14.2)	47.2 (13.6)*
Women	52 (56%)	155 (62%)	255 (66%)
Ethnicity†			
American Indian and Alaska Native	1 (1%)	4 (2%)	2 (1%)
Asian	16 (17%)	35 (14%)	16 (4%)
African American	3 (3%)	27 (11%)	89 (23%)
White	64 (69%)	155 (62%)	255 (66%)
Native Hawaiian or other Pacific Islander	0 (0%)	3 (2%)	0 (0%)
Mixed race	5 (5%)	17 (7%)	25 (6%)
Unknown/refused to answer	4 (4%)	8 (3%)	0 (0%)
BMI (kg/m ²)	25.4 (5.5)	28.9 (6.9)*	32.3 (8.4)*‡
Use of ICS	..	141 (57%)	345 (89%)‡
Percentage with BMI >30 kg/m ²	15 (16)	82 (33)	207 (53)
Spirometry data			
FEV ₁ (% predicted)	99.4 (13.8)	83.8 (18.3)*	72.3 (20.5)*‡
FVC (% predicted)	102.6 (12.7)	97.7 (15.4)*	85.2 (18.2)*‡
FEV ₁ /FVC	0.97 (0.10)	0.85 (0.12)*	0.83 (0.13)*
ACT score§	..	18.9 (3.9)	16.8 (4.9)†
Blood markers of atopy¶			
Blood eosinophil cell count (×10 ⁶ /L)	133 (106)	292 (220)*	292 (275)*
Serum IgE concentration (IU/mL)	51 (74)	360 (474)*	342 (581)*
FeNO (ppm)	17 (13)	46 (41)*	30 (30)*‡
Asthma exacerbations**			
≥1 exacerbation in past 2 years	..	88 (35%)	..
≥1 exacerbation in past year	..	..	194 (50%)
Necessitating admittance to hospital in past year	..	..	46 (12%)
Necessitating ER visit in past year	..	..	95 (25%)

Data reported as mean (SD) unless otherwise indicated. UCSF=University of California San Francisco. SARP=Severe Asthma Research Program. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. ICS=inhaled corticosteroids. ACT=asthma control test. FeNO=fractional exhaled nitric oxide. PPM=parts per million. ER=emergency room. *Indicates statistically different from UCSF healthy cohort (p<0.05). †Race was significantly different between SARP cohort and either UCSF healthy or UCSF asthma cohorts. ‡Indicates statistically different between UCSF asthma and SARP asthma cohorts (p<0.05). §Scores on ACT range from 5 to 25 with lower scores indicating worse asthma control; 98 of the UCSF patients did not have this score recorded. ¶Two of the UCSF healthy participants and 18 of the UCSF asthma patients did not have blood eosinophils measured; four of the UCSF healthy participants, 18 of the UCSF asthma patients, and four of the SARP asthma patients did not have serum immunoglobulin E levels measured. ||23 of the UCSF healthy participants, 60 of the UCSF asthma patients, and three of the SARP asthmatics did not have FeNO measured. **The UCSF asthma patients completed a questionnaire that asked about asthma exacerbations requiring treatment with prednisone in the past 2 years; the SARP asthma patients completed a questionnaire that asked about asthma exacerbations requiring treatment with prednisone in the past year and also captured information about hospitalisations and ER visits for treatment of asthma in the past year.

Table 1: Baseline characteristics

participants had plasma IL-6 measured, not all participants had data for every study outcome, and analyses used available data (missing data is described in the table footnotes and summarised in the appendix p 6). Ethics approval for this study was obtained by the institutional review boards for each centre.

Role of the funding source

The funding source was research grants awarded by the US National Institutes of Health Heart, Lung, and Blood Institute (NHLBI) and the Parker B Francis Foundation.

