METHODS

SARP patients

Adult asthmatic patients were recruited to SARP from November 1, 2012, to October 1, 2015, by 7 clinical research centers (including UCSF) across the United States. Recruitment techniques varied by study site and included use of physician-to-physician letters, physician-to-patient letters, newspaper advertisements, direct mail, television advertising, poster flyers in the local community and clinics, mass e-mail to students/faculty, and use of recruitment databases.

The SARP protocol included 2 to 3 baseline characterization visits in which patients underwent detailed characterization and provided a sample of venous blood and induced sputum. Inclusion criteria mandated that at least 60% of patients met the American Thoracic Society/European Respiratory Society definition for severe asthma. The medication requirements for this definition included treatment with either continuous or near-continuous systemic corticosteroids or high-dose ICSs (>880 μg of fluticasone daily or equivalent) for at least half of the past year and continuously for the prior 3 months, plus a second controller medication (ie, a long-acting β-agonist or leukotriene modifier). In addition to the medication requirement, patients with severe asthma had to meet at least 1 minor criterion that indicated persistence of significant asthma impairment or report that their symptoms deteriorated immediately after a medication taper. The remaining 40% of patients with asthma did not meet the criteria for severe asthma and were classified as having nonsevere asthma. All patients were nonsmokers (<10 pack-years of tobacco use if >30 years of age; <5 pack-years if <30 years of age) and required to have evidence of bronchial hyperresponsiveness (defined as a PC_{20} methacholine value <16 mg/mL) or reversible airflow obstruction, as evidenced by an increase in FEV_1 of 12% or greater after albuterol inhalation (≤720 μg), ipratropium bromide inhalation (136 μg), or both.

Patients were excluded if they were pregnant or breast-feeding during the initial characterization period, had a history of premature birth (<35 weeks gestation), or had a diagnosis of any other chronic pulmonary disorder. SARP patients younger than 18 years of age were excluded from analysis.

Patients completed comprehensive phenotypic characterization similar to patients recruited at UCSF, including a physician-directed history, Asthma Control Test, spirometry, maximum bronchodilator reversibility (see below), complete blood count with cell differential, induced sputum cell counts, serum IgE measurements, and FENO measurements. In addition, patients completed extensive questionnaires that characterized asthma symptoms, quality of life, medication use, and health care use. The SARP protocol included 2 baseline visits in which patients underwent detailed characterization studies and provided samples of venous blood and induced sputum. Data reported here are from these 2 baseline visits. All patients signed an informed consent form approved by their local institutional review board.

Procedures for withholding asthma and allergy medications

SARP asthmatic patients were also asked to hold their bronchodilator medications before spirometric testing. The medication holds for SARP were as follows: short-acting β-agonists, 4 hours; short-acting anticholinergics, 6 hours; long-acting β-agonists, 12 hours; long-acting muscarinic antagonists, 24 hours; and leukotriene modifiers, 24 hours.

Maximum bronchodilator reversibility test (SARP)

Patients were evaluated for baseline spirometry, after which 4 puffs of albuterol (360 μg) were administered. Spirometry was then repeated after 15 minutes. An additional 2 puffs of albuterol (180 μg) were then administered, and spirometry was repeated again after 15 minutes. If the change in FEV_1 from the spirometric maneuver performed after 4 puffs and after 6 puffs was greater than 5%, an additional 2 puffs of albuterol were administered with repeat spirometry after an additional 15 minutes. If the change was less than 5%, the procedure was stopped, and the last maneuver was taken to be the highest achievable measure. No more than 8 puffs of albuterol were administered as part of the maximum bronchodilator reversibility procedure.

