

Race is associated with differences in airway inflammation in patients with asthma

Sharmilee M. Nyenhuis, MD,^{a,b} Jerry A. Krishnan, MD, PhD,^{a,b} Alalia Berry, MD,^c William J. Calhoun, MD,^d Vernon M. Chinchilli, PhD,^e Linda Engle, BS,^e Nicole Grossman, MD,^f Fernando Holguin, MD, MPH,^g Elliot Israel, MD,^f Rick A. Kittles, PhD,^h Monica Kraft, MD,^h Stephen C. Lazarus, MD,ⁱ Erik B. Lehman, MS,^e David T. Mauger, PhD,^e James N. Moy, MD,^j Stephen P. Peters, MD, PhD,^k Wanda Phipatanakul, MD, MS,^l Lewis J. Smith, MD,^m Kaharu Sumino, MD, MPH,ⁿ Stanley J. Szeffler, MD,^o Michael E. Wechsler, MD, MMSc,^{f,p} Sally Wenzel, MD,^g Steven R. White, MD,^q and Steven J. Ackerman, PhD^a

Chicago, Ill, Madison, Wis, Galveston, Tx, Hershey and Pittsburgh, Pa, Boston, Mass, Tucson, Ariz, San Francisco, Calif, Winston-Salem, NC, St Louis, Mo, and Aurora and Denver, Colo

Background: African American subjects have a greater burden from asthma compared with white subjects. Whether the pattern of airway inflammation differs between African American and white subjects is unclear.

Objective: We sought to compare sputum airway inflammatory phenotypes of African American and white subjects treated or

not with inhaled corticosteroids (ICSs; ICS+ and ICS−, respectively).

Methods: We performed a secondary analysis of self-identified African American and white subjects with asthma enrolled in clinical trials conducted by the National Heart, Lung, and Blood Institute–sponsored Asthma Clinical Research Network and

From ^athe Department of Medicine, University of Illinois at Chicago; ^bthe University of Illinois Hospital & Health Sciences System, Chicago; ^cthe Division of Allergy, Pulmonary and Critical Care, Department of Medicine, the University of Wisconsin School of Medicine and Public Health, University of Wisconsin, Madison; ^dthe Division of Pulmonary Critical Care & Sleep Medicine, Department of Internal Medicine, University of Texas Medical Branch, Galveston; ^ethe Department of Public Health Sciences, Pennsylvania State University, Hershey; ^fPulmonary and Critical Care Medicine, Department of Medicine, Brigham and Women's Hospital, Boston; ^gthe Division of Pulmonary, Allergy and Critical Care Medicine, Department of Medicine, University of Pittsburgh School of Medicine; ^hthe University of Arizona College of Medicine, Tucson; ⁱthe Division of Pulmonary and Critical Care, Department of Medicine, University of California, San Francisco; ^jStroger Hospital of Cook County, Chicago; ^kthe Division of Pulmonary, Critical Care, Allergy and Immunologic Diseases, Department of Medicine, Wake Forest School of Medicine, Winston-Salem; ^lthe Division of Allergy and Immunology, Department of Pediatrics, Boston Children's Hospital; ^mthe Division of Pulmonary and Critical Care, Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago; ⁿthe Division of Pulmonary and Critical Care Medicine, Department of Medicine, Washington University School of Medicine, St Louis; ^othe Division of Pulmonary Medicine, Department of Pediatrics, Children's Hospital of Colorado, Aurora; ^pthe Division of Pulmonary, Critical Care and Sleep Medicine, Department of Medicine, National Jewish Health, Denver; and ^qthe Division of Pulmonary/Critical Care, Department of Medicine, University of Chicago.

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Corresponding author: Sharmilee M. Nyenhuis, MD, 920N-CSB MC 719, 840 S Wood St, Chicago, IL 60612. E-mail: snyenhui@uic.edu.

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AsthmaNet. Demographics, clinical characteristics, and sputum cytology after sputum induction were examined. We used a sputum eosinophil 2% cut point to define subjects with either an eosinophilic ($\geq 2\%$) or noneosinophilic ($< 2\%$) inflammatory phenotype.

Results: Among 1018 participants, African American subjects ($n = 264$) had a lower FEV₁ percent predicted (80% vs 85%, $P < .01$), greater total IgE levels (197 vs 120 IU/mL, $P < .01$), and a greater proportion with uncontrolled asthma (43% vs 28%, $P < .01$) compared with white subjects ($n = 754$). There were 922 subjects in the ICS+ group (248 African American and 674 white subjects) and 298 subjects in the ICS− group (49 African American and 249 white subjects). Eosinophilic airway inflammation was not significantly different between African American and white subjects in either group (percentage with eosinophilic phenotype: ICS+ group: 19% vs 16%, $P = .28$; ICS− group: 39% vs 35%, $P = .65$; respectively). However, when adjusted for confounding factors, African American subjects were more likely to exhibit eosinophilic airway inflammation than white subjects in the ICS+ group (odds ratio, 1.58; 95% CI, 1.01–2.48; $P = .046$) but not in the ICS− group ($P = .984$).

Conclusion: African American subjects exhibit greater eosinophilic airway inflammation, which might explain the greater asthma burden in this population. (J Allergy Clin Immunol 2016;■■■:■■■–■■■.)

Key words: Asthma, race, eosinophil, airway inflammation, African American, body mass index, corticosteroid, induced sputum, clinical trial

African American subjects have a higher prevalence of asthma than white subjects and a greater burden of morbidity and mortality, with rates of asthma-related emergency department visits, hospitalizations, and death being approximately 2 to 3 times the rates observed in white subjects.^{1,2} Many factors, including asthma severity, differences in access to health care, and environmental exposures, have been implicated as causes of race-related variations in asthma burden.^{3–8} Studies that have controlled for these factors have still found higher asthma-related emergency department visits, hospitalizations, and death from asthma among African American subjects.^{9–11} Results of some studies suggest that African American subjects can have reduced responsiveness to some asthma therapies, including inhaled corticosteroids (ICSs), compared with white subjects, suggesting biological differences in asthma between these groups.^{12–15} The basis for race-related differences in asthma burden and treatment responsiveness is not well understood.

Airway inflammation is a key cornerstone of asthma pathogenesis and is mediated by numerous inflammatory cells that infiltrate the airways, including eosinophils, neutrophils, type 2 lymphocytes, basophils, and mast cells.¹⁶ By counting these inflammatory cells in induced sputum, airway inflammation in asthmatic patients has been characterized as either eosinophilic ($\geq 2\%$ eosinophils) or noneosinophilic ($< 2\%$ eosinophils).^{17–19} Emerging evidence suggests that differences in airway inflammatory phenotype can affect response to asthma therapies.^{20–22} In one study subjects with noneosinophilic asthma had an impaired response to treatment with oral and high-dose ICSs compared with those with eosinophilic asthma.²⁰ Relatively few African American patients with asthma (21/158 patients) were included in this study, and therefore it remains unclear

Abbreviations used

ACQ:	Asthma Control Questionnaire
ACRN:	Asthma Clinical Research Network
ACT:	Asthma Control Test
BMI:	Body mass index
ICS:	Inhaled corticosteroid
SLIMSIT:	Salmeterol and Leukotriene Modifiers vs. Salmeterol and ICS Treatment
SMOG:	Smoking Modulates Outcomes of Glucocorticoid Therapy in Asthma
SOCS:	Salmeterol Off Corticosteroids

whether differences in airway inflammation could explain, at least in part, the observed race-related disparities in asthma burden.²⁰

These considerations led us to ask the following question: Are there differences in the prevalence of eosinophilic versus noneosinophilic airway inflammatory phenotypes in African American versus white patients with asthma? Because ICS use can improve asthma control and modify the observed airway inflammatory phenotype, we compared the clinical characteristics and airway inflammation phenotypes in African American and white subjects separately in patients receiving (ICS+) and off (ICS−) ICS treatment.

METHODS

Study population

The study population consisted of self-reported African American or white patients with asthma enrolled in 10 clinical trials conducted by the National Heart, Lung, and Blood Institute–sponsored Asthma Clinical Research Network (ACRN) and AsthmaNet (see Fig E1 and Table E1 in this article's Online Repository at www.jacionline.org) that included at least 1 sputum induction as part of the study protocol.^{23–32} The data were collected as part of the clinical trials that had been reviewed and approved by institutional review boards at all participating ACRN and AsthmaNet centers, and all subjects provided written informed consent. The University of Illinois Institutional Review Board deemed the secondary analyses in this report to be exempt from human subject review.

All subjects were aged 12 years or older; met the criteria for mild or moderate persistent asthma, as defined by the National Asthma Education and Prevention Program Guidelines for the Diagnosis and Management of Asthma; were current nonsmokers with a lifetime history of smoking no greater than 10 pack years; and had not smoked within the past 12 months (see Table E1).³³ In addition, all subjects had to have either (1) a positive methacholine challenge result with a provocative concentration for a 20% decrease in FEV₁ of less than or equal to 16 mg/mL in ICS+ subjects or less than or equal to 12 mg/mL in ICS− subjects or (2) a postbronchodilator increase in FEV₁ of 12% or greater. Sputum eosinophils were the chosen inflammatory marker for this study because evidence supports that differences in airway inflammatory phenotype affect asthma exacerbations and response to asthma therapies.^{20,21} Sputum induction data and corresponding clinical data were only included when ICS use or nonuse was known and standardized as part of the study protocols. Subjects in the ICS+ group were receiving ICSs (fluticasone equivalent dose range, 80–400 μ g/d) for at least 4 weeks before sputum assessment. Those in the ICS− group had not been treated with ICSs for at least 6 weeks before the time of sputum assessment. In the ICS− group patients using a leukotriene-modifying agent in the 4 weeks before sputum induction or clarithromycin in the 6 weeks before sputum induction were not included in the analysis because of potential effects on airway inflammation.^{34–36} Only unique subjects were included within the ICS+ or ICS− groups. Subjects meeting the criteria for both groups either at different time points in a study or participating in a different study were included in the analyses.