	UCSF asthma cohort			SARP cohort		
	IL-6 low (n=213)	IL-6 high (n=36)	p value	IL-6 low (n=285)	IL-6 high (n=102)	p value
Age (years)	36.6 (14.2)	41.6 (13.4)	0.05	46.5 (13.9)	49.1 (12.7)	0.09
Women	133 (62%)	22 (61%)	0.99	176 (62%)	79 (77%)	0.005
Obesity measures						
BMI (kg/m ²)	27.6 (5.7)	36.3 (8.6)	<0.0001	30.3 (6.8)	37.9 (9.8)	<0.0001
BMI ≥30	54 (25%)	28 (78%)	<0.0001	124 (44%)	83 (81%)	<0.0001
Waist circumference (cm)*	..	..	..	96 (16)	111 (18)	<0.0001
Use of ICS	113 (53%)	28 (78%)	0.006	251 (88%)	94 (92%)	0.35
ACT score†	19.2 (3.7)	17.6 (4.5)	0.07	17.4 (4.8)	15.4 (4.8)	0.0002
Spirometry data‡						
FEV ₁ (% predicted)	85.9 (17.3)	71.3 (19.4)	<0.0001	74.4 (20.5)	66.5 (19.6)	0.0008
FEV ₁ bronchodilator reversibility	..	..	..	17.5 (16.7%)	22.2 (20.2%)	0.02
FVC (% predicted)	99.6 (14.2)	86.8 (17.9)	<0.0001	87.9 (18.0)	77.7 (16.8)	<0.0001
FVC bronchodilator reversibility	..	..	..	10.3 (11.6%)	14.1 (13.3%)	0.007
FEV ₁ /FVC	0.86 (0.12)	0.81 (0.13)	0.05	0.83 (0.12)	0.83 (0.14)	0.98
Blood cell counts (×10 ⁶ /L)§						
Total white blood cells	6676 (1641)	8891 (1981)	<0.0001	6868 (2006)	9139 (3161)	<0.0001
Neutrophils	3806 (1324)	5442 (1784)	<0.0001	3992 (1740)	5671 (2600)	<0.0001
Eosinophils	289 (225)	309 (190)	0.62	286 (281)	308 (257)	0.48
Serum IgE measures¶						
Total IgE (IU/mL)	385 (586)	437 (587)	0.63	308 (567)	439 (611)	0.05
≥1 positive ImmunoCap number	..	..	..	228 (81%)	82 (82%)	0.88
FeNO (ppm)	45 (38)	52 (57)	0.44	31 (31)	29 (28)	0.67
Sputum cell counts**						
Eosinophils	4.1 (6.4%)	3.5 (3.8%)	0.65	4.6 (10.2%)	3.1 (5.9%)	0.22
Neutrophils	40.1 (21.4%)	33.7 (20.3%)	0.19	54.2 (24.9%)	57.4 (26.3%)	0.35
Outcomes of metabolic dysfunction††						
History of hypertension	19 (9%)	13 (36%)	<0.0001	77 (27%)	53 (52%)	<0.001
Systolic BP (mm Hg)	115 (11)	122 (13)	0.007	123 (15)	128 (16)	0.005
Diastolic BP (mm Hg)	77 (9)	80 (8)	0.02	77 (10)	78 (11)	0.17
History of diabetes	..	..	..	19 (7%)	14 (14%)	0.04
Asthma exacerbations‡‡						
≥1 exacerbation in past 2 years	68 (32%)	20 (56%)	0.008	..	..	..
≥1 exacerbation in past year	..	..	..	125 (44%)	69 (68%)	<0.0001
Necessitating admittance to hospital in past year	..	..	..	25 (9%)	21 (21%)	0.004
Necessitating ER visit in past year	..	..	..	59 (21%)	36 (35%)	0.005
Asthma severity§§						
Severe	..	..	..	150 (53%)	71 (70%)	..

Data are reported as mean (SD) unless otherwise indicated. UCSF=University of California San Francisco. SARP=Severe Asthma Research Program. IL6=interleukin-6. ICS=inhaled corticosteroids. ACT=asthma control test. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. IgE=immunoglobulin E. FeNO=fractional exhaled nitric oxide. BP=blood pressure. ER=emergency room. *Waist circumference was not available for the UCSF asthma patients. †ACT was not measured in 98 of the UCSF asthma patients. ‡In the SARP asthma patients FEV₁ and FVC were measured before and after a maximum reversibility test using up to eight puffs of albuterol, as detailed in the text; one of the SARP patients did not have this data recorded. §Blood cell counts were not measured in 18 of the UCSF asthma patients. ¶Total serum IgE was not measured in 18 of the UCSF asthma patients and four of the SARP asthma patients. ImmunoCAP Specific IgE is reported as the number and percentage of patients with at least one positive result when tested for 15 specific allergens. ImmunoCap measures were not performed in four SARP asthma patients. ||Measures of FeNO were not made in 60 of the UCSF asthma patients, and three of the SARP asthma patients. **Cell counts in induced sputum cells were not available in 102 UCSF asthma patients and 94 SARP asthma patients. ††Hypertension history data was not recorded in seven of the UCSF asthma patients. Blood pressure measures were not recorded in two of the UCSF asthma patients, and the UCSF patients did not have data for history of diabetes available. ‡‡One UCSF asthma patient did not have exacerbation data recorded. §§The classification of asthma severity was determined using criteria from the European Respiratory Society/American Thoracic Society guidelines.