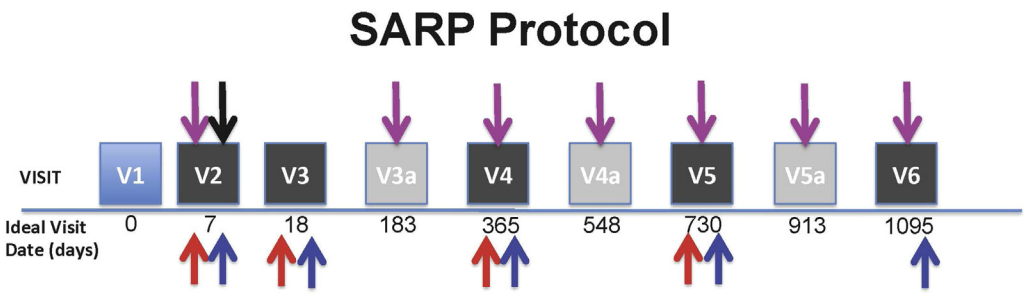


FIG E1. Schematic of the SARP protocol (6 visits over 3 years). *V1*, Characterization of asthma severity (lung function measures and asthma questionnaires); *V2-V6*, disease characterization, lung function (before/after bronchodilation), blood and sputum collection, asthma disease questionnaires; *V3a-V5a*, telephone interview and asthma disease questionnaires. *Black arrow*, Visit with an intramuscular injection of triamcinolone acetonide (40 mg); *red arrows*, visits that included sputum induction and blood draw; *blue arrows*, visits that measured FEV_1 before and after bronchodilation with albuterol; *purple arrows*, visits that documented exacerbation history.