The following measures were collected by trained research staff using standardized procedures: self-reported race (African American or white subjects), anthropometrics (age, sex, and body mass index [BMI]), atopic status (≥ 1 positive allergy test result), prebronchodilator FEV₁, asthma symptom control (Asthma Control Questionnaire [ACQ] or Asthma Control Test [ACT] score, depending on the trial protocol), maximum bronchodilator responsiveness (percentage change in FEV₁ after 4 puffs of albuterol), total blood eosinophil counts, and total serum IgE levels.^{37,38} We defined uncontrolled asthma as a score of 1.5 or greater by using the ACQ or 19 or less by using the ACT. ACQ or ACT data were not collected in 2 studies (Salmeterol Off Corticosteroids [SOCS] and SMOG), and maximum bronchodilator responsiveness data were not collected in 2 studies (SOCS and Salmeterol and Leukotriene Modifiers vs. Salmeterol and ICS Treatment [SLIMSIT]). Blood eosinophil counts and total serum IgE levels were used as secondary markers of inflammation because recent studies have shown an association with asthma severity.^{39,40} Blood eosinophil counts are also a promising biomarker for eosinophilic airway burden.⁴¹ Blood eosinophil and total serum IgE data were not collected in 4 studies (SMOG, Measuring Inhaled Corticosteroid Efficacy [MICE], SOCS, and Vitamin D in Asthma [VIDA]). Blood eosinophil data were available but not used in 1 study (SLIMSIT) because subjects were not receiving standardized treatment at the time of blood collection.

Two induced sputum samples

We examined a subgroup of participants who underwent at least 2 sputum inductions (8 studies; see Fig E1) because a previous study found that sputum eosinophilia is intermittent.²⁰ Two sputum samples from 2 different time points when the participant was taking the same treatment regimen for at least 4 weeks in the ICS+ group and at least 6 weeks in the ICS− group were analyzed for sputum eosinophilia. Eosinophilia of 2% or greater occurring on 1 or both sputum inductions defined eosinophilic airway inflammation.

Sputum induction and processing

Sputum was collected by using methods validated for consistency and quality control, as previously described.^{42,43} Briefly, subjects had spirometry performed before and 10 minutes after 360 mg of inhaled albuterol to ensure postbronchodilator FEV₁ of greater than 50% of predicted value. A 12-minute sputum induction was then performed, and cell differentials (eosinophils, neutrophils, macrophages, epithelial cells, and lymphocytes) were calculated as the percentage of cells in the whole sputum expectorate. Sputum samples with 80% or greater squamous epithelial cells were not included in the analysis. The other cell types in the sputum were calculated as the percentage of nonsquamous cells. The presence or absence of eosinophilic inflammation was determined by using a 2% cutoff based on published reference values for eosinophils in induced sputum from healthy subjects; subjects with 2% or greater sputum eosinophils were classified as having eosinophilic airway inflammation, and subjects with less than 2% sputum eosinophils were classified as having noneosinophilic airway inflammation.^{17,18} The presence of neutrophilic inflammation was determined by using a 61% cutoff based on published reference values for neutrophils in induced sputum.⁴⁴ Subjects with greater than 61% sputum neutrophils were classified as having neutrophilic airway inflammation, and subjects with 2% or greater sputum eosinophils and greater than 61% sputum neutrophils were classified as having mixed eosinophilic/neutrophilic phenotype.

Analyses

Continuous variables are presented as means and SDs or medians and interquartile ranges, as appropriate. Categorical variables are reported by using frequencies and percentages. Differences between groups were assessed by using 2-sample *t* tests, Wilcoxon rank sum tests, Fisher exact tests, or χ^2 tests, as appropriate. In the subgroup of participants who underwent a second sputum induction, we calculated the intraclass correlation coefficient to assess the within-individual reliability of

percentage eosinophils in separate sputum inductions in unique subjects. We used multivariable logistic regression models to assess the adjusted association between race and eosinophilic airway inflammation after accounting for potential confounders separately in the ICS+ and ICS− groups. The variables for the multivariable logistic regression model were selected if they were significantly different between African American and white subjects. To avoid collinearity in the regression models, we did not include total serum IgE levels (related to atopy) and sputum neutrophilia (related to BMI).

Analyses were performed with SAS (version 9.3; SAS Institute, Cary, NC) and Systat (version 11; Systat, San Jose, Calif) statistical software. A 2-tailed *P* value of less than .05 was used to denote statistically significant differences.

RESULTS

Subjects' characteristics

Of the 1018 subjects, 264 (26%) self-identified as African American. Compared with white subjects (*n* = 754 [74%]), African American subjects were significantly older, had a higher BMI, had a lower FEV₁ percent predicted, were more likely to have uncontrolled asthma, and had higher total IgE level (Table I). In the large majority of both African American and white subjects, the age at asthma onset was before age 40 years (Table I).

ICS+ subjects

Of 922 ICS+ subjects, 248 (27%) were African American (Table II). African American subjects were older and less atopic; had a higher BMI, lower FEV₁ percent predicted, and higher total serum IgE levels; and were more likely to have uncontrolled asthma compared with white subjects. There was no significant difference in age at asthma onset, total blood eosinophil counts, or percentage of sputum eosinophils/neutrophils in African American and white subjects (Table II). Only 17% of ICS+ subjects had eosinophilic airway inflammation. There were no significant differences between the African American and white subgroups (19% vs 16%, *P* = .28; Fig 1), even when stratified by age, age of asthma onset, atopy, and presence or absence of uncontrolled asthma (see Table E2 in this article's Online Repository at www.jacionline.org). When stratified by BMI, the proportion with eosinophilic airway inflammation was similar among African American and white subjects, except for those with a BMI of 30 kg/m² or greater (see Table E2). In the subgroup with a BMI of 30 kg/m² or greater, African American subjects were more likely to have eosinophilic airway inflammation than white subjects (16% vs 8%). Of those subjects with eosinophilic airway inflammation, there was no difference in the percentage of sputum eosinophils in African American versus white subjects (6.8% vs 5.9%, *P* = .95, respectively). African American subjects with eosinophilic airway inflammation were twice as likely to have a mixed granulocytic airway inflammation ($\geq 2\%$ eosinophils and $>61\%$ neutrophils) compared with white subjects (7% vs 3%, *P* = .02). In noneosinophilic subjects almost three quarters had neutrophilic airway inflammation ($>61\%$ neutrophils). African American subjects were less likely to exhibit this airway inflammatory pattern (71% vs 75%, *P* = .02).

ICS− subjects

Of ICS− subjects, 16% (*n* = 49) were African American (Table III). African American subjects had a higher BMI. As in

TABLE I. Subjects' characteristics: All subjects

	African American subjects (n = 264)	White subjects (n = 754)	P value
Age (y)	38 ± 12	35 ± 12	<.01
Female sex, no. (%)	186 (70)	478 (63)	.04
Age at asthma onset, no. (%)			.67
<40 y	248 (94)	699 (94)	
≥40 y	15 (6)	48 (6)	
Atopy*	226 (87)	674 (91)	.03
BMI (kg/m ²)	34 ± 10	28 ± 7	<.01
Prebronchodilator FEV ₁ (% predicted)	80 ± 13	85 ± 14	<.01
Bronchodilator responsiveness (% change in FEV ₁)	14 ± 12	13 ± 9	.21
Uncontrolled asthma, no. (%)†‡	102 (43)	180 (28)	<.01
Sputum eosinophils (%), median (IQR)	0.4 (0.0-1.3)	0.4 (0.0-1.4)	.56
Sputum neutrophils (%), median (IQR)	43 (23-68)	40 (24-57)	.07
Blood eosinophils/mm ³ , median (IQR)§	169 (100-291)	200 (108-300)	.20
Total serum IgE (IU/mL), median (IQR)	197 (111-497)	120 (48-282)	<.01

Values above represent means ± SDs, unless otherwise indicated.

IQR, Interquartile range.

*One or more positive skin test results.

†Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

‡Asthma control data are available for 869 subjects: 235 African American subjects and 634 white subjects.

§Blood eosinophil data are available for 463 subjects: 97 African American subjects and 366 white subjects.

||Total serum IgE data are available for 543 subjects: 115 African American subjects and 428 white subjects.

TABLE II. Subjects' characteristics: ICS+ group

	African American subjects (n = 248)	White subjects (n = 674)	P value
Age (y)	38 ± 12	36 ± 12	<.01
Female sex, no. (%)	177 (71)	434 (64)	.05
Age at asthma onset, no. (%)			.56
<40 y	234 (94)	626 (93)	
≥40 y	14 (6)	45 (7)	
Atopy*	211 (86)	601 (91)	.03
BMI (kg/m ²)	34 ± 10	28 ± 7	<.01
Prebronchodilator FEV ₁ (% predicted)	80 ± 13	86 ± 14	<.01
Bronchodilator responsiveness (change in FEV ₁)	14 ± 11	13 ± 9	.10
Uncontrolled asthma, no. (%)†‡	100 (45)	147 (27)	<.01
ICS dose (μg/d), median (IQR)	320 (80-400)	176 (80-400)	.04
Sputum eosinophils (%), median (IQR)	0.4 (0.0-1.2)	0.3 (0.0-1.1)	.45
Sputum neutrophils (%), median (IQR)	42 (23-65)	40 (24-59)	.26
Blood eosinophils/mm ³ , median (IQR)§	190 (100-228)	182 (100-260)	.75
Total serum IgE (IU/mL), median (IQR)	197 (103-497)	120 (48-293)	<.01

Values above represent means ± SDs, unless otherwise indicated. The total number of patients in Tables II and III do not equal the total number of unique patients shown in Table I because 203 (20%) patients met the criteria to be included (at different times) for both the ICS+ and ICS− groups. Patients were treated with ICSs for at least 4 weeks to be in the ICS+ group.