Table 2: Characteristics of the IL-6 high and IL-6 low asthma cohorts

The NHLBI provided funding for the Severe Asthma Research Program (SARP) and programme officers from NHLBI designed the structure of the SARP. An NHLBI

programme officer is also a member of the SARP steering committee. The NHLBI was not involved in patient recruitment, data collection, data analysis, data

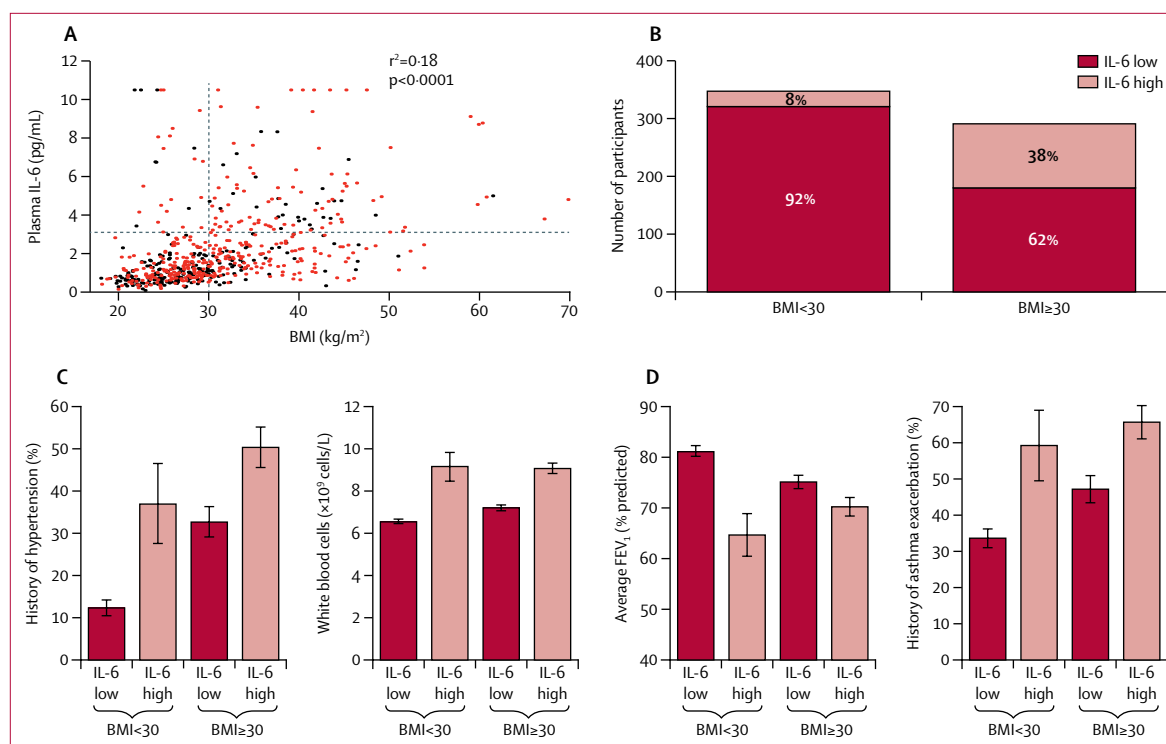


Figure 2: Association between plasma IL-6, metabolic dysfunction, and severity of asthma in both obese and non-obese patients

(A) Correlation between plasma IL-6 levels and BMI in UCSF asthma patients (black dots) and SARP asthma patients (red dots). Pearson coefficient was calculated with the cohorts combined. (B) Proportion of asthma patients with high plasma IL-6 levels and low plasma IL-6 levels stratified by BMI <30 kg/m² and BMI ≥30 kg/m². (C) Metabolic dysfunction in patients with IL-6 high asthma and IL-6 low asthma in both obese and non-obese subgroups. (D) Asthma severity in patients with IL-6 high asthma and IL-6 low asthma in both obese and non-obese subgroups. Error bars represent standard error for binary variables, and standard error of mean for continuous variables.

IL-6=interleukin-6. FEV₁=forced expiratory volume in 1 s.

interpretation, or manuscript preparation. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit the report for publication with the permission of all coauthors.

Results

93 healthy control participants and 249 asthma participants were recruited at UCSF, and 387 asthma participants were recruited from the SARP programme. The UCSF healthy cohort and the UCSF asthma cohort had a similar mean age, but the SARP cohort was older and weighed more than both the UCSF healthy cohort and the UCSF asthma cohort (table 1). The older age and higher bodyweight of the SARP cohort reflects the recruitment methods for SARP, which emphasised inclusion of patients with severe asthma who are known to be older and heavier.