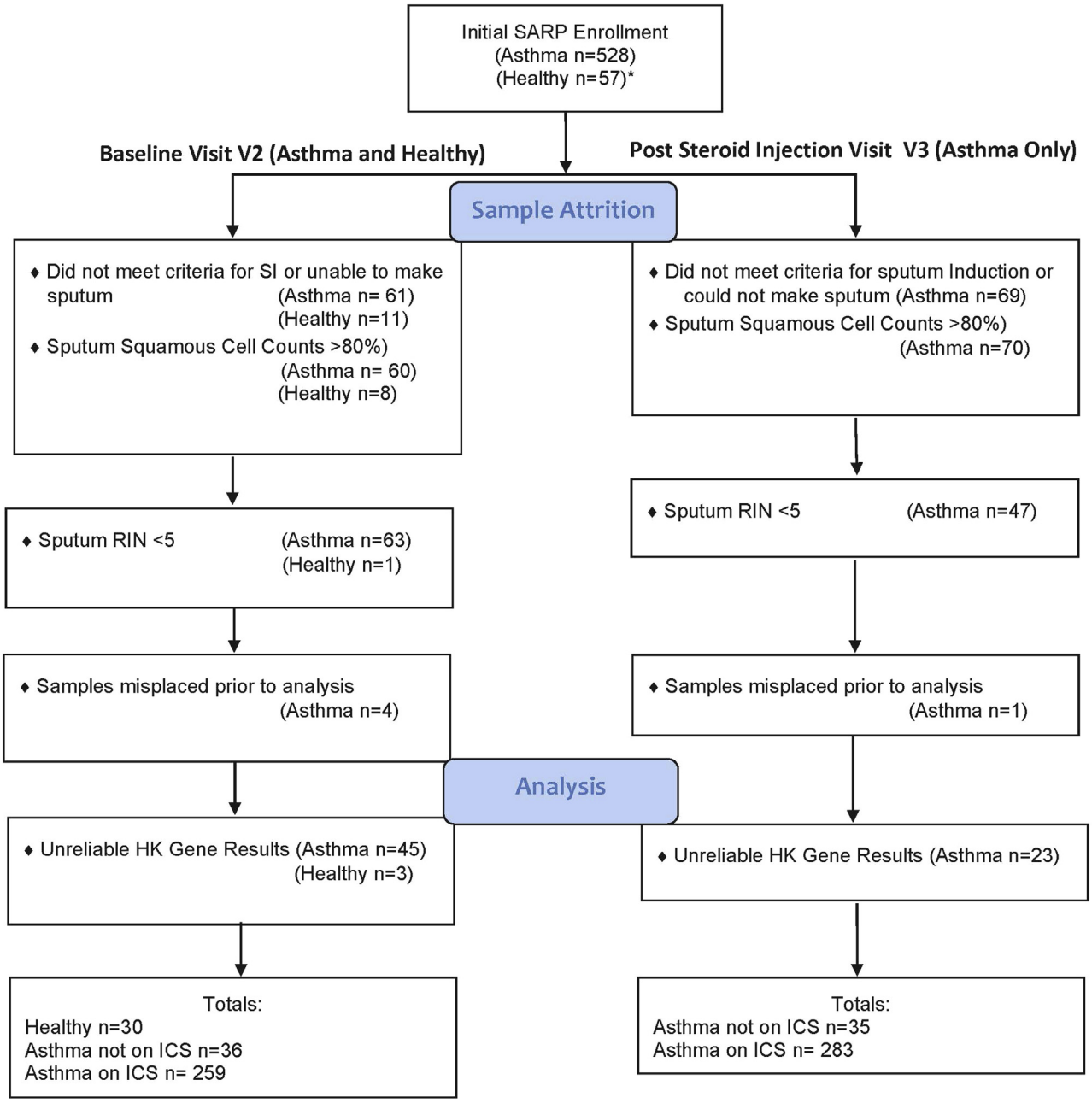


FIG E2. Sample attrition: numbers of samples excluded from our study. *Four healthy subjects were excluded for having evidence of significant atopy (>5 positive test results for common aeroallergens on ImmunoCAP immunologic testing). *HK*, Housekeeping genes.

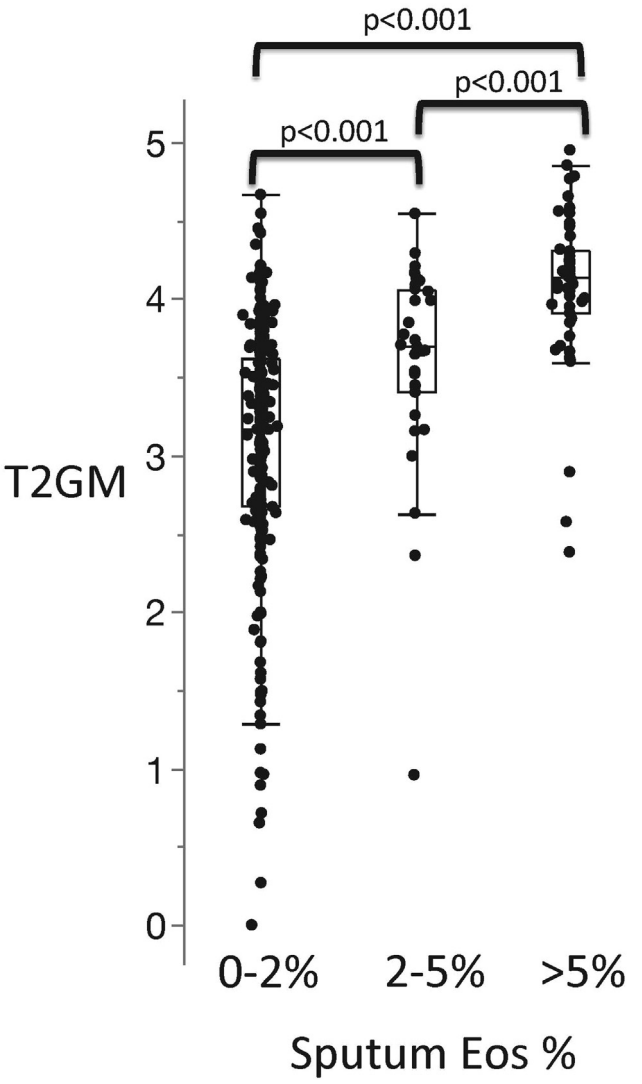


FIG E3. Asthmatic patients with sputum eosinophil *(Eos)* percentages of between 2% and 5% (n = 31) or greater than 5% (n = 51) have greater sputum T2GM values compared with patients with sputum eosinophil percentages of less than 2% (n = 213).