IQR, Interquartile range.

*One or more positive skin test results.

†Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

‡Asthma control data are available for 775 subjects: 222 African American subjects and 553 white subjects.

§Blood eosinophil data are available for 378 subjects: 85 African American subjects and 293 white subjects.

||Total serum IgE data are available for 466 subjects: 104 African American subjects and 362 white subjects.

the ICS+ group, there was no difference in the percentage of sputum eosinophils/neutrophils in African American and white subjects (Table III). Only 36% of ICS− subjects had eosinophilic airway inflammation, with no significant difference between the African American and white subgroups (39% vs 35%, $P = .65$; Fig 1), even when stratified by BMI, age, age of asthma onset, atopy, and uncontrolled asthma (see Table E3 in this article's Online Repository at www.jacionline.org). In contrast to ICS+ subjects ICS− eosinophilic subjects showed a trend toward a lower percentage of sputum eosinophils in African American subjects compared with white subjects (5.4% vs 9.4%, $P = .07$, respectively). African American subjects with eosinophilic

airway inflammation were 4 times as likely to have mixed granulocytic airway inflammation compared with white subjects (14% vs 3%, $P = .003$). African American subjects with noneosinophilic airway inflammation were twice as likely to exhibit neutrophilic inflammation (>61% neutrophils; 35% vs 17%, $P = .004$).

Multivariable logistic regression

A multivariable logistic regression analysis was performed in the ICS+ and ICS− subgroups to investigate whether race was associated with eosinophilic airway inflammation after accounting for potential confounders (age, sex, atopic status, BMI, FEV₁

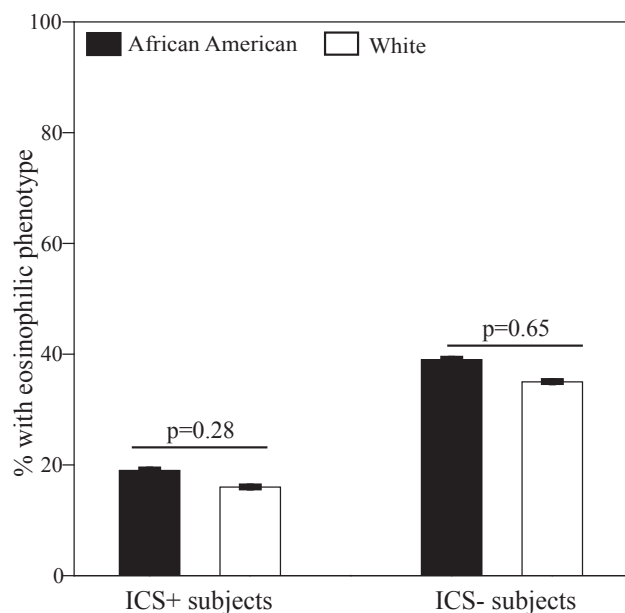


FIG 1. Distribution of eosinophilic airway inflammation ($\geq 2\%$ eosinophils) in ICS+ and ICS- African American (black bars) and white (white bars) patients with asthma. A similar distribution of inflammatory phenotype in African American and white patients with asthma is shown. ICS+ group: 248 African American and 674 white patients; ICS- group: 49 African American and 249 white patients.

percent predicted, and uncontrolled asthma; see Table E4 in this article's Online Repository at www.jacionline.org). Interestingly, race was associated with eosinophilic airway inflammation in the ICS+ group only. African American subjects had a 58% greater odds of eosinophilic airway inflammation than white subjects (odds ratio, 1.58; 95% CI, 1.01-2.48; $n = 757$; $P = .046$; Fig 2). There were no race-related differences in eosinophilic airway inflammation in the ICS- stratum, although there were fewer subjects in this stratum, and the CIs were wide.

Analyses of 2 induced sputum samples

Four hundred seventy-seven (47%) subjects had at least 2 induced sputum samples that were included in the analysis. The interval between the sputum inductions varied from 2 to 53 weeks (see Fig E2 in this article's Online Repository at www.jacionline.org). African American subjects who had at least 2 induced sputum samples were younger (ICS- group only) and had a higher BMI and lower FEV₁ percent predicted (ICS+ group only, see Tables E5 and E6 in this article's Online Repository at www.jacionline.org). When compared with subjects with 1 induced sputum sample, subjects with 2 induced sputum samples were older and had a higher BMI, lower FEV₁ percent predicted, and greater bronchodilator reversibility (see Table E7 in this article's Online Repository at www.jacionline.org). When results from 2 induced sputum samples were used, the percentage of subjects (ICS+ and ICS- groups) with eosinophilic airway inflammation increased, supporting the intermittent nature of eosinophilia that occurs in the asthmatic airway (Fig 3). There were no significant race-related differences in the proportion of subjects with eosinophilic airway inflammation when results of 2 induced sputum samples from the same subject were used (within the ICS+ and ICS- groups: $P = .29$ and $.78$, respectively; Fig 3). The induced sputum pattern of inflammation within a

subject remained eosinophilic or noneosinophilic in 80.1% (339/423) of unique subjects when sputum induction was repeated; the intraclass correlation coefficient for percentage sputum eosinophils between 2 induced sputum samples was 0.31 in ICS+ group (fair agreement) and 0.68 in the ICS- group (moderate agreement).

DISCUSSION

The principal findings from this study were (1) only a minority of subjects with mild or moderate persistent asthma had an eosinophilic sputum airway inflammatory phenotype and (2) there are race-related differences in eosinophilic airway inflammation in asthmatic patients treated with ICSs.

The current report significantly contributes to the current body of literature on the influence of race on airway inflammatory patterns in asthmatic patients. A previous study, which included only small numbers of African American subjects (25 patients), suggested potential variations in airway inflammation based on race.⁴⁵ In this previous study African American subjects with asthma had a greater number of sputum neutrophils, but the analysis did not take into account ICS use.⁴⁵ The current study includes a 10-fold higher number of African American patients with asthma ($n = 264$) and examined eosinophilic and noneosinophilic airway inflammation separately in patients treated with or without ICSs.

We did not observe race-related differences in eosinophilic and noneosinophilic airway inflammation until we accounted for potential confounders. After accounting for baseline differences in age, sex, atopic status, BMI, FEV₁ percent predicted, and uncontrolled asthma, we observed an increased odds of eosinophilic airway inflammation in African American subjects taking ICSs. Significant race differences in neutrophilic and mixed granulocytic airway inflammation were also found. Neutrophilic airway inflammation is commonly seen in patients with severe disease.⁴⁶ In our population of patients with mild-to-moderate asthma, it was frequently seen both in African American and white subjects in the ICS+ group, likely because of ICS use. However, in the ICS- group African American subjects were twice as likely to have neutrophilic inflammation, which might identify a group of patients that could be less responsive to ICS treatment.⁴⁷ Mixed granulocytic airway inflammation is associated with poorer asthma control, greater asthma symptoms and health care use, and lower lung function. Our findings of higher numbers of African American subjects with this inflammatory pattern in both the ICS+ and ICS- groups suggests a mechanism that can account for more severe and difficult-to-control asthma in African American subjects. Further cluster analyses in African American patients with asthma might identify whether the mixed granulocytic inflammatory pattern identifies patients with severe asthma, as has been reported previously in a predominantly white population.⁴⁸

We identified a number of race-related differences in clinical characteristics. African American subjects had lower lung function (FEV₁ percent predicted) and higher BMI and total serum IgE levels, and they were more likely to have uncontrolled asthma. These findings are consistent with previous reports indicating lower lung function and increased total serum IgE levels are associated with a greater burden of asthma among African American compared with white subjects.^{13,39,48,49}

TABLE III. Subjects' characteristics: ICS– group

	African American subjects (n = 49)	White subjects (n = 249)	P value
Age (y)	31 ± 10	32 ± 10	.32
Female sex, no. (%)	31 (63)	145 (58)	.51
Age at asthma onset, no. (%)			.65
<40 y	46 (96)	236 (97)	
≥40 y	2 (4)	7 (3)	
Atopy*	45 (92)	235 (95)	.50
BMI (kg/m ²)	30 ± 9	26 ± 5	<.01
Prebronchodilator FEV ₁ (% predicted)	83 ± 14	86 ± 13	.14
Bronchodilator responsiveness (% change in FEV ₁)	11 ± 13	12 ± 9	.55
Uncontrolled asthma, no. (%)†‡	6 (21)	44 (28)	.47
Sputum eosinophils (%), median (IQR)	0.7 (0.2-3.1)	1.1 (0.2-3.9)	.50
Sputum neutrophils (%), median (IQR)	43 (24-73)	37 (22-60)	.12
Blood eosinophils/mm ³ , median (IQR)§	140 (80-300)	200 (130-300)	.11
Total serum IgE (IU/mL), median (IQR)	178 (78-464)	128 (64-258)	.13

Values above represent means ± SDs, unless otherwise indicated. The total number of subjects in Tables II and III do not equal the total number of unique subjects shown in Table I because 203 (20%) subjects met the criteria to be included (at different times) for both the ICS+ and ICS– groups. Patients were off ICSs for at least 6 weeks to be in the ICS– group.

IQR, Interquartile range.

*One or more positive skin test results.

†Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

‡Asthma control data are available for 185 subjects: 28 African American subjects and 157 white subjects.

§Blood eosinophil data are available for 156 subjects: 23 African American subjects and 133 white subjects.

||Total serum IgE data are available for 146 subjects: 21 African American subjects and 125 white subjects.