14% (36/249) of the UCSF asthma patients and 26% (102/387) of the SARP asthma patients were IL-6 high—ie, plasma IL-6 higher than 3.1 pg/mL (figure 1A, table 2). Patients with asthma with plasma IL-6 levels lower than 3.1 pg/mL were classified as IL-6 low. Among patients with asthma we noted no significant correlation between plasma IL-6 measurements and sputum IL-6 gene expression measurements ($r^2=0.003$; $p=0.45$;

figure 1B) and sputum IL-6 protein measurements ($r^2=0.02$; $p=0.10$; figure 1C), making airway cells an unlikely source of the high systemic IL-6.

Median CRP concentrations were significantly higher in patients with asthma than in healthy controls (median 1.6 mg/L [IQR 0.5–5.0] vs 0.5 mg/L [0.3–1.9]; $p=0.004$). CRP concentrations were also higher in IL-6 high asthmatics than in IL-6 low asthmatics (median 12.9 mg/L [IQR 2.2–20.9] vs 1.1 mg/L [0.5–3.4]; $p<0.0001$) and significantly correlated with plasma IL-6 levels ($r^2=0.29$; $p<0.0001$; figure 1D). Plasma IL-6 measures were also significantly correlated with blood neutrophils ($r^2=0.16$; $p<0.0001$; figure 1E).

In the SARP cohort, significantly more of the participants with IL-6 high asthma were female (table 2); but ethnicity did not differ between IL-6 high and IL-6 low subgroups in either asthma cohort. Compared to those with IL-6 low asthma, patients with IL-6 high asthma in both the UCSF asthma and SARP cohorts had significantly higher BMI, a higher prevalence of hypertension, and higher values for systolic blood pressure and blood leucocytes (table 2, appendix p 7). Diabetes in the SARP cohort was recorded more frequently in the IL-6 high subgroup than in the IL-6 low subgroup (history of diabetes was not measured in UCSF

cohort). FEV₁ and FVC values in both asthma cohorts were lower in the IL-6 high subgroups than the IL-6 low subgroups (table 2), and the maximum bronchodilator reversibility test in the SARP cohort showed large increases in FEV₁ and FVC after treatment with bronchodilators (table 2; appendix p 8). The percentage of patients who had severe asthma according to ERS/ATS criteria was higher in the IL-6 high subgroup than the IL-6 low subgroup in the SARP cohort. Furthermore, asthma exacerbations were more frequent and ACT scores were lower in the IL-6 high subgroups in both cohorts than in the IL-6 low subgroups (table 2, appendix p 8). Sputum eosinophil percentage, blood eosinophil numbers, sputum neutrophil percentage, fractional exhaled nitric oxide (FeNO), and serum concentrations of immunoglobulin E in both cohorts did not differ significantly in the IL-6 high and IL-6 low subgroups (table 2).

Plasma IL-6 was significantly and positively correlated with BMI in both cohorts (figure 2A), and 78% (28/36) and 81% (83/102) of the IL-6 high subgroups in the UCSF and SARP asthma patients were obese, respectively (table 2). But 62% (178/289) of obese patients had low plasma IL-6 (figure 2B), and this prompted us to explore more deeply the association between IL-6 inflammation, metabolic dysfunction, and asthma severity in obese and non-obese patients. To do this, we compared outcomes of metabolic dysfunction and

asthma severity in patients with IL-6 high and IL-6 low asthma, and we stratified patients into obese and non-obese subgroups.

We studied associations between IL-6 levels, metrics of asthma severity, including pulmonary function, frequency of asthma exacerbations, and criteria for severe asthma, while controlling for BMI in regression models. Compared with obese IL-6 low asthma patients, we noted that obese IL-6 high asthma patients had characteristics of metabolic dysfunction, including a frequent history of hypertension, and increases in total blood leucocytes and blood neutrophils (table 3). Asthma outcomes were also consistently worse in obese IL-6 high asthma patients than in obese IL-6 low asthma patients (figure 2 C, D, table 3). Furthermore, we noted that indicators of metabolic dysfunction (hypertension and increased blood leucocytes) and more severe asthma (worse pulmonary function and increased exacerbations) were also more prevalent among non-obese IL-6 high asthmatics compared with non-obese IL-6 low asthmatics (table 3), suggesting that IL-6 levels are associated with metabolic dysfunction and severe asthma, even in the absence of obesity.

We found that the relationship between increased IL-6 and worse asthma remained robust even after controlling for multiple covariates including (BMI, age, blood eosinophil cell counts, and chronic systemic corticosteroid use; table 4).

