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**Refractory Airway Type-2 Inflammation in a Large Subgroup of Asthmatics
treated with Inhaled Corticosteroids**

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ABSTRACT

Background: Airway type 2 inflammation is usually corticosteroid sensitive, but the role of type 2 inflammation as a mechanism of asthma in patients on high dose inhaled corticosteroids (ICS) is uncertain.

Objective: To determine if airway type 2 inflammation persists in patients treated with ICS and to evaluate the clinical features of patients with steroid resistant airway type-2 inflammation.

Methods: We used qPCR to generate a composite metric of type-2 cytokine gene expression (type 2 Gene Mean, "T2GM") in induced sputum cells from healthy controls, severe asthma patients on ICS (n=174), and non-severe asthma patients on ICS (n=85). We explored relationships between asthma outcomes and the T2GM, and the utility of non-invasive biomarkers of the airway T2GM.

Results: The sputum cell T2GM in asthma subjects was significantly increased in asthma subjects and remained high following treatment with intramuscular triamcinolone. We used the median value for the T2GM as a cutoff to classify "steroid-treated type 2-low" (stT2-low) and "steroid-resistant type 2-high" (srT2-high) subgroups. Compared to patients with stT2-low asthma, those with srT2-high asthma were older age and had more severe asthma. Blood eosinophil cell counts predicted srT2-high asthma when BMI was < 40 , but not when it was ≥ 40 , whereas, blood IgE strongly predicted srT2-high asthma when age was < 34 years but not when it was ≥ 34 .

69

70 **Conclusion:** Despite ICS therapy many asthmatics have persistent airway type 2
71 inflammation, (srT2-high asthma) and these patients are older and have more severe
72 disease. Body weight and age modify the performance of blood-based biomarkers of
73 airway type-2 inflammation.

74

75 **Key Messages:**

76 - Inhaled or systemic corticosteroids do not fully suppress airway type-2 inflammation in
77 a large sub-group of severe asthma patients.

78 - Patients with steroid resistant T2-high asthma are characterized by older age and
79 more severe disease.

80 -Body weight and age modify the performance of blood-based biomarkers of airway
81 type-2 inflammation

82

83

84 **Keywords:** Severe asthma, type-2 inflammation, steroid resistance, biomarkers,

Capsule Summary:

Airway type-2 inflammation is not suppressed in many asthma patients treated with corticosteroids. These steroid resistant type 2-high asthma subjects are characterized by older age and have more severe asthma than patients with steroid treated type 2-low disease.

INTRODUCTION

Asthma affects 300-400 million people worldwide (1) and the 5-10% of asthmatics who have severe disease account for much of the public health burden of asthma (2). Airway type 2 inflammation mediated by type 2 cytokines (IL-4, IL-5, and IL-13) is a key pathologic mechanism of asthma (3), but the role of type 2 inflammation in severe asthma is not fully understood. Inhaled corticosteroids (ICS) are a mainstay treatment for asthma because they decrease airway type 2 inflammation (4), but many patients continue to experience asthma exacerbations, asthma symptoms, and airflow obstruction despite ICS treatment(5). One possibility is that some patients have persistent airway type 2 inflammation despite ICS therapy. Alternatively, some patients may have a type 2-low asthma endotype that is not effectively treated by ICS. There are few studies to guide thinking about which of these two possibilities is operating in severe asthma.

We have recently shown that it is feasible to quantify airway type 2 inflammation using PCR-based measures of expression of type-2 cytokines (IL-4, IL-5, and IL-13) in induced sputum cells(6). We also proposed the “type 2 gene mean” (T2GM) as a composite metric of gene expression for IL-4, IL-5, and IL-13 in sputum cells (6). The sputum cell T2GM provides a sensitive and specific measure of airway type 2 inflammation, and it allows quantitative analysis of type 2 inflammation in large patient cohorts(6). The Severe Asthma Research Program (SARP) cohort includes patients with non-severe and severe forms of asthma who undergo detailed characterization studies and collection of biospecimens, including induced sputum(7,8). The majority of

patients in the cohort are treated with ICS usually in high doses(8). To determine if airway type 2 inflammation persists in patients with asthma who are treated with ICS and the clinical features of the subgroup with steroid resistant airway type 2 inflammation, we measured T2GM values in induced sputum in the SARP cohort in an analysis restricted to patients taking ICS.