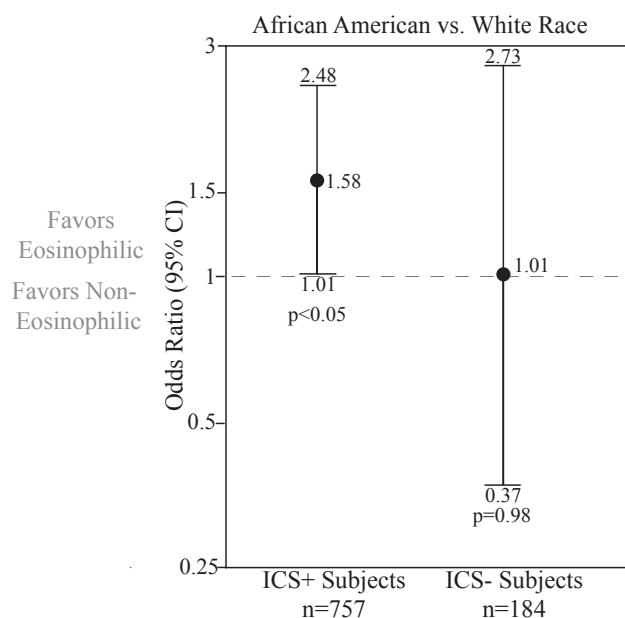


FIG 2. Odds ratio estimates and 95% CIs for eosinophilic airway inflammation (≥2% eosinophils) in African American versus white subjects stratified by ICS status. Estimated odds ratios with 95% CIs are shown for eosinophilic airway inflammation after adjusting for age, sex, atopy, BMI, FEV₁ percent predicted, and uncontrolled asthma. In the ICS+ group African American subjects were significantly more likely to have eosinophilic airway inflammation ($P = .046$) compared with white subjects. In the ICS– group no significant race-related differences in eosinophilic airway inflammation were observed, although the CIs were wide and the number of subjects in the ICS– stratum was approximately 25% of the number of subjects in the ICS+ stratum. Depending on the protocol in the parent clinical trials, some subjects contributed to the ICS+ and ICS– strata at different points in the trial ($n = 89$ subjects); a total of 852 unique subjects contributed to the analyses. See Table E4 for adjusted odds ratios for other covariates in the analyses.

Our study suggests that differences in eosinophilic airway inflammation can contribute to race-related differences in asthma burden. This increased risk of eosinophilic airway inflammation in African American subjects receiving ICSs might be due to higher rates of corticosteroid insensitivity and reduced cellular sensitivity to corticosteroids in African American patients with asthma.^{14,50,51} However, the biologic mechanisms for steroid insensitivity are unclear and can include a decreased number of steroid receptors, reduced steroid receptor binding, increased nuclear transcription factor levels, or other pharmacogenomic effects.^{15,52-54} Although differential adherence might account for differences in patterns of airway inflammation, a previous study examining race effects on treatment failures in ACRN trials showed similar medication adherence in African American and white subjects (87.5% vs 91%, respectively).¹³ Therefore differential adherence likely does not affect the findings of this current study. In this analysis we found that BMI among African American subjects was about 6 kg/m² higher than among white subjects (ICS+ and ICS– groups combined). Some studies suggest that obesity is associated with more difficult-to-control asthma and a poor response to asthma treatment or corticosteroid insensitivity.^{52,55} Whether the combination of African American race and obesity leads to greater corticosteroid insensitivity is yet to be determined. Obesity (BMI ≥30 kg/m²) in this study was found to be associated with an eosinophilic phenotype only in African American subjects taking ICSs. This might indicate a race interaction in airway inflammation, although larger studies that adjust for the higher rates of obesity in this particular subgroup of asthmatic patients are needed to confirm our finding. Because obesity is associated with worse asthma control, efforts directed at obesity prevention and weight loss might help reduce race-related asthma disparities.⁵⁶⁻⁵⁸

The major strengths of this study include the large number of African American and white subjects with asthma enrolled in

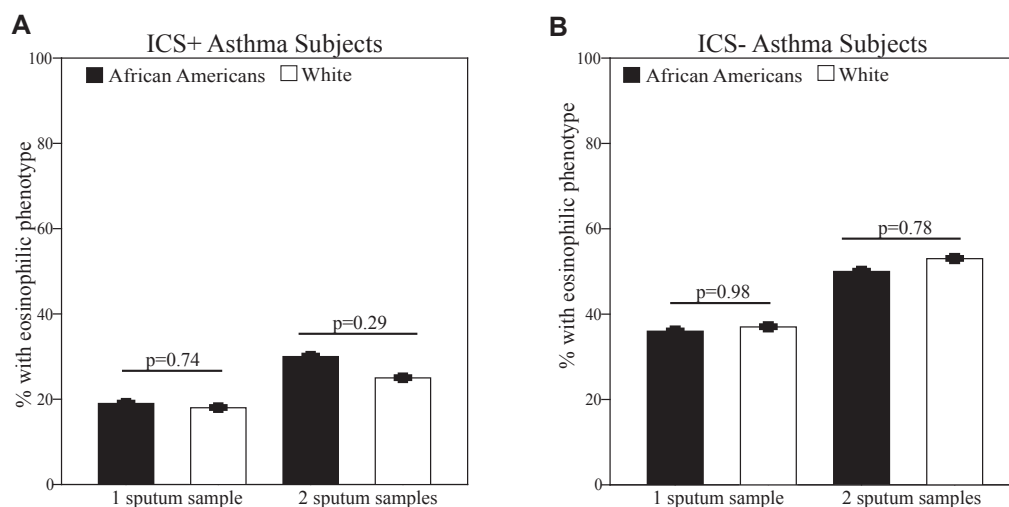


FIG 3. Frequency of eosinophilic airway inflammation ($\geq 2\%$ eosinophils on one or both sputum inductions) in subjects with 2 sputum samples. **A**, Sputum inflammatory cell profile assessed by the first versus 2 sputum samples in African American (black bars; $n = 99$) and white (white bars; $n = 266$) subjects in the ICS+ subgroup with 2 sputum samples analyzed. **B**, Sputum inflammatory cell profile assessed by the first versus 2 sputum samples in African American (black bars; $n = 22$) and white (white bars; $n = 90$) subjects in the ICS- subgroup with 2 sputum samples analyzed. Overall, there were no significant differences by race observed in each ICS stratum.

these clinical trials conducted across the United States and the analysis of data from multiple studies using standardized protocols and quality controls for sputum induction and cytology measurements.

However, there are a number of potential limitations to the study. Notably, our study was conducted among clinical trial participants with mild or moderate persistent asthma. It is not known whether our findings are generalizable to patients with intermittent or severe persistent asthma. Results of studies in clinical trial participants might not apply to all patients with asthma because only a minority of these patients are eligible and enroll in clinical trials. Moreover, we used self-reported race in this study; further studies using genetic ancestry markers to classify patients' race are recommended. Use of genetic ancestry markers will provide the opportunity to examine the extent to which the airway inflammatory phenotype varies with the percentage of African ancestry (rather than a dichotomous "race" variable).⁵⁹

We assessed 2 broad phenotypes of airway inflammation: eosinophilic and noneosinophilic. We recognize that other inflammatory phenotypes exist: neutrophilic and paucigranulocytic. The relationship of these inflammatory phenotypes with a clinical phenotype is not clearly defined and is an area for further research.

Furthermore, our analysis relied mostly on a single assessment of airway inflammation using induced sputum. Although induced sputum is a direct and noninvasive measure of airway inflammation, other measures, including blood eosinophil counts, exhaled nitric oxide levels, and total serum IgE levels, have been associated with greater asthma severity, yet have not consistently been shown to predict ICS treatment responsiveness to the same degree as sputum eosinophils have.⁶⁰⁻⁶² Blood eosinophil counts and total serum IgE levels were measured in a subset of patients in this analysis. No difference was found in blood eosinophil counts, although African American subjects had significantly higher total

serum IgE levels compared with white subjects. Larger studies examining additional measures of airway inflammation, such as blood eosinophil counts, activated airway eosinophil counts, exhaled nitric oxide levels, and total serum IgE levels, should be pursued to fully address airway inflammatory differences that might exist in African American and white patients with asthma.

The analyses we conducted in the subset of patients with 2 induced sputum samples demonstrated no significant race-related differences in the proportion of patients with eosinophilic (or noneosinophilic) asthma. These analyses included sputum samples with highly variable time periods between the 2 induced sputum samples (up to 53 weeks, depending on the study). Additionally, subjects included might not be representative of the entire sample because we reported differences in the clinical characteristics of subjects with 1 versus 2 induced sputum samples. These differences are likely due to variations in eligibility criteria used in the different study protocols (some of which included 2 induced sputum samples).

The findings from our study have important clinical implications. In both African American and white subjects with mild-to-moderate asthma, we found that noneosinophilic airway inflammation was the predominant pattern. Patients with noneosinophilic airway inflammation might not benefit from treatment intensification that focuses on targeting eosinophils, such as ICSs. Other treatments, such as the addition of β -agonists and/or antimuscarinic agents, should be considered.^{20,21} In a recent *post hoc* analysis, African American subjects without atopic inflammation (history of eczema and increased blood eosinophil counts and total serum IgE levels) had a better response to adding a long-acting β -agonist compared with increasing the dose of the ICS or adding a leukotriene modifier.⁶³

Another important finding from this study is that African American subjects taking ICSs were more likely to have eosinophilic airway inflammation. There are multiple factors

that might account for this novel finding, including greater disease severity, uncontrolled asthma, obesity, and glucocorticoid resistance, in African American subjects in the ICS+ group.^{14,50,51} Although we did not see a race difference in those not taking ICSs, this could be due to the small sample size in the ICS– group. Additional studies in African American adults need to be performed to determine whether race and atopic status affect the response to therapy in those on and off ICSs. There is an ongoing AsthmaNet study evaluating the most efficacious add-on treatment options in African American patients with asthma, which will begin to address our knowledge gaps in this field.²²

In summary, in our study of more than 1000 patients with asthma (a quarter of whom were African American), we found evidence of worse asthma impairment (lower lung function, higher total serum IgE levels, and worse symptom control) among African American compared with white subjects and a greater risk of eosinophilic airway inflammation in African American subjects receiving ICS treatment. We conclude that differences in eosinophilic airway inflammatory phenotype in the context of ICS use might contribute to the greater burden of asthma in African American compared with white subjects. Additional studies are needed to address the mechanisms underlying this novel finding, particularly the role of ICSs in this patient population.