	BMI <30 kg/m ²			BMI ≥30 kg/m ²		
	IL-6 low (n=320)	IL-6 high (n=27)	p value	IL-6 low (n=178)	IL-6 high (n=111)	p value
Age (years)	41.1 (15.4)	49.0 (17.6)	0.01	44.3 (13.2)	46.7 (12.1)	0.12
Women	199 (62%)	11 (41%)	0.04	110 (62%)	90 (81%)	0.0006
BMI (kg/m ²)	25.4 (2.9)	25.5 (2.3)	0.80	36.0 (5.5)	40.4 (8.2)	<0.0001
Spirometry data						
FEV ₁ (% predicted)	81.1 (20.1)	64.2 (22.7)	<0.0001	76.0 (19.4)	68.6 (18.7)	0.001
FVC (% predicted)	95.6 (16.9)	81.9 (21.3)	<0.0001	88.1 (17.4)	79.7 (16.5)	<0.0001
FEV ₁ /FVC	0.84 (0.12)	0.76 (0.14)	0.002	0.85 (0.12)	0.84 (0.13)	0.85
Use of ICS	223 (70%)	23 (85%)	0.12	141 (79%)	99 (89%)	0.04
ACT score*	18.5 (4.1)	16.6 (4.7)	0.03	16.9 (5.1)	15.6 (4.8)	0.04
≥1 asthma exacerbation in the past 1–2 years†	108 (34%)	16 (59%)	0.01	85 (48%)	73 (66%)	0.003
Blood cell counts (×10 ⁶ /L)‡						
Total blood leucocytes	6557 (1750)	9137 (3532)	<0.0001	7191 (1992)	9064 (2755)	<0.0001
Neutrophils	3734 (1479)	5804 (3117)	<0.0001	4232 (1710)	5568 (2230)	<0.0001
Eosinophils	305 (276)	367 (270)	0.19	268 (245)	293 (233)	0.39
Outcomes of metabolic dysfunction§						
History of hypertension	39 (12%)	10 (37%)	0.002	57 (33%)	56 (51%)	0.004
Systolic BP (mm Hg)	117 (13)	122 (16)	0.05	125 (14)	127 (15)	0.22
Diastolic BP (mm Hg)	75 (9)	75 (9)	0.96	80 (10)	80 (11)	0.79

Data are mean (SD), unless otherwise indicated. IL-6=interleukin-6. UCSF=University of California San Francisco. SARP=Severe Asthma Research Program. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. ICS=inhaled corticosteroids. ACT=asthma control test. BP=blood pressure. *ACT scores were not available for 98 patients. †See footnote to table 1 for details about questionnaire data for asthma exacerbations. ‡Blood cell counts were not available for 18 patients. §Hypertension history data were not available for seven patients.

Table 3: Clinical variables in IL-6 high and IL-6 low asthma subgrouped by obesity

	UCSF asthma cohort (n=249)				SARP cohort (n=387)				
	Unadjusted	Model 1	Model 2	Model 3*	Unadjusted	Model 1	Model 2	Model 3	Model 4
FEV ₁ (% predicted)	-6.7% (-9.5 to -4.0); p<0.0001	-6.0% (-9.2 to -2.8); p=0.0003	-4.5% (-7.6 to -1.4); p=0.0051	-4.4% (-7.4 to -1.3); p=0.0050	-8.1% (-10.5 to -5.6); p<0.0001	-8.3% (-11.1 to -5.5); p<0.0001	-6.4% (-9.2 to -3.6); p<0.0001	-6.2% (-9.1 to -3.4); p<0.0001	-4.9% (-7.6 to -2.1); p=0.0006
FVC (% predicted)	-6.2% (-8.5 to -4.0); p<0.0001	-4.7% (-7.3 to -2.0); p=0.0006	-3.5% (-6.1 to -0.9); p=0.0087	-3.3% (-5.9 to -0.7); p=0.0133	-8.4% (-10.6 to -6.3); p<0.0001	-7.6% (-10.0 to -5.2); p<0.0001	-5.4% (-7.8 to -3.0); p<0.0001	-5.7% (-8.1 to -3.2); p<0.0001	-4.6% (-7.0 to -2.2); p=0.0003
Asthma exacerbations									
≥1 exacerbation past 2 years†	1.4 (1.0 to 1.9); p=0.04	1.4 (1.0 to 2.1); p=0.07	1.5 (1.0 to 2.1); p=0.06	1.5 (1.0 to 2.4); p=0.04	..	..	..	..	..
≥1 exacerbation past year	..	..	..	..	2.2 (1.7 to 2.9); p<0.0001	2.0 (1.4 to 2.7); p<0.0001	1.9 (1.4 to 2.6); p=0.0001	2.0 (1.4 to 2.7); p=0.0001	1.7 (1.2 to 2.4); p=0.0022
≥1 admittance to hospital for asthma in past year	..	..	..	..	2.0 (1.4 to 3.0); p=0.0005	2.0 (1.3 to 3.0); p=0.0029	2.0 (1.3 to 3.2); p=0.0021	2.2 (1.4 to 3.4); p=0.0009	1.8 (1.1 to 3.0); p=0.0248
≥1 visit to ER for asthma in past year	..	..	..	..	1.9 (1.4 to 2.5); p=0.0001	1.5 (1.1 to 2.1); p=0.0179	1.6 (1.1 to 2.3); p=0.0083	1.7 (1.2 to 2.5); p=0.0033	1.5 (1.0 to 2.2); p=0.0325
Severe asthma‡	..	..	..	..	2.3 (1.7 to 3.1); p<0.0001	2.2 (1.6 to 3.0); p<0.0001	1.9 (1.4 to 2.7); p=0.0001	2.2 (1.5 to 3.3); p<0.0001	1.8 (1.3 to 2.6); p=0.0013