Materials and Methods:

Study Design

Subjects studied included a reference (healthy) cohort and a cohort of adult asthma patients recruited by seven clinical centers in the National Health and Blood Institutes Severe Asthma Research Program-3 (SARP-3) (7). 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 (5). The visit structure of the SARP protocol is outlined in Supplementary Figure S1. The first baseline visit included completion of medical history and asthma control questionnaires, spirometry, measurement of nitric oxide in exhaled breath (FeNO), immunologic tests (ImmunoCAP[®]), and collection of biospecimens (induced sputum and blood). In addition, maximum bronchodilator reversibility tests (MBRT's) (spirometry before and after 4-8 puffs of albuterol) were performed on baseline visits 2 and 3, and participants also underwent a systemic corticosteroid response test (SCRT). The SCRT involved an intramuscular injection of triamcinolone acetonide (40mg) on baseline visit 2 and repeat characterization (including MBRT, sputum induction, and blood draw) on visit 3 (2-4 weeks later).

Healthy Subjects

Healthy control subjects (n=57) were recruited between November 1, 2012 and October 1, 2015. All healthy control subjects had no history of pulmonary disease, allergic rhinitis or any other atopic disease. Each of the 7 centers was required to recruit a minimum of 7 healthy control patients. To ensure that the demographic characteristics of the healthy control group resembled those of the asthma patients, the healthy control subjects recruited across all centers were required to be >55% female sex; >40% older than 45 years of age; and >20% African American race. The recruitment strategy for the healthy controls cohort was continuously monitored by the SARP data coordinating center to ensure appropriate recruitment. In pulmonary function tests, all healthy controls had normal airway responses to inhaled methacholine and normal FEV₁ (FEV₁% predicted >80%). Four healthy control subjects were excluded for having evidence of significant atopy (>5 positive tests for common aeroallergens on ImmunoCAP[®] immunologic testing).

Asthma Subjects

528 adult (age≥18) asthma subjects were recruited by SARP-3 centers between November 1, 2012 and October 1, 2015. The SARP protocol included two baseline visits, with a 40 mg triamcinolone steroid injection at the second visit, and a follow up visit 2 weeks later. At each visit patients underwent detailed disease characterization and provided samples of blood and induced sputum(7). Of the 528 adult subjects enrolled into the SARP-3 protocol 55 were not taking inhaled corticosteroids. We restricted our analysis to the subgroup of asthma subjects taking ICS (n=473).

Additional details about recruitment methods, subject enrollment, study measurements, and study procedures are provided in the supplementary appendix.

Sputum induction and processing

Sputum induction and processing was performed utilizing a standard procedure in all subjects (9). Briefly, subjects inhaled nebulized 3% saline through a mouthpiece for 12 minutes, as previously described (9). Subjects interrupted inhalation at two minute intervals to spit saliva into a saliva cup and induced sputum into a sputum cup. Saliva was discarded and induced sputum was processed. A 10% solution of Sputolysin (EMD Millipore) was added at a 1:1 g:ml (sputum weight : Sputolysin) ratio to the induced sputum, mixed using a serological pipette, and placed in a 37°C shaking water bath for 15 minutes. Samples were removed at 5, 10, and 15 minute intervals for additional mixing with the pipette, and a portion of this sample was used to determine total and differential cell counts, as previously described (9). The sample was then centrifuged in the cold (4°C) at 2000 rpm for 10 minutes. The cell pellet was then re-suspended in 1mL of Qiagen RNeasy Protect Saliva Reagent. All pellets were stored at -80°C.

RNA Extraction

RNA was extracted from sputum cells using the RNeasy Qiagen kits (Qiagen Inc.) using methods previously described(6). RNA quality was measured using the Agilent 2100 bioanalyzer (Biogen Weston MA, USA), which performs electrophoretic separations according to molecular weight. Each sample was assigned an RNA Integrity Number (RIN) based on extent of RNA degradation. An RIN of 10 indicates

intact RNA where as an RIN of 1 indicates totally degraded RNA (10). Samples with RIN values less than 5 were excluded from the analysis (Supplemental Fig S2)(11). Purified RNA was aliquoted and stored at -80°C.

Acceptable sputum sample

Samples were considered acceptable for PCR analysis if they had a squamous cell % < 80 and a RIN value ≥ 5 .