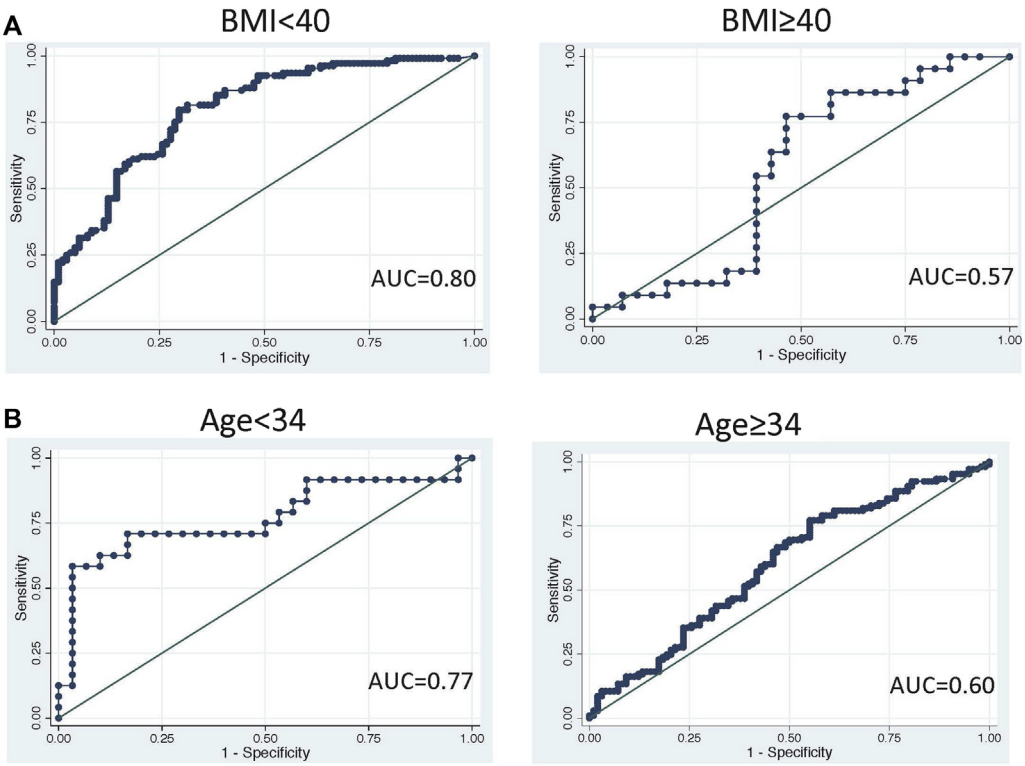
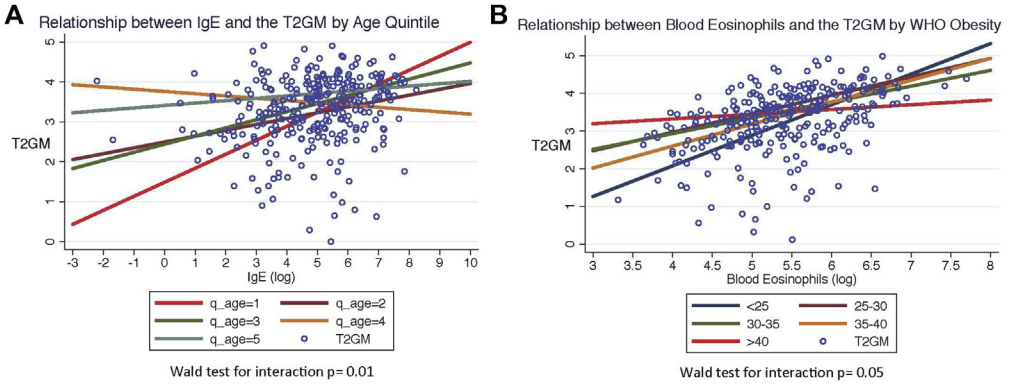


FIG E4. ROC curves for blood eosinophil cell counts as a predictor of refractory T2-high asthma in asthmatic patients taking ICSs with a BMI of less than 40 kg/m^2 (n = 210) and a BMI of 40 kg/m^2 or greater (n = 50; **A**) and blood IgE levels as a predictor of refractory T2-high asthma in asthmatic patients taking ICSs with age of less than 34 years (n = 52) and age of 34 years or greater (n = 208; **B**).