Data are the effect per one natural log increase in plasma IL-6 levels (pg) on % predicted measures of FEV₁ and FVC and odds ratio for asthma exacerbation and severe asthma (95% CI) and p value. UCSF=University of California San Francisco. SARP=Severe Asthma Research Program. ICS=inhaled corticosteroids. ER=emergency room. OR=odds ratio. FEV₁=forced expiratory volume in 1 s. FVC=forced vital capacity. *Effect size per one natural log increase in plasma IL-6 concentrations (pg/mL). Linear and logistic regression models for UCSF and SARP datasets. 18 UCSF asthma participants had blood cell counts missing. †One UCSF asthma participant did not have exacerbation data recorded. ‡Severe asthma determined by criteria from the European Thoracic Society/American Thoracic Society guidelines. Model 1 adjusts for the covariate of BMI. Model 2 adjusts for the covariates BMI and age. Model 3 adjusts for the covariates BMI, age, blood eosinophil cell counts, and ICS use. Model 4 adjusts for the covariates BMI, age, blood eosinophil cell counts, and chronic systemic corticosteroid use.

Table 4: Association between continuous plasma IL-6 measures and outcomes of asthma severity, controlled for confounding variables

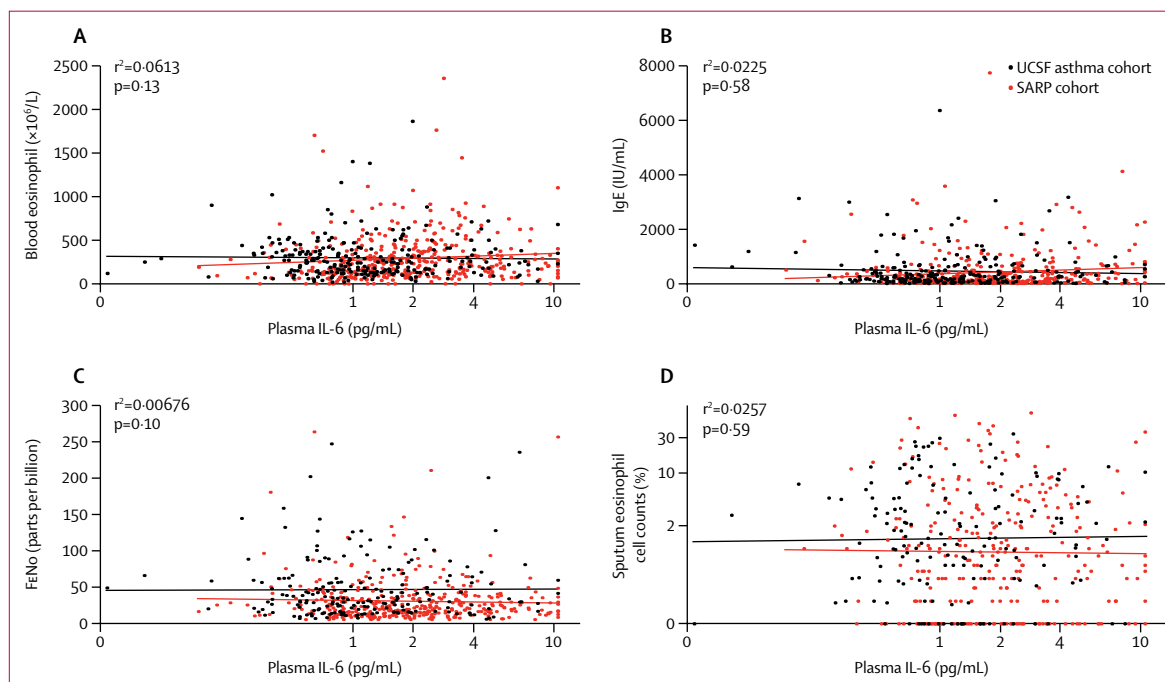


Figure 3: Association between plasma IL-6 measures and measures of type 2 inflammation in asthma