PCR based measures of Type-2 Inflammation

Using previously described methods (6), real-time Taqman-based quantitative PCR (qPCR) was performed on RNA extracted from induced sputum cell pellets. The genes for three type-2 cytokines (IL-4, IL-5, and IL-13) were measured and the genes for interferon gamma and IL-17 were measured for comparison. The expression levels of four housekeeping genes (*GAPDH*, *PPIA*, *YWHAZ*, and *PSMB2*) were also measured. Some reactions for the genes of interest did not yield a cycle threshold (CT) value after 40 cycles, and here we assigned a CT value equal to the highest CT value detected in other samples for that gene. All CT values were in the linear portion of the amplification curve. Gene expression data was normalized to the geometric mean of the 4 housekeeping genes. Gene expression data is displayed as log2 normalized. Details of the primers and probes are provided in supplementary table S1. As a composite metric of airway type-2 gene expression, we averaged the gene expression of IL-4, IL-5, and IL-13 to generate the T2 gene mean ("T2GM"), as previously described (6).

Statistical Methods

Statistical analyses were performed using JMP 12 software package (SAS Institute, Cary NC) and Stata 15.0 (StataCorp College Station TX), and p-values less than 0.05 were taken as statistically significant. Two group comparisons for continuous variables were made utilizing the Student t-test when normality assumptions were met, or Wilcoxon rank-sum when normality assumptions were not met. Two group comparisons for dichotomous variables were made using a Pearson's chi-square test. Tests for trends across ordered groups were performed using a Wilcoxon-type test for trend (12). To assess the relationship between biomarkers of airway type-2 inflammation and the T2GM we used ordinary least squares (OLS) linear regression models with the T2GM as the dependent variable. In these models, the predictor variables were log transformed blood eosinophil cells counts, log transformed sputum eosinophil %, log transformed serum IgE levels, and fractional exhaled nitric oxide measures. Interaction terms were constructed between body mass index and each of the biomarkers of airway type-2 inflammation on the dependent variable of the T2GM. This was repeated for the interaction of age and each of the biomarkers of airway type-2 inflammation. Age and body mass index were divided into 5 categories for the OLS regression models. To assess the statistical significance of interaction terms Wald tests were performed using the testparm command in STATA(13). Marginal effects for statistically significant interactions were assessed using the margins command in STATA(13). As a sensitivity analysis we repeated all of the OLS linear regression models using quantile regression models with robust standard errors. Receiver operating characteristic (ROC) curves and

tests of equality of the ROC area under the curve (AUC) were made using the rocgold command in STATA(13).

RESULTS

Subjects:

Acceptable sputum samples were available from 259 of the 473 adult asthma patients on ICS at the baseline visit, 283 of the 473 adult asthma patients on ICS at the post steroid baseline visit, and 30 of the healthy control subjects (Supplemental Figure S2). Subject demographics did not differ significantly between subjects with sputum samples compared to subjects without sputum samples (Supplemental Table S2,S3).

Cycle threshold exclusion criteria for housekeeping genes:

Gene expression for type 2 cytokines was 8 CT values higher on average than the gene expression for the housekeeping genes. Based on this, we excluded data from sputum cell samples where the cycle threshold (CT) for any two housekeeping gene was greater than 20 (Supplemental Figure S2). In our initial publication describing methods of qPCR-based gene profiling in sputum cells from a smaller number of patients, we had excluded data from sputum cell samples where the cycle threshold (CT) for any two housekeeping genes was greater than 35 (6). Based on data from the larger patient cohort studied here, we consider the CT of 20 to be better, because it ensures that all measures of type-2 cytokine gene expression are in the linear portion of the amplification curve.

Refractory airway type-2 inflammation in a large subgroup of asthmatics treated with ICS.

The mean gene expression levels in induced sputum cells for individual IL-4, IL-5, and IL-13 cytokines was significantly higher in asthmatics than in healthy controls (Fig 1A, B, C), and the type 2 gene mean (T2GM) value was also significantly increased (Fig 1D). In contrast, the mean gene expression levels in induced sputum cells for interferon gamma and IL-17 was not significantly different in asthmatics than in healthy controls (Fig 1E,F). Treatment with intramuscular triamcinolone did not significantly decrease the gene expression of IL-4, IL-5, and IL-13 cytokines (Fig 1 A-C). The triamcinolone injection had a minimal impact on the T2GM value, which decreased by 3% on average and did not reach the level of statistical significance ($p=0.19$) (Fig 1 D). Despite taking higher doses of inhaled corticosteroids, severe asthma patients had significantly higher T2GM values than non-severe asthma patients (Fig 1F). The mean gene expression levels in induced sputum cells for interferon gamma and IL-17 was not significantly different in asthmatics than in healthy controls and did not change after the triamcinolone injection (Fig 1 F, G).