Age Quintile (year range)	N (%)	dy/dx	P-value
1 (18.0-33.7)	52 (20)	0.35	ref
2 (33.8-45.1)	52 (20)	0.15	0.07
3 (45.3-52.9)	52 (20)	0.20	0.26
4 (52.9-61.6)	52 (20)	-0.06	0.002
5 (62.0-84.4)	51 (20)	0.06	0.019

WHO Obese Category (BMI range)	N (%)	dy/dx	P-value
1 (18-24.9)	42 (16)	0.81	0.003
2 (25-29.9)	71 (27)	0.49	0.06
3 (30-34.9)	62 (24)	0.42	0.17
4 (35-39.9)	34 (13)	0.58	0.06
5 (40-69.8)	50 (19)	0.13	ref

FIG E5. Sensitivity analysis using quantile regression models as opposed to OLS models. **A,** Relationship between serum IgE levels and T2GM values is modified by age (Wald test for interaction, $P = .01$). Five quantile regression lines demonstrate the relationship between log-transformed serum IgE levels and T2GM values subgrouped by age quintiles. Asthmatic patients in the youngest quintile have serum IgE levels that strongly correlate with airway T2GM measures *(red line)*, whereas this relationship decreases in the older quintiles (*green*, *blue*, *maroon*, and *orange lines*). **B,** Relationship between blood eosinophil counts and T2GM values is modified by BMI (Wald test for interaction, $P = .05$). Five quantile regression lines demonstrate the relationship between log-transformed blood eosinophil counts and T2GM values subgrouped by World Health Organization *(WHO)* BMI categories. Although asthmatic patients in BMI categories of less than 40 kg/m^2 have blood eosinophil counts that correlate with airway T2GM measures (*green*, *blue*, *maroon*, and *orange lines*), this relationship decreases in patients with BMI of 40 kg/m^2 or greater *(red line)*. *dy/dx*, Marginal effect of serum IgE levels on T2GM values stratified by age quintiles or the marginal effect of blood eosinophil counts on T2GM values stratified by BMI WHO classification. *P* values are for (1) comparison of the average marginal effects of the 4 oldest age quintiles compared with the youngest (reference) quintile and (2) comparison of the average marginal effects of the 4 lowest BMI WHO categories compared with the highest (reference) BMI WHO category.

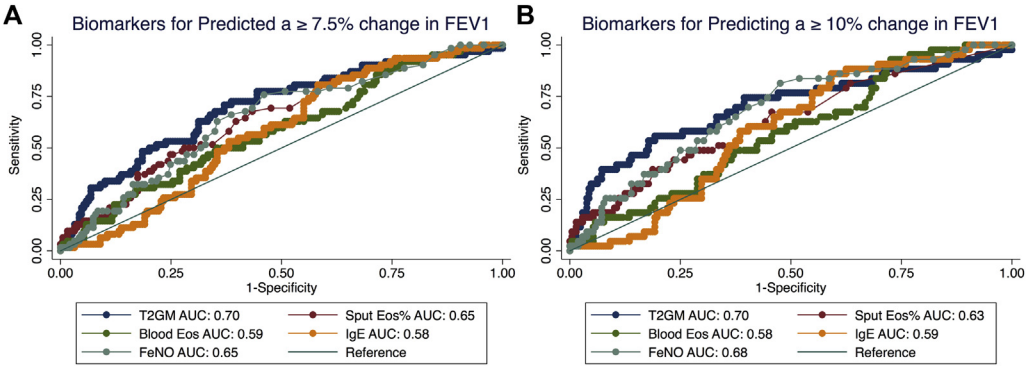


FIG E6. ROC curves demonstrating the T2GM value as a superior predictor of a 7.5% absolute change in FEV_1 percentage **(A)** and 10% absolute change in FEV_1 percentage **(B)** 2 weeks after a triamcinolone injection compared with other biomarkers of airway type 2 inflammation. *Eos,* Eosinophils.