Correlation between (A) Plasma IL-6 concentrations and blood eosinophil cell counts. (B) Plasma IL-6 concentrations and serum IgE concentrations. (C) Plasma IL-6 concentrations and exhaled nitric oxide levels. (D) Plasma IL-6 concentrations and sputum eosinophil cell percentages. Best-fit lines were created in each cohort separately and the Pearson coefficient was calculated with the cohorts combined. UCSF asthma cohort is represented by black symbols and black best-fit lines and the SARP cohort is represented by red symbols and red best-fit lines. IgE=immunoglobulin E. IL-6=interleukin-6. UCSF=University of California San Francisco. SARP=Severe Asthma Research Program. FeNO=fraction of nitric oxide in exhaled air.

Using a Pearson correlation coefficient, we found no significant association between plasma IL-6 and biomarkers of type 2 inflammation in blood or airway biospecimens, including blood eosinophils ($r^2=0.004$; $p=0.13$), blood immunoglobulin E concentrations ($r^2=0.0005$; $p=0.58$), FeNO ($r^2=0.046$; $p=0.10$), and sputum eosinophil concentrations ($r^2=0.0007$; $p=0.59$; figure 3A–D).

The associations that are supported by our findings are shown in the modified directed acyclic graph in the appendix (p 9), which shows that IL-6 is not related to type 2 inflammation.

Discussion

An important concept in asthma is that mechanisms of disease that originate in the lung drive airway pathology and airway dysfunction and explain the marked heterogeneity in phenotypes. In particular, there has been a strong emphasis on research into mechanisms of type 2 inflammation in asthma, which has led to new treatments targeting various members of the type 2 pathway. However, many patients with asthma do not have type 2 inflammation and these patients will not benefit from treatments directed at type 2 inflammation.^{12,13} New insights into mechanisms of non-type 2 inflammation are now needed as a first step towards developing treatment options for patients with type-2 low asthma. Unfortunately, efforts so far to uncover new disease mechanisms using experimental approaches that focus on analyses of lung biospecimens have not been very productive. Here, we report that systemic IL-6 inflammation occurs in a large subgroup of patients with asthma, most of whom are obese. We also report that systemic IL-6 inflammation is a biomarker for patients who have both metabolic dysfunction and severe asthma. Taken together, our findings help to explain the heterogeneous effects of obesity on disease severity in asthma and point to treatment of systemic IL-6 inflammation and metabolic dysfunction as novel therapeutic strategies for asthma.

More than 80% of the IL-6 high asthma patients across our two asthma cohorts were obese, but only 38% of obese asthma patients had high plasma IL-6 concentrations. The subset of obese asthma patients with high IL-6 concentrations were more likely to have hypertension and systemic leucocytosis, which are indicators of metabolic dysfunction.^{14,15} We recorded the highest signals for asthma severity in this IL-6 high subset of obese asthmatics with metabolic dysfunction. Taking IL-6 as a biomarker of metabolic dysfunction,¹⁶ our findings show that asthma in obese patients with metabolic dysfunction is more severe than in obese patients without metabolic dysfunction. This finding helps to explain why obesity is associated with severe asthma in only some patients, and it emphasises the importance of metabolic and inflammatory complications of obesity over complications arising from chest wall

loading from excessive body mass. Our data also suggest that asthma in non-obese patients with metabolic dysfunction is more severe than in non-obese patients without metabolic dysfunction. This interpretation is consistent with our results showing that the relationships between plasma IL-6 and measures of asthma severity remain significant when BMI is controlled for in regression models.

We noted no association between plasma measures of IL-6 and measures of type 2 inflammation (airway and systemic) in the two asthma cohorts and no relation between measures of plasma IL-6 protein and measures of IL-6 gene transcripts or IL-6 protein in the airway. These findings suggest that the increase in systemic IL-6 concentrations in IL-6 high asthma does not happen as a result of upstream type 2 inflammation or of spillover of IL-6 inflammation from the lungs. Overall, our findings suggest a so-called outside-in mechanism of lung dysfunction in IL-6 high asthma in which the pro-inflammatory mediators driving disease severity originate in extrapulmonary organs. For example, the origin of the increased IL-6 in IL-6 high asthma is most likely inflammatory macrophages in white adipose tissue.¹⁷ Circulating IL-6 could mediate airway pathology through effects on endothelial cells, epithelial cells, and other structural airway cells.^{18,19} It could also affect T-cell function, including regulatory T cells and Th17 cells.^{20,21}

Another outside-in mechanism of lung dysfunction in IL-6 high asthma could include insulin resistance, a consequence of metabolic dysfunction. Insulin resistance has been linked to poor lung function in asthma.²² It has previously been suggested that the upregulation of insulin-related metabolic signalling cascades has adverse consequences for smooth muscle cells and other airway structural cells.²³ Finally, we acknowledge that IL-6 is not the only cytokine that is upregulated in patients with metabolic dysfunction. Cytokines such as TNF- α or leptin might also be increased in patients with IL-6 high asthma, and the activities of these cytokines could be a mechanism of disease in IL-6 high asthma.