Clinical Features of Steroid Treated Type 2-Low (stT2-Low) and Steroid Treated Type 2-High (stT2-high) Asthma.

The median split value for the T2GM value in the asthma subgroup approximated the 90th centile value for the T2GM in the healthy subgroup (Fig 1D), and we used the 50th centile value in asthma as a cutoff to classify “steroid-treated type 2-low” (stT2-low) and “steroid-resistant type 2-high” (srT2-high) subgroups. In effect, this means that 50% of

the asthmatics had refractory type 2 inflammation in their airways despite treatment with ICS. Subjects with srT2-high asthma were significantly older than those with stT2-low asthma (Table 1). To further explore the effect of age on T2GM, we divided asthmatics into quintiles based upon age (quintile 1, 18.3 to 33.6 years, n=52; quintile 2, 33.8 to 45.0 years, n=52; quintile 3, 45.3 to 52.9 years, n=52; quintile 4, 52.9 to 61.6 years, n=52; quintile 5, 62.0 to 84.4 years, n=51). We found that T2GM was significantly higher in the older age quintiles than in the younger age quintiles (Fig 2A). In contrast to the positive relationships we found between age and T2GM, we found that subgroups of subjects with srT2-high and stT2-low asthma had similar BMIs (Table 1) and that the T2GM did not increase significantly from low to high BMI classes, as defined by World Health Organization (WHO) guidelines (14) (Fig 2B).

Compared to stT2-low asthma, subjects with srT2-high asthma had more severe asthma as evidenced by lower FEV₁, higher frequency of asthma exacerbations in the prior year, and higher frequency of a severe asthma diagnosis (Table 1). Subjects with srT2-high asthma also had higher blood and sputum eosinophil cell counts (Table 1, Figure S6), higher fractional exhaled nitric oxide (FeNO) measures, higher blood IgE measures (Table 1), and slightly increased sputum cell gene expression for IFN γ and a trend for higher IL-17 (Table 1).

The numbers of patients with srT2-high asthma differed among patients with non-severe and severe asthma in the SARP cohort. Whereas 40% of the non-severe asthmatics had srT2-high asthma, 55% of the severe asthmatics had srT2-high disease ($p = 0.02$).

Although the majority of patients with severe asthma had srT2-high asthma, 45% of severe asthmatics did not have an increase in their sputum cell T2GM. Among this large srT2-low subgroup, we noted that 54% had an FEV1 < 80% and 20% were exacerbation prone (>2 exacerbations in the past year)(Table 1)(8). As a sensitivity analysis we repeated these comparisons using quantile regression models and found similar results (Supplemental Table S4).

Utility of non-invasive biomarkers as tests of refractory airway type 2 inflammation.

Blood IgE, blood eosinophils, sputum eosinophils, and FeNO are non-invasive biomarkers of airway type 2 inflammation (15). To compare the performance of these tests as biomarkers of refractory airway type-2 inflammation, we used receiver-operating characteristic (ROC) analysis. Sputum eosinophils and blood eosinophils outperformed blood IgE and FENO for predicting srT2-high asthma (Fig 3). Specifically, the area under the curve (AUC) values for sputum eosinophils (0.79, 95% CI 0.72-0.84) and blood eosinophils (0.76, 95% CI 0.70-0.82), were statistically higher than the AUC values for IgE (0.62, 95% CI 0.55-0.69, $p < 0.002$ for both comparisons) and trended towards being higher for FeNO (0.72, 95% CI 0.66-0.78), ($p = 0.09$ for comparison of FeNO to sputum eosinophils, and $p = 0.34$ for comparison of FeNO to blood eosinophils) (Fig 3).

Because type 2 inflammation increased with age (above) and because prior reports show that obesity modifies the relationships between systemic and airway measures of type 2 inflammation in asthma (16), we explored whether age or BMI influenced the