TABLE E1. Sequences of primers and probes used for sputum cell gene expression

Gene primers	Sequence
PPIA, outer forward	ATGAGAACTTCATCCTAAAGCATACG
PPIA, outer reverse	TTGGCAGTGCAGATGAAAAACT
PPIA, inner forward	ACGGGTCCTGGCATCTTGT
PPIA, probe	ATGGCAAATGCTGGACCCAACACA
PPIA, inner reverse	GCAGATGAAAAACTGGGAACCA
GAPDH, outer forward	CAATGACCCCTTCATTGACCTC
GAPDH, outer reverse	CTCGCTCCTGGAAGATGGTGAT
GAPDH, inner forward	GATTCCACCCATGGCAAATTC
GAPDH, probe	CGTTCTCAGCCTTGACGGTGCCA
GAPDH, inner reverse	GGGATTTCCATTGATGACAAGC
YWHAZ, outer forward	CTTCTGTCTTGTCACCAACCATTC
YWHAZ, outer reverse	CAACTAAGGAGAGATTTGCTGCAG
YWHAZ, inner forward	TGGAAAAAGGCCGCATGAT
YWHAZ, probe	TGGCTCCACTCAGTGTCTAAGGCACCCT
YWHAZ, inner reverse	TCTGTGGGATGCAAGCAAAG
PSMB2, outer forward	CCATATCATGTGAACCTCCTCCT
PSMB2, outer reverse	GTCGAGGATACTGAGAGTCAGGAA
PSMB2, inner forward	TCCTCCTGGCTGGCTATGAT
PSMB2, probe	ACAGCGCTGGCCCTTCATGCTC
PSMB2, inner reverse	GGCTGCCAGGTAGTCCATGT
IL4, outer forward	GGGTCTCACCTCCCAACTGC
IL4, outer reverse	TGTCTGTTACGGTCAACTCGGT
IL4, inner forward	GCTTCCCCCTCTGTTCTTCCT
IL4, probe	TCCACGGACACAAGTGCGATATCACC
IL4, inner reverse	GCTCTGTGAGGCTGTTCAAAGTT
IL5, outer forward	GCCATGAGGATGCTTCTGCA
IL5, outer reverse	GAATCCTCAGAGTCTCATTGGCTATC
IL5, inner forward	AGCTGCCTACGTGTATGCCA
IL5, probe	CCCCACAGAAATTCCCACAAGTGCA
IL5, inner reverse	GTGCCAAGGTCTCTTTCACCA
IL13, outer forward	CAACCTGACAGCTGGCATGT
IL13, outer reverse	CCTTGTGCGGGCAGAATC
IL13, inner forward	GCCCTGGAATCCCTGATCA
IL13, probe	TCGATGGCACTGCAGCCTGACA
IL13, inner reverse	GCTCAGCATCCTCTGGGTCTT
IL17, outer forward	ACTGCTACTGCTGCTGAGCCT
IL17, outer reverse	GGTGAGGTGGATCGGTTGTAGT
IL17, inner forward	CAATCCCACGAAATCCAGGA
IL17, probe	CCCAAATTCTGAGGACAAGAACTTCCCC
IL17, inner reverse	TTCAGGTTGACCATCACAGTCC
IFNG, outer forward	GTAACTGACTTGAATGTCCAACGC
IFNG, outer reverse	GACAACCATTACTGGGATGCTC
IFNG, inner forward	CCAACGCAAAGCAATACATGA
IFNG, probe	TCCAAGTGATGGCTGAACTGTCGCC
IFNG, inner reverse	TTTTCGCTTCCCTGTTTTAGCT

TABLE E2. Comparison of asthmatic patients receiving ICSs with sputum samples at visit 2 compared with those without sputum samples at visit 2