Because IL-6 is a pro-inflammatory factor, differentiation factor, and growth factor,²⁴ it is a therapeutic target in rheumatoid arthritis,²⁵ systemic juvenile arthritis,²⁶ and Crohn's disease.²⁷ Our data provide a rationale for also testing the efficacy of IL-6 inhibitors in severe asthma.²⁸ Additionally, because IL-6 is a marker of metabolic dysfunction, we provide a rationale for exploring the efficacy of treatments for metabolic dysfunction as strategies to lessen disease severity in asthma.

Our findings suggest a role for systemic IL-6 inflammation as a mediator of disease severity in a subgroup of patients with asthma. This role might be causal according to Hill's criteria for causality,²⁹ based on the large effect size, the consistent finding of an association between IL-6 and asthma severity in two asthma cohorts, the specific clinical features of the IL-6 high subgroup, the clear dose response for IL-6

measures and outcomes of asthma severity, and the biological plausibility for IL-6 as a mediator of asthma pathology. However, for definitive conclusions to be made about whether IL-6 has a causal role in mediating asthma severity, the results of randomised controlled trials testing the efficacy of IL-6 inhibition in asthma will be needed. We provide a rationale for such trials here and our findings suggest that plasma IL-6 could be used as a biomarker to identify patients with IL-6 high asthma.

Contributions

MCP and JVF conceived and designed the study. KWM, GAH, ATH, BDL, EI, BRP, DTM, SAC, SCE, MWJ, NNJ, AMC, MC, FH, SEW, PGW, and ERB made substantial contributions to the design and analysis plan of the study. MCP, JVF, and BRP analysed the data. MCP and JVF prepared the first draft of the report and all authors revised the draft for important intellectual content.

Declaration of interests

MCP has served as a speaker for Amgen. EI has received research support from Genentech; non-financial support from Boehringer Ingelheim, GlaxoSmithKline, Merck, Sunovion, and Teva; served as a speaker for Merck; provided expert testimony on behalf of Campbell, Campbell, Edwards and Conroy, Crammer, Bishop and O'Brien, Ficksman and Conley, Fox Rothchild, LLP, Ryan Ryan Deluca LLP; served as a consultant for AstraZeneca, Novartis, Philips Respiromics, and Regeneron Pharmaceuticals; and received royalties from UpToDate, and received travel and meeting expenses from Research in Real Life and TEVA Specialty Pharmaceuticals. DTM has received non-financial support from Boehringer Ingelheim, GlaxoSmithKline, Merck, Sunovion, and TEVA. SCE is Chair of the American Board of Internal Medicine Pulmonary Board. MC has received research support from Genentech, Amgen, Teva, Novartis, GlaxoSmithKline, Sanofi-Aventis, Vectura, Medimmune, Johnson & Johnson, Invivo, Pfizer, and KaloBios; served as consultant for Boston Scientific, Holaira, Neostem, and Teva; served as speaker for GlaxoSmithKline, Genentech, Boston Scientific, Boehringer Ingelheim, and Teva; has received royalties from Elsevier; and holds stock in Sparo. SEW has received research support from Amgen, AstraZeneca, GlaxoSmithKline, Sanofi-Aventis, and Boehringer Ingelheim; and has received personal fees from Actelion, AstraZeneca, GlaxoSmithKline, Novartis, and Boehringer Ingelheim. PGW has received research support from Genentech, served on advisory boards for Genentech, Johnson & Johnson, and Neostem; and served as consultant for Roche, AstraZeneca, and Novartis. ERB has served as consultant for Amgen, AstraZeneca, Medimmune, Boehringer Ingelheim, Pfizer, Forest, Genentech, Novartis, Roche, GlaxoSmithKline, Merck, Regeneron, and Sanofi-Aventis; received research support from Amgen, AstraZeneca, Medimmune, Boehringer Ingelheim, Pfizer, Forest, Genentech, Roche, Novartis, Roche, GlaxoSmithKline, Teva, Jenssen, Johnson & Johnson, and Sanofi-Aventis. JVF has served as consultant for Boehringer Ingelheim and Dynavax and Medimmune and has received research support from Pfizer and Genentech. All other authors declare no competing interests.

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