performance of non-invasive biomarkers as predictors of sputum cell T2GM values. For this analysis, we built linear regression models. In these models the outcome variable was the T2GM and the predictor variables were age (by age quintile) and each of four type-2 biomarkers (blood IgE, blood eosinophils, sputum eosinophils, and FeNO). We included an interaction terms for age and each of the four type 2 biomarkers, and we constructed linear regression models with predictor variables of BMI (by WHO obesity categories) and each of the four type 2 biomarkers and interaction terms for BMI and each of the four type 2 biomarkers. We did not find any effect of age or body mass index on the performance of sputum eosinophil cell percentages or FeNO as predictive biomarkers for the sputum cell T2GM (Supplemental Tables S5,S6). However, we found a strong modifying effect of age on the relationship between blood IgE levels and sputum cell T2GM values (p-value test for interaction 0.004). Specifically, the correlation between blood IgE levels and sputum cell T2GM values was strong in patients in age quintile 1 (age < 33.6) but decreased in the older patients in age quintiles 2-5 (Fig 4A). In addition, we found a strong modifying effect of BMI on the relationship between blood eosinophils and the sputum cell T2GM values (p-value test for interaction, 0.02). Specifically, although blood eosinophil cell counts correlated well with the sputum cell T2GM values in patients with a BMI less than 40, the correlation decreased with increasing BMI and was very weak in morbidly obese patients (BMI>40) (Fig 4B). To illustrate this point more clearly, we used a receiver operating curves analysis. Whereas blood eosinophils are a strong predictor stT2-high asthma in asthmatics with BMI<40 (AUC 0.80), they are a poor predictor of stT2-high asthmatics with a BMI $\geq$ 40 (AUC

0.57) (Supplemental Fig S3). As a sensitivity analysis we repeated these OLS models using quantile regression models and found similar results (Supplemental Fig S4).

Treatment responses in srT2-high and stT2-low asthma

The treatment response to bronchodilators and systemic corticosteroids was better in srT2-high than in stT2-low asthma. Specifically, for the maximum bronchodilator reversibility test, which involved measurement of spirometry before and after 4-8 puffs of albuterol, the FEV1 response in the srT2-high asthmatics was significantly larger than in the stT2-low asthmatics (Fig 5A). Similarly, for the systemic corticosteroid response test, which involved measurement of spirometry before and 2-4 weeks after an intramuscular injection of triamcinolone acetonide (40mg), the FEV1 response in the srT2-high asthmatics was significantly larger than in the stT2-low asthmatics (Fig 5B). To compare the discriminating ability of the T2GM to predict improvement in FEV1 with corticosteroid treatment, we again used an ROC analysis. We found that the sputum cell T2GM performed better than blood eosinophil cell counts for predicting a 5% absolute change in FEV1% following triamcinolone (AUC 0.71 vs. AUC 0.59, $p = 0.001$) (Fig 5C). The T2GM also performed better than sputum eosinophils (AUC 0.63, $p = 0.01$), FeNO (0.61, $p = 0.02$), and blood IgE (0.56, $p = 0.003$) (Fig 5C). As a sensitivity analysis we also evaluated the discriminating ability of the T2GM for predicting a 7.5% absolute change and a 10% absolute change in FEV1% following the triamcinolone injection. In these analyses the T2GM was also a superior predictor than the other type-2 biomarkers (Supplemental Fig S5).

Clinical Features of Persistently Steroid Resistant T2-High asthma

196 of the 259 asthma subjects (76%) had paired T2GM measurements before and after the triamcinolone injection. We divided these subjects into four groups based upon the change of the T2GM after the triamcinolone injection. T2GM measurements were initially low and remained below the T2 high threshold value after the triamcinolone injection in 71 (36%) (group 1); T2GM measurements were initially low and increased above the T2 high threshold after triamcinolone in 25 subjects (13%) (group 2); T2GM measurements were initially high and decreased below the T2 high threshold after triamcinolone in 41 subjects (21%)(group 3); T2GM measurements were initially high and remained above the T2 high threshold after the triamcinolone injection in 59 of subjects (30%)(group 4) (Supplemental Table S6). Compared to groups 1-3, subjects in group 4 (resistant T2-high) were older and had more severe asthma (Supplemental Table S7).

DISCUSSION

In this study we report that airway type-2 inflammation is not suppressed in approximately half of asthmatics treated with inhaled corticosteroids, and we describe how patients with steroid resistant type 2-high asthma are older and have more severe asthma than patients with steroid treated type 2-low disease. We also show that age and BMI influence the performance of blood- and breath-based biomarkers of refractory airway type-2 inflammation. Our data have implications for understanding the numbers of patients who are eligible for treatment with specific inhibitors of the type 2 inflammation pathway and for how biomarkers may be used to identify such patients.