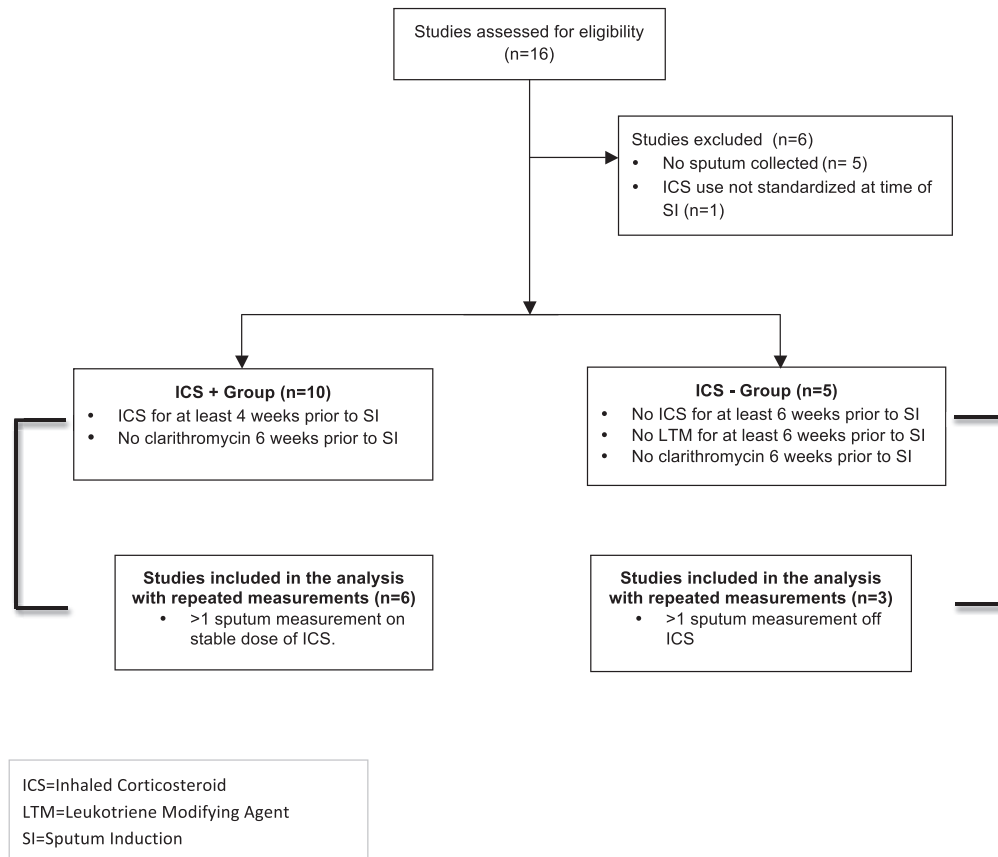
We acknowledge the study participants, the ACRN and AsthmaNet clinical research coordinators, and the data coordinating center.

Clinical implications: African American patients with asthma carry a greater disease burden than white patients. Race-related differences in eosinophilic airway inflammation might contribute to the disparities in adults with asthma on ICSs.

REFERENCES

- Moorman JE, Rudd RA, Johnson CA, King M, Minor P, Bailey C, et al. National surveillance for asthma—United States, 1980–2004. *MMWR Surveill Summary* 2007;56:1–54.
- Akinbami L. Asthma prevalence, health care use and mortality: United States 2003–05. CDC National Center for Health Statistics; 2006. Available at: <http://www.cdc.gov/nchs/data/hestat/asthma03-05/asthma03-05.htm>. Accessed October 21, 2015.
- Grant EN, Lyttle CS, Weiss KB. The relation of socioeconomic factors and racial/ethnic differences in US asthma mortality. *Am J Public Health* 2000;90:1923–5.
- Joseph CLM, Ownby DR, Peterson EL, Johnson CC. Racial differences in physiologic parameters related to asthma among middle-class children. *Chest* 2000;117:1336–44.
- Stewart KA, Higgins PC, McLaughlin CG, Williams TV, Granger E, Croghan TW. Differences in prevalence, treatment, and outcomes of asthma among a diverse population of children with equal access to care: findings from a study in the military health system. *Arch Pediatr Adolesc Med* 2010;164:720–6.
- Nishimura KK, Galanter JM, Roth LA, Oh SS, Thakur N, Nguyen EA, et al. Early-life air pollution and asthma risk in minority children: the GALA II and SAGE II studies. *Am J Respir Crit Care Med* 2013;188:309–18.
- Thakur N, Oh SS, Nguyen EA, Martin M, Roth LA, Galanter J, et al. Socioeconomic status and childhood asthma in urban minority youths: the GALA II and SAGE II studies. *Am J Respir Crit Care Med* 2013;188:1202–9.
- Akinbami LJ, Moorman JE, Simon AE, Schoendorf KC. Trends in racial disparities for asthma outcomes among children 0 to 17 years, 2001–2010. *J Allergy Clin Immunol* 2014;134:547–53.e5.
- Zoratti EM, Havstad S, Rodriguez J, Robens-Paradise Y, Lafata JE, McCarthy B. Health service use by African Americans and Caucasians with asthma in a managed care setting. *Am J Respir Crit Care Med* 1998;158:371–7.
- Boudreaux ED, Emond SD, Clark S, Camargo CA. Race/ethnicity and asthma among children presenting to the emergency department: differences in disease severity and management. *Pediatrics* 2003;111:E615–21.
- Ford JG, McCaffrey L. Understanding disparities in asthma outcomes among African Americans. *Clin Chest Med* 2006;27:423–30.
- Haselkorn T, Lee JH, Mink DR, Weiss ST. TENOR Study Group. Racial disparities in asthma-related health outcomes in severe or difficult-to-treat asthma. *Ann Allergy Asthma Immunol* 2008;101:256–63.
- Wechsler ME, Castro M, Lehman E, Chinchilli VM, Sutherland ER, Denlinger L, et al. Impact of race on asthma treatment failures in the Asthma Clinical Research Network. *Am J Respir Crit Care Med* 2011;184:1247–53.
- Chan MT, Leung DY, Szefer SJ, Spahn JD. Difficult-to-control asthma: clinical characteristics of steroid-insensitive asthma. *J Allergy Clin Immunol* 1998;101:594–601.
- Ortega VE, Meyers DA. Pharmacogenetics: implications of race and ethnicity on defining genetic profiles for personalized medicine. *J Allergy Clin Immunol* 2014;133:16–26.
- Drazen JM, Arm JP, Austen KF. Sorting out the cytokines of asthma. *J Exp Med* 1996;183:1–5.
- Spanevello A, Confalonieri M, Sulotto F, Romano F, Balzano G, Migliori GB, et al. Induced sputum cellularity—reference values and distribution in normal volunteers. *Am J Respir Crit Care Med* 2000;162:1172–4.
- Belda J, Leigh R, Parameswaran K, O'Byrne PM, Sears MR, Hargreave FE. Induced sputum cell counts in healthy adults. *Am J Respir Crit Care Med* 2000;161:475–8.
- Szefer SJ, Wenzel S, Brown R, Erzurum SC, Fahy JV, Hamilton RG, et al. Asthma outcomes: biomarkers. *J Allergy Clin Immunol* 2012;129(suppl):S9–23.
- McGrath KW, Icitovic N, Boushey HA, Lazarus SC, Sutherland ER, Chinchilli VM, et al. A large subgroup of mild-to-moderate asthma is persistently non-eosinophilic. *Am J Respir Crit Care Med* 2012;185:612–9.
- Iwamoto H, Yokoyama A, Shiota N, Shoda H, Haruta Y, Hattori N, et al. Tiotropium bromide is effective for severe asthma with noneosinophilic phenotype. *Eur Respir J* 2008;31:1379–80.
- Sutherland ER, Busse WW. Designing clinical trials to address the needs of childhood and adult asthma: the National Heart, Lung, and Blood Institute's AsthmaNet. *J Allergy Clin Immunol* 2014;133:34–8.e1.
- Lazarus SC, Boushey HA, Fahy JV, Chinchilli VM, Lemanski RF Jr, Sorkness CA, et al. Long-acting 2-agonist monotherapy vs continued therapy with inhaled corticosteroids in patients with persistent asthma: a randomized controlled trial. *JAMA* 2001;285:2583–93.
- Szefer SJ, Martin RJ, King TS, Boushey HA, Cherniack RM, Chinchilli VM, et al. Significant variability in response to inhaled corticosteroids for persistent asthma. *J Allergy Clin Immunol* 2002;109:410–8.
- Boushey HA, Sorkness CA, King TS, Sullivan SD, Fahy JV, Lazarus SC, et al. Daily versus as-needed corticosteroids for mild persistent asthma. *N Engl J Med* 2005;352:1519–28.
- Martin RJ, Szefer SJ, King TS, Kraft M, Boushey HA, Chinchilli VM, et al. The Predicting Response to Inhaled Corticosteroid Efficacy (PRICE) trial. *J Allergy Clin Immunol* 2007;119:73–80.
- Lazarus SC, Chinchilli VM, Rollings NJ, Boushey HA, Cherniack R, Craig TJ, et al. Smoking affects response to inhaled corticosteroids or leukotriene receptor antagonists in asthma. *Am J Respir Crit Care Med* 2007;175:783–90.
- Deykin A, Wechsler ME, Boushey HA, Chinchilli VM, Kunselman SJ, Craig TJ, et al. Combination therapy with a long-acting beta-agonist and a leukotriene antagonist in moderate asthma. *Am J Respir Crit Care Med* 2007;175:228–34.
- Peters SP, Kunselman SJ, Icitovic N, Moore WC, Pascual R, Ameredes BT, et al. Tiotropium bromide step-up therapy for adults with uncontrolled asthma. *N Engl J Med* 2010;363:1715–26.
- Sutherland ER, King TS, Icitovic N, Ameredes BT, Bleecker E, Boushey HA, et al. A trial of clarithromycin for the treatment of suboptimally controlled asthma. *J Allergy Clin Immunol* 2010;126:747–53.
- Calhoun WJ, Ameredes BT, King TS, Icitovic N, Bleecker ER, Castro M, et al. Comparison of physician-, biomarker-, and symptom-based strategies for adjustment of inhaled corticosteroid therapy in adults with asthma: the BASALT randomized controlled trial. *JAMA* 2012;308:987–97.
- Castro M, King TS, Kunselman SJ, Cabana MD, Denlinger L, Holguin F, et al. Effect of vitamin D3 on asthma treatment failures in adults with symptomatic asthma and lower vitamin D levels: the VIDA randomized clinical trial. *JAMA* 2014;311:2083–91.
- National Asthma Education and Prevention Program. Full report of the expert panel: guidelines for the diagnosis and management of asthma. (EPR-3; Source Document). National Heart, Lung and Blood Institute; 2007. Available at: <http://www.nhlbi.nih.gov/guidelines/asthma/asthgdln.htm>. Accessed October 21, 2015.
- Simpson JL, Powell H, Boyle MJ, Scott RJ, Gibson PG. Clarithromycin targets neutrophilic airway inflammation in refractory asthma. *Am J Respir Crit Care Med* 2008;177:148–55.