Characteristics	No sputum (n = 214)	Sputum (n = 259)	*P* value
Age (y)	48.7 (12.9)	50.3 (14.0)	.76
Female sex, no. (%)	141 (66)	177 (68)	.57
Race, no. (%)			.59
American Indian and Alaska Native	1 (0)	1 (0)	
Asian	6 (3)	12 (5)	
African American	61 (29)	59 (23)	
White	133 (62)	170 (66)	
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	
Mixed race	13 (6)	17 (7)	
BMI (kg/m^2)	32.6 (8.0)	32.7 (8.5)	.90
ATS/ERS criteria, severe asthma	138 (65)	174 (67)	.54

ATS, American Thoracic Society; *ERS*, European Respiratory Society.

TABLE E3. Comparison of asthmatic patients receiving ICSs with sputum samples at visit 3 compared with those without sputum samples at visit 3

Characteristics	No sputum (n = 190)	Sputum (n = 283)	*P* value
Age (y)	48.01 (13.5)	48.7 (13.5)	.65
Female sex, no. (%)	121 (64)	197 (69)	.25
Race, no. (%)			.69
American Indian and Alaska Native	0 (0)	2 (1)	
Asian	6 (3)	12 (4)	
African American	52 (27)	68 (24)	
White	120 (64)	183 (64)	
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	
Mixed race	12 (6)	18 (6)	
BMI (kg/m^2)	31.9 (7.8)	33.2 (8.6)	.08
ATS/ERS criteria, severe asthma	130 (68)	182 (64)	.36

ATS, American Thoracic Society; *ERS*, European Respiratory Society.

TABLE E4. Quantile regression models evaluating the effect of a 1-unit change in each characteristic on the T2GM value*

Characteristics	β Coefficient	95% CI	*P* value
Age (y)	0.01	−0.00 to 0.19	.05
Male sex	−0.28	−0.54 to −0.03	.03
BMI (kg/m^2)	0.00	−0.12 to 0.01	.44
Spirometry			
FEV_1 (% predicted)	−0.01	−0.02 to −0.00	.001
FVC (% predicted)	−0.01	−0.01 to 0.00	.15
FEV_1/FVC ratio	−0.02	−0.03 to −0.01	<.001
Blood cell counts ($\times 10^6$/L)			
Neutrophils (100 cell change)	0.00	−0.00 to 0.01	.65
Eosinophils (100 cell change)	0.10	0.06 to 0.14	<.001
Serum IgE (IU/L, 100-IU change)	0.02	0.00 to 0.04	.03
FENO (ppm)	0.02	0.01 to 0.02	<.001
Sputum cell counts (%)			
Eosinophils	0.04	0.03 to 0.05	<.001
Neutrophils	0.00	0.00 to 0.01	.34
Sputum *IL17* gene expression†	0.05	0.00 to 0.10	.05
Sputum *IFNG* gene expression†	0.05	0.00 to 0.10	.04
ACT score‡	−0.01	−0.03 to 0.02	.55
Severity ATS/ERS criteria			
Mild	Reference	Reference	Reference
Moderate	0.10	−0.34 to 0.55	.65
Severe	0.37	0.00 to 0.75	.05
Asthma exacerbations, no. (%)			
No exacerbation in past year	Reference	Reference	Reference
One to 2 exacerbations in past year	0.27	−0.03 to 0.57	.08
Greater than 2 exacerbations in past year	0.41	0.12 to 0.70	.006
Age of asthma onset (y)	0.01	0.00 to 0.02	.12
Maximal BD response test (FEV_1 %)	0.03	0.02 to 0.04	<.001
Systemic Corticosteroid Response Test (FEV_1 %)	0.03	0.02 to 0.04	<.001

The β coefficient is reported as a 1-unit change in T2GM value for each 1-unit change in the predictor, unless otherwise indicated.
ACT, Asthma Control Test; *ATS*, American Thoracic Society; *FVC*, forced vital capacity.
*T2GM is a scaled measure of airway type 2 inflammation on a $\log_2$ scale.
†Gene expression data are in $\log_2$ scale normalized to 4 housekeeping genes.
‡ACT scores range from 5 to 25, with lower scores indicating worse asthma control.