The proportion of patients with srT2-high asthma was higher than those with severe asthma than in those with non-severe asthma. This is consistent with the idea that type-2 airway inflammation is refractory to treatment with corticosteroids in a subgroup of asthmatics, and that incompletely suppressed type 2 inflammation is a mechanism of severe asthma. An argument against this interpretation would be that patients with severe asthma may not be compliant with corticosteroids and that their disease could be brought under control with proper compliance or with higher doses of corticosteroids. This argument is not supported by the fact that systemic corticosteroid treatment did not significantly decrease the sputum cell T2GM values in these patients. Thus, it is more likely that corticosteroids do not fully suppress type 2 inflammation in many patients and that these patients stand to benefit from adjunctive treatment with specific inhibitors of type 2 inflammation, including inhibitors of type 2 cytokine or inhibitors of the prostaglandin D2 receptor 2 (17–21).

We found that treatment with intramuscular triamcinolone did not significantly decrease airway gene expression for type 2 cytokines in induced sputum cells. Although unexpected, our data are not the first to suggest that corticosteroids have limited effects on airway inflammation in severe forms of asthma. For example, it has been reported that dexamethasone only weakly decreases IL-13 production in lung lymphocytes from patients with moderate severe asthma whereas it strongly inhibits IL-13 production in lung lymphocytes in mild asthma(22). And we have previously shown that systemic corticosteroids has limited effects on airway inflammation and lung function in the same SARP cohort studied here (23). For example, we previously showed that the effect of

intramuscular triamcinolone on sputum eosinophils and FeNO in the SARP cohort (most of whom were taking inhaled or oral corticosteroids) was relatively modest. Specifically, the absolute triamcinolone-associated reduction in sputum eosinophil % was 0.2 and in FeNO was 2 PPM in adult severe and patients. Furthermore, the effect of triamcinolone on lung function was also modest (only 20% of severe asthma patients had an improvement in FEV1 of 10% or greater with parenteral corticosteroid and that the overall steroid-associated improvement in FEV1 was only 3%)(23). Taken together, these data indicate that the effect of additional systemic corticosteroid is blunted in severe patients already on high dose inhaled steroids and that airway type-2 inflammation can remain elevated in asthma in spite of high doses of corticosteroids.

We found that steroid resistant airway type-2 inflammation was significantly higher in older asthmatics when compared to younger asthmatics, a finding that provides a clue for how corticosteroid resistance develops. One possibility is that immunosenescence results in decreased regulatory T cell activity that renders older patients more susceptible to type 2 inflammation (24). Alternatively, prior work has suggested that lung lymphocyte production of the type-2 cytokines are not suppressed by corticosteroid treatment in severe asthma (22). Thus, prolonged exposure to high doses of inhaled corticosteroids over time may render lymphocytes resistant to the suppressive affects of corticosteroid treatment.

In examining the utility of non-invasive biomarkers as tests of refractory airway type 2 inflammation, we found that sputum eosinophils and blood eosinophils outperformed

blood IgE measures and FENO as predictors of refractory type 2-high asthma. But we also noted that age and BMI were significant modifiers of the performance of non-invasive biomarkers of airway type 2 inflammation. Most importantly, blood eosinophils performed poorly as a biomarker in morbidly obese patients and blood IgE levels performed surprisingly well in patients under the age of 35. These data are highly relevant to decisions for when to treat patients with severe asthma with protein therapeutics. In particular, care is needed in ruling out adjunctive treatment with inhibitors of type 2 inflammation in morbidly obese patients with low eosinophil counts. According to our data, the blood eosinophil number may be a poor indicator of airway type 2 inflammation in this asthma subgroup.

Our data provide new insights about bronchodilator responsiveness and airway type 2 inflammation. Specifically, we show that the treatment response to albuterol is better in the srT2-high asthma subgroup than in stT2-low asthma subgroup. We do not believe that this effect of type 2 inflammation has been shown before, although eosinophilic inflammation has been more strongly associated with reversibility than other measures of lung function(25). The mechanism of this effect is not clear but could be related to the role of IL-13 to induce proliferation and hypercontractility of airway smooth muscle cells (26).

Based on the sputum cell T2GM, 45% of severe asthmatics did not have increased type 2 inflammation in their airways. But many of them did have abnormal FEV1 or were exacerbation prone. We propose that these patients are less likely to benefit from

treatment with inhibitors of type-2 inflammation. In exploring other mechanisms of disease in this subgroup, we found no increases in gene expression for IL17 or interferon γ in their sputum cells, and we are unable to point to the mechanism of persistent airway disease in these patients.

Our study adds to the growing number of studies that have used gene expression data from sputum cells to explore patterns of airway inflammation in asthma (27–29). In general, these studies find a subgroup of asthma patients with high type-2 gene expression measures who are phenotypically distinct from patients with low type-2 gene expression (27–29), but not all studies report this