35. Pizzichini E, Leff JA, Reiss TF, Hendeles L, Boulet LP, Wei LX, et al. Montelukast reduces airway eosinophilic inflammation in asthma: a randomized, controlled trial. *Eur Respir J* 1999;14:12-8.
36. Minoguchi K, Kohno Y, Minoguchi H, Kihara N, Sano Y, Yasuhara H, et al. Reduction of eosinophilic inflammation in the airways of patients with asthma using montelukast. *Chest* 2002;121:732-8.
37. Juniper EF, O'Byrne PM, Guyatt GH, Ferrie PJ, King DR. Development and validation of a questionnaire to measure asthma control. *Eur Respir J* 1999;14:902-7.
38. Nathan RA, Sorkness CA, Kosinski M, Schatz M, Li JT, Marcus P, et al. Development of the asthma control test: a survey for assessing asthma control. *J Allergy Clin Immunol* 2004;113:59-65.
39. Naqvi M, Choudhry S, Tsai HJ, Thyne S, Navarro D, Nazario S, et al. Association between IgE levels and asthma severity among African American, Mexican, and Puerto Rican patients with asthma. *J Allergy Clin Immunol* 2007;120:137-43.
40. Tran TN, Khatry DB, Ke X, Ward CK, Goassage D. High blood eosinophil count is associated with more frequent asthma attacks in asthma patients. *Ann Allergy Asthma Immunol* 2014;113:19-24.
41. Wagener AH, de Nijs SB, Lutter R, Sousa AR, Weersink EJ, Bel EH, et al. External validation of blood eosinophils, FE(NO) and serum periostin as surrogates for sputum eosinophils in asthma. *Thorax* 2015;70:115-20.
42. Martin RJ, Kephart DK, Dyer AM, Fahy JV, Kraft M. Asthma Clinical Research Network. Quality control within the Asthma Clinical Research Network. *Control Clin Trials* 2001;22:207S-21S.
43. Gershman NH, Wong HH, Liu JT, Mahlmeister MJ, Fahy JV. Comparison of two methods of collecting induced sputum in asthmatic subjects. *Eur Respir J* 1996;9:2448-53.
44. Simpson JL, Scott R, Boyle MJ, Gibson PG. Inflammatory subtypes in asthma: assessment and identification using induced sputum. *Respirology* 2006;11:54-61.
45. Woodruff PG, Khashayar R, Lazarus SC, Janson S, Avila P, Boushey HA, et al. Relationship between airway inflammation, hyperresponsiveness, and obstruction in asthma. *J Allergy Clin Immunol* 2001;108:753-8.
46. Wenzel SE. Asthma: defining of the persistent adult phenotypes. *Lancet* 2006;368:804-13.
47. Green RH, Brightlung 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.
48. Hastie AT, Moore WC, Meyers DA, Vestal PL, Li HS, Peters SP, et al. Analyses of asthma severity phenotypes and inflammatory proteins in subjects stratified by sputum granulocytes. *J Allergy Clin Immunol* 2010;125:1028-36.e13.
49. Kumar R, Seibold MA, Aldrich MC, Williams LK, Reiner AP, Colangelo L, et al. Genetic ancestry in lung-function predictions. *N Engl J Med* 2010;363:321-30.
50. Goleva E, Li LB, Eves PT, Strand MJ, Martin RJ, Leung DYM. Increased glucocorticoid receptor beta alters steroid response in glucocorticoid-insensitive asthma. *Am J Respir Crit Care Med* 2006;173:607-16.
51. Federico MJ, Covar RA, Brown EE, Leung DYM, Spahn JD. Racial differences in T-lymphocyte response to glucocorticoids. *Chest* 2005;127:571-8.
52. Sutherland ER, Goleva E, Strand M, Beuther DA, Leung DYM. Body mass and glucocorticoid response in asthma. *Am J Respir Crit Care Med* 2008;178:682-7.
53. Goleva E, Li LB, Leung DYM. IFN-gamma reverses IL-2- and IL-4-mediated T-cell steroid resistance. *Am J Respir Cell Mol Biol* 2009;40:223-30.
54. Li LB, Leung DYM, Martin RJ, Goleva E. Inhibition of histone deacetylase 2 expression by elevated glucocorticoid receptor beta in steroid-resistant asthma. *Am J Respir Crit Care Med* 2010;182:877-83.
55. Peters-Golden M, Swern A, Bird SS, Hustad CM, Grant E, Edelman JM. Influence of body mass index on the response to asthma controller agents. *Eur Respir J* 2006;27:495-503.
56. Dixon AE, Holguin F, Sood A, Salone CM, Pratley RE, Beuther DA, et al. An official American Thoracic Society Workshop report: obesity and asthma. *Proc Am Thorac Soc* 2010;7:325-35.
57. Schatz M, Zeiger RS, Yang SJ, Chen W, Sajjan S, Allen-Ramey F, et al. Prospective study on the relationship of obesity to asthma impairment and risk. *J Allergy Clin Immunol Pract* 2015;3:560-5.e1.
58. Camargo CA Jr, Boulet LP, Sutherland ER, Busse WW, Yancey SW, Emmett AH, et al. Body mass index and response to asthma therapy: fluticasone propionate/salmeterol versus montelukast. *J Asthma* 2010;47:76-82.
59. Nyenhuis SN, Du J, Ackerman SJ, Krishnan JA, Kittles R. Impact of self-identified race and genetic ancestry on airway inflammation in asthma [abstract]. *J Allergy Clin Immunol* 2015;135:AB86.
60. Pizzichini E, Pizzichini MM, Efthimiadia A, Dolovich J, Hargreave FE. Measuring airway inflammation in asthma: eosinophils and eosinophilic cationic protein in induced sputum compared with peripheral blood. *J Allergy Clin Immunol* 1997;99:539-44.
61. Smith AD, Cowan JO, Filsell S, McLachlan C, Monti-Sheehan G, Jackson P, et al. Diagnosing asthma: comparisons between exhaled nitric oxide measurements and conventional tests. *Am J Respir Crit Care Med* 2004;169:473-8.
62. Korevaar DA, Westerhof GA, Wang J, Cohen JF, Spijker R, Sterk PJ, et al. Diagnostic accuracy of minimally invasive markers for detection of airway eosinophilia in asthma: a systematic review and meta-analysis. *Lancet Respir Med* 2015;3:290-300.
63. Malka J, Mauger DT, Covar R, Rabinovitch N, Lemanske RF Jr, Spahn JD, et al. Eczema and race as combined determinants for differential response to step-up asthma therapy. *J Allergy Clin Immunol* 2014;134:483-5.

**FIG E1.** CONSORT flow diagram of studies assessed for eligibility.

ICS- Group**SMOG** (Non-smoker group, ICS and LTRA groups)

4 sputum inductions (SI) over 22 weeks, 2 SIs at week 10 and 24 were used for analysis. Subjects were on placebo for at least 6 weeks prior to SI.

SOCS (Placebo and LABA only groups)

7 SIs over 28 weeks, 2 consecutive SIs at week 14 and 22 were used for analysis. Subjects were on placebo or LABA only for at least 6 weeks prior to SI.

IMPACT (Placebo group)

2 SIs over 53 weeks, beginning at least 10 weeks post-treatment

ICS+ Group**SOCS** (ICS group)

7 SIs over 28 weeks, 2 consecutive SIs at week 6 and 8 were used for analysis. Subjects were on ICS for at least 4 weeks prior to SI.

VIDA

2 SIs over 13 weeks, beginning 4 weeks post-ICS treatment

BASALT (guideline-based and biomarker-based groups)

2 SIs over 40 weeks, beginning 4 weeks post-ICS treatment

TALC (ICS + LABA group)

4 SIs over 46 weeks, 2 SIs at week 4 and either week 18, 34 or 50 were used for analysis. Subjects were on ICS for at least 4 weeks prior to SI.

MIA (ICS + placebo group)

3 SIs over 29 weeks, 2 consecutive SIs at week 4 and 25 were used for analysis. Subjects were on ICS for at least 4 weeks prior to SI.

SLIMSIT

4 SIs over 32 weeks, 2 consecutive SIs on same treatment (ICS+LTRA) at week 4 and 22. Subjects were on ICS for 4 weeks prior to SI.

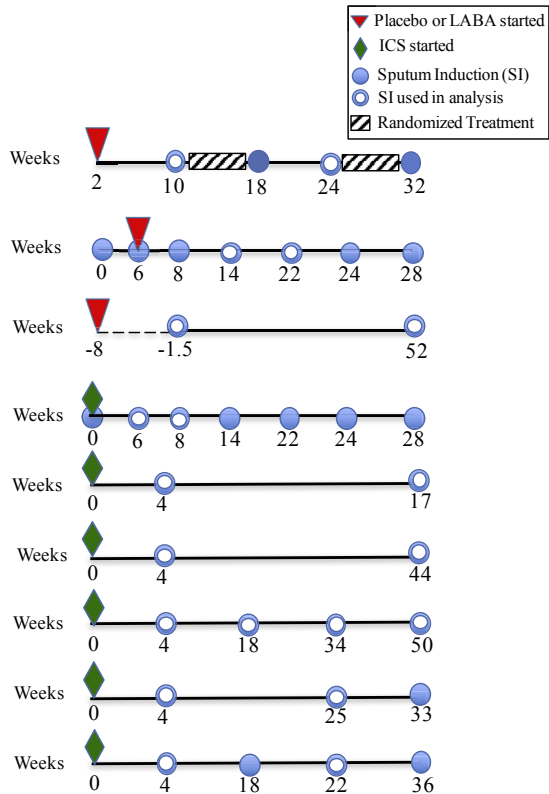


FIG E2. Clinical studies and 2 consecutive sputum inductions used in analyses of sputum inflammatory phenotypes. The blue and white circles indicate the sputum samples used for analysis. The week 18 and 32 sputum induction for the SMOG nonsmoker group was not included because subjects were receiving treatment. The week 8 sputum induction for the SOCS steroid-untreated group was not included because it occurred less than 6 weeks after discontinuation of ICS treatment. Weeks 24 and 28 in the SOCS steroid-untreated group and weeks 14, 22, and 22 in the SOCS steroid-treated group were not used because only the first 2 consecutive sputum samples were analyzed. Because of the crossover design in Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroid (TALC), the second sputum sample used for analysis was taken from either week 18, 34, or 50, which was based on when the subject was taking the same ICS dose because they were on when the first sputum induction occurred (week 4). The week 18 and 36 sputum induction for SLIMSIT was not included because of treatment differences. *BASALT*, Best Adjustment Strategy for Asthma over Long Term; *IMPACT*, Improving Asthma Control Trial; *MIA*, Macrolides in Asthma; *TALC*, Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroid; *VIDA*, Vitamin D in Asthma.

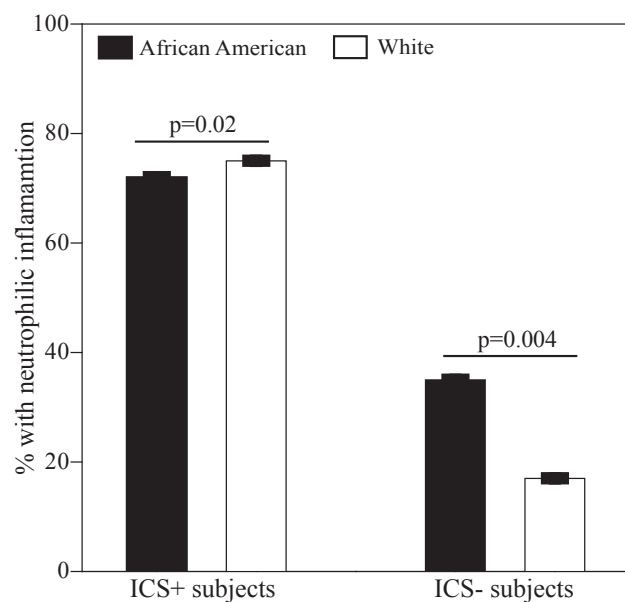


FIG E3. Distribution of neutrophilic airway inflammation (>61% neutrophils) in ICS+ and ICS- African American (*black bars*) and white (*white bars*) patients with asthma. White subjects in the ICS+ group had more neutrophilic airway inflammation than African American subjects, whereas African American subjects in the ICS- group were twice as likely to have neutrophilic airway inflammation than white subjects. ICS+ group: 248 African American subjects and 674 white subjects; ICS- group: 49 African American subjects and 249 white subjects.

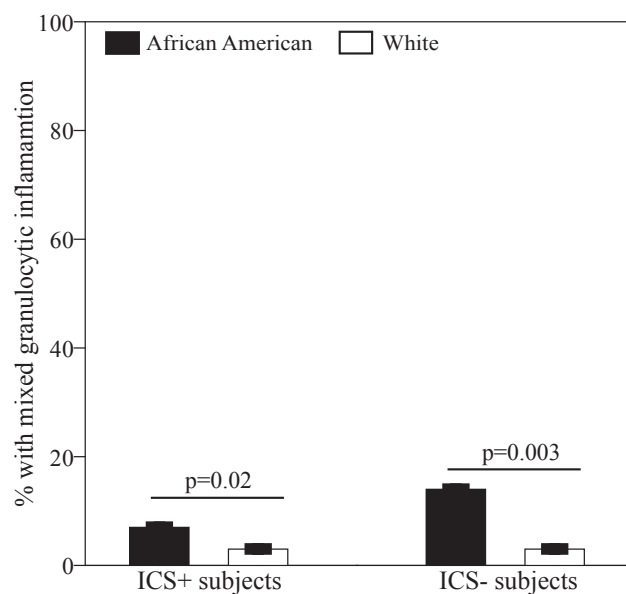


FIG E4. Distribution of mixed granulocytic airway inflammation ($\geq 2\%$ sputum eosinophils and $>61\%$ sputum neutrophils) in ICS+ and ICS- African American (*black bars*) and white (*white bars*) patients with asthma. African American subjects in both the ICS+ and ICS- groups were more likely to have mixed granulocytic airway inflammation than white subjects. ICS+ group: 248 African American subjects and 674 white subjects; ICS- group: 49 African American subjects and 249 white subjects.

TABLE E1. Inclusion/exclusion criteria for each study

	SOCS (n = 164) ¹⁸	MICE (n = 30) ¹⁹	IMPACT (n = 225) ²⁰	PRICE (n = 72) ²¹	SMOG (n = 83) ²²	SLIMSIT (n = 192) ²³	TALC (n = 210) ²⁴	MIA (n = 92) ²⁵	BASALT (n = 342) ²⁶	VIDA (n = 408) ²⁷
Inclusion criteria	(1) Asthma confirmed by: (A) β -Agonist reversibility of FEV ₁ $\geq$ 12% OR (B) PC ₂₀ $\leq$ 8 mg/mL if ICS– or $\leq$ 16 mg/mL if ICS+ (2) If ICS+ and baseline FEV ₁ >80%, then PC ₂₀ $\leq$ 8 mg/mL (3) Age 12-65 y	(1) History of asthma with baseline FEV ₁ 55% to 85% (2) Reversible airflow obstruction ($\geq$ 12% and $\geq$ 200 cc FEV ₁ change after albuterol) OR PC ₂₀ $\leq$ 8 mg/mL (3) Exercise-induced bronchospasm ($\geq$ 12% decrease in FEV ₁ after exercise challenge) (4) Age 18-55 y	(1) History of asthma (2) Reversible airflow obstruction $\geq$ 12% after albuterol OR PC ₂₀ $\leq$ 8 mg/mL (3) Age 18-65 y	(1) History of asthma with baseline FEV ₁ 55% to 85% (2) PC ₂₀ $\leq$ 12 mg/mL (3) Age 18-55 y	(1) History of asthma, as defined by ATS criteria (2) Reversible airflow obstruction FEV ₁ $\geq$ 12% after albuterol OR PC ₂₀ $\leq$ 8 mg/mL (3) Age 18-50 y	(1) Clinical history consistent with asthma (2) Asthma confirmed by: (A) β -Agonist reversibility of FEV ₁ $\geq$ 12% in those with FEV ₁ $\geq$ 40% to <55% OR (B) PC ₂₀ $\leq$ 8 mg/mL if FEV ₁ $\geq$ 55% (3) Age 12-65 y	(1) Clinical history consistent with asthma (2) Asthma confirmed by: (A) β -Agonist reversibility of FEV ₁ $\geq$ 12% OR (B) PC ₂₀ $\leq$ 8 mg/mL if ICS– or $\leq$ 16 mg/mL if ICS+ (3) Need for daily controller therapy (4) $\geq$ 18 y	(1) Physician diagnosed asthma (2) Asthma confirmed by: (A) β -Agonist reversibility of FEV ₁ $\geq$ 12% OR (B) PC ₂₀ $\leq$ 16 mg/mL if $\geq$ 1.5 if no controller medications (3) ACQ score $\geq$ 1.25 if on controller medications (4) Age 18-60 y	(1) Clinical history consistent with asthma (2) Asthma confirmed by: (A) β -Agonist reversibility of FEV ₁ $\geq$ 12% OR (B) PC ₂₀ $\leq$ 8 mg/mL if ICS– or $\leq$ 16 mg/mL if ICS+ (3) Need for daily controller therapy (4) $\geq$ 18 y	(1) Physician-diagnosed asthma for $\geq$ 12 mo (2) Asthma confirmed by: (A) β -Agonist reversibility of FEV ₁ $\geq$ 12% OR (B) PC ₂₀ $\leq$ 8 mg/mL if ICS– or $\leq$ 16 mg/mL if ICS+ (3) $\geq$ 18 y
Exclusion criteria	(1) Oral steroid use in past 6 wk (2) FEV ₁ <50% (3) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 1 y (2) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 6 wk (2) FEV ₁ <50% (3) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 4 wk (2) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 6 wk (2) FEV ₁ <70%	(1) Oral steroid use in past 6 wk (2) FEV ₁ $\leq$ 40% (3) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 6 wk (2) FEV ₁ $\leq$ 40% (3) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 6 wk (2) FEV ₁ $\leq$ 60% (3) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 4 wk (2) FEV ₁ $\leq$ 40% (3) >10 pack-year use of tobacco and use in past year	(1) Oral steroid use in past 4 wk (2) FEV ₁ <50% (3) >10 pack-year use of tobacco and use in past year
Asthma trial network	ACRN	ACRN	ACRN	ACRN	ACRN	ACRN	ACRN	ACRN	ACRN	AsthmaNet
No. included in current study	119	21	145	29	28	110	112	28	168	257

ATS, American Thoracic Society; BASALT, Best Adjustment Strategy for Asthma over Long Term; IMPACT, Improving Asthma Control Trial; MIA, Macrolides In Asthma; MICE, Measuring Inhaled Corticosteroid Efficacy; PRICE, Predicting Responses for Inhaled Corticosteroid Efficacy; TALC, Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid on Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroids; VIDA, Vitamin D and Asthma.

TABLE E2. Eosinophilic phenotype (as a percentage) stratified by selected subjects' characteristics in the ICS+ group

	African American subjects (n = 248)		White subjects (n = 674)		<i>P</i> value
	No.	Eosinophilic phenotype, no. (%)	No.	Eosinophilic phenotype, no. (%)	
Age (y)					
12-17	4	3 (75)	9	3 (33)	.27
18-44	167	30 (18)	504	81 (16)	.57
45-64	77	15 (19)	153	25 (16)	.55
≥65	0	0 (0)	8	1 (13)	NA
Age of asthma onset (y)					
<40	234	44 (30)	626	103 (16)	.42
≥40	14	4 (29)	45	7 (16)	.43
Atopic status*					
Positive	34	6 (18)	58	9 (16)	.79
Negative	211	42 (20)	601	99 (17)	.26
BMI (kg/m ²)					
<18.5	1	0 (0)	6	0 (0)	NA
18.5-24.9	34	10 (29)	270	55 (10)	.23
25.0-29.9	61	14 (23)	206	39 (19)	.49
≥30.0	152	24 (16)	192	16 (8)	.03
Uncontrolled asthma†					
Uncontrolled	100	23 (23)	147	26 (18)	.30
Controlled	122	19 (16)	406	59 (15)	.78
ICS dose‡					
Low	121	22 (18.2)	397	55 (13.9)	.241
Moderate	127	26 (20.5)	277	55 (19.9)	.886

The χ^2 test or Fisher exact test was used.

NA, Not applicable.

*One or more positive skin test results.

†Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

‡Low dose = ≤246 µg/d, moderate dose = 265-440 µg/d.

TABLE E3. Eosinophilic phenotype (as a percentage) stratified by selected subjects' characteristics in ICS– subjects

	African American subjects (n = 49)		White subjects (n = 249)		<i>P</i> value
	No.	Eosinophilic phenotype, no. (%)	No.	Eosinophilic phenotype, no. (%)	
Age (y)					
12-17	3	1 (33)	8	3 (38)	1.0
18-44	40	15 (38)	213	76 (36)	.83
45-64	6	3 (50)	28	9 (32)	.64
≥65	0	0 (0)	0	0 (0)	NA
Age of asthma onset (y)					
<40	46	18 (39)	236	86 (36)	.73
≥40	2	1 (50)	7	1 (14)	.42
Atopic status*					
Positive	4	1 (25)	13	3 (23)	1.0
Negative	45	18 (40)	235	85 (36)	.63
BMI (kg/m ²)					
<18.5	0	0 (0)	5	0 (0)	NA
18.5-24.9	17	8 (47)	123	57 (46)	.96
25.0-29.9	11	5 (46)	78	21 (27)	.29
≥30.0	21	6 (29)	43	10 (23)	.65
Uncontrolled asthma†					
Uncontrolled	6	3 (50)	44	20 (46)	1.0
Controlled	22	5 (23)	113	26 (23)	.98

The χ^2 test or Fisher exact test was used.

NA, Not applicable.

*One or more positive skin test results.

†Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

TABLE E4. OR and 95% CI for eosinophilic airway inflammation in asthmatic patients

Variable studied*	OR	95% CI of OR	P value
Race			
ICS+ group	1.58	1.01-2.48	.046
ICS− group	1.01	0.37-2.73	.984
Age			
ICS+ group	1.00	0.99-1.02	.741
ICS− group	1.01	0.97-1.04	.714
Sex			
ICS+ group	1.01	0.67-1.54	.949
ICS− group	0.69	0.34-1.33	.253
Atopy†			
ICS+ group	0.99	0.53-1.84	.965
ICS− group	4.48	0.53-37.7	.168
BMI (kg/m ²)			
ICS+ group	0.95	0.92-0.98	<.001
ICS− group	0.98	0.92-1.04	.449
Prebronchodilator FEV ₁ (% predicted)			
ICS+ group	0.99	0.98-1.01	.442
ICS− group	0.96	0.94-0.99	.010
Uncontrolled asthma‡			
ICS+ group	1.44	0.95-2.19	.087
ICS− group	2.63	1.26-5.46	.010

Data are available for 757 subjects of the ICS+ group and 184 subjects of the ICS− group.

NA, Not applicable; OR, odds ratio.

*From multivariable logistic regression model with race, age, sex, atopy, BMI, FEV₁ percent predicted, and uncontrolled asthma as independent variables.

†One or more positive skin test results.

‡Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

TABLE E5. ICS+ subjects' characteristics with 2 induced sputum samples

	African American subjects (n = 99)	White subjects (n = 266)	P value
Age (y)	40 ± 11	38 ± 13	.23
Female sex, no. (%)	72 (73)	177 (67)	.26
Age at asthma onset, no. (%) [*]			.31
<40 y	93 (94)	240 (91)	
≥40 y	6 (6)	25 (9)	
BMI (kg/m ²)	36 ± 10	29 ± 8	<.01
Prebronchodilator FEV ₁ (% predicted)	77 ± 14	83 ± 15	<.01
Bronchodilator responsiveness (% change in FEV ₁)	18 ± 13	16 ± 10	.25
Uncontrolled asthma, no. (%) ^{†‡}			
At time of first induced sputum	43 (46)	81 (34)	.03
At time of second induced sputum	29 (31)	61 (25)	.27

Values above represent means ± SDs, unless otherwise indicated.

^{*}Age at asthma onset data are available for 364 subjects: 99 African American subjects and 265 white subjects.

[†]Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

[‡]Asthma control data are available for 335 subjects: 93 African American subjects and 242 white subjects.

TABLE E6. ICS— subjects' characteristics with 2 induced sputum samples

	African American subjects (n = 22)	White subjects (n = 90)	P value
Age (y)	27 ± 9	32 ± 9	.02
Female sex, no. (%)	15 (68)	49 (54)	.24
Age at asthma onset, no. (%) [*]			.62
<40 y	21 (100)	84 (97)	
≥40 y	0 (0)	3 (3)	
BMI (kg/m ²)	28 ± 7	26 ± 5	.15
Prebronchodilator FEV ₁ (% predicted)	86 ± 11	86 ± 13	.85
Bronchodilator responsiveness, (% change in FEV ₁)	14 ± 11	11 ± 7	.37
Uncontrolled asthma, no. (%) ^{†‡}			
At time of first induced sputum	0 (0)	8 (27)	.18
At time of second induced sputum	0 (0)	1 (3)	1.0

Values above represent means ± SDs, unless otherwise indicated.

^{*}Age at asthma onset data are available for 108 subjects: 21 African American subjects and 87 white subjects.

[†]Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

[‡]Asthma control data are available for 37 subjects: 7 African American subjects and 30 white subjects.

TABLE E7. Subjects' characteristics with 1 versus 2 IS samples analyzed

	1 IS samples (n = 595)	2 IS samples (n = 423)	P value
Age (y)	35 ± 12	38 ± 12	<.01
Female sex, no. (%)	385 (64.7)	79 (65.9)	.68
Age at asthma onset, no. (%) [*]			.07
<40 y	562 (95)	385 (92)	
≥40 y	30 (5)	33 (8)	
Atopy [†]	533 (91)	367 (89)	.42
BMI (kg/m ²)	28 ± 8	31 ± 9	<.01
Prebronchodilator FEV ₁ (% predicted)	86 ± 14	82 ± 15	<.01
Bronchodilator responsiveness (% change in FEV ₁)	12 ± 9	16 ± 11	<.01
Uncontrolled asthma, no. (%) [‡]	150 (30)	132 (36)	.08
Eosinophilic, no. (%)	127 (21)	83 (20)	.50
Blood eosinophils/mm ³ , median (IQR) [§]	190 (100-289)	200 (100-300)	.44
Total serum IgE (IU/mL), median (IQR)	140 (62-323)	126 (65-352)	.96
Studies from which participants were drawn	1-10	1,3,5-10	<.01

Values above represent means ± SDs, unless otherwise indicated.

IS, Induced sputum.

^{*}Age at asthma onset data are available for 592 subjects with 1 IS and 418 subjects with 2 IS.

[†]One or more positive skin test results.

[‡]Uncontrolled asthma = ACT score ≤19 or ACQ score ≥1.5.

[§]Asthma control data are available for 500 subjects with 1 IS sample and 370 subjects with 2 IS samples.

^{||}1 = SOCS, 2 = Measuring Inhaled Corticosteroid Efficacy [MICE], 3 = IMPACT, 4 = PRICE, 5 = SMOG, 6 = SLIMSIT, 7 = Tiotropium Bromide as an Alternative to Increased Inhaled Corticosteroid in Patients Inadequately Controlled on a Lower Dose of Inhaled Corticosteroid [TALC], 8 = MIA, 9 = BASALT, and 10 = Vitamin D in Asthma [VIDA].