TABLE E5. Interaction between age quintiles on the relationship between biomarkers of type 2 inflammation and T2GM values

Type 2 biomarkers	*P* value, Wald test for interaction
Blood IgE (IU)	.004
Blood eosinophils ($\times 10^6$/L)	.59
Sputum eosinophils (%)	.09
FENO (ppm)	.20

TABLE E6. Interaction between BMI World Health Organization categories on the relationship between biomarkers of type 2 inflammation and T2GM values

Type 2 biomarkers	*P* value, Wald test for interaction
Blood IgE (IU)	.79
Blood eosinophils ($\times 10^6$/L)	.02
Sputum eosinophils (%)	.35
FENO (ppm)	.27

TABLE E7. Clinical characteristics of subjects with persistent or fluctuating T2GM measures before and after triamcinolone injection

Characteristics	Persistently low T2GM (n = 71)	T2GM increased after injection (n = 25)	T2GM decreased after injection (n = 41)	Persistently high T2GM (n = 59)	*P* value*
Age (y)	44.7 (13.1)	45.8 (12.6)	48.4 (13.7)	52.5 (14.6)	.01
Female sex, no. (%)	47 (66)	15 (60)	34 (83)	44 (75)	.14
Race, no. (%)					.06
American Indian and Alaska Native	1 (1)	0 (0)	0 (0)	0 (0)	
Asian	0 (0)	0 (0)	5 (12)	5 (8)	
African American	15 (21)	4 (16)	10 (24)	14 (24)	
White	54 (76)	18 (72)	24 (59)	34 (58)	
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	0 (0)	0 (0)	
Mixed race	1 (1)	3 (12)	2 (5)	6 (10)	
BMI (kg/m^2)	33.1 (9.4)	36.0 (7.5)	30.7 (7.4)	34.0 (9.4)	.10
Spirometry (baseline)					
FEV_1 (% predicted)	79.1 (14.6)	72.6 (19.5)	76.6 (17.1)	68.5 (17.0)	.003
FVC (% predicted)	88.1 (13.5)	84.2 (13.8)	90.0 (16.1)	83.3 (15.1)	.09
Spirometry (after steroid)					
FEV_1 (% predicted)	78.1 (16.4)	73.2 (20.2)	81.5 (15.3)	72.9 (19.3)	.07
FVC (% predicted)	87.1 (14.7)	84.9 (15.7)	93.3 (15.5)	85.8 (15.3)	.06
Severity ATS/ERS criteria, no. (%)					.034
Mild	16 (23)	4 (16)	8 (20)	1 (2)	
Moderate	15 (21)	20 (20)	6 (15)	11 (19)	
Severe	40 (56)	16 (64)	27 (66)	47 (80)	
Change after SCRT					
T2GM	−0.11 (0.89)	1.04 (0.59)	−0.86 (0.54)	−0.13 (0.41)	<.001
Blood eosinophils ($\times 10^6$/L)†	−30 (146)	−7 (106)	−230 (335)	−232 (341)	.001
Sputum eosinophils (%)	−0.2 (1.2)	0.4 (2.5)	−2.7 (4.6)	−4.6 (10.0)	.01
F_{ENO} (ppm)	−3 (16)	2 (20)	−12 (20)	−6 (17)	<.001
FEV_1 (% predicted)	−1.0 (6.8)	0.6 (9.6)	4.9 (8.1)	4.4 (9.1)	<.001

Data are reported as means (SDs), unless otherwise indicated.

ATS, American Thoracic Society; *ERS*, European Respiratory Society; *FVC*, forced vital capacity; *SCRT*, systemic corticosteroid response test.

**P* value comparisons use ANOVA for continuous variables and the Pearson χ^2 test for categorical variables.

†Blood eosinophil data were available for 101 asthmatic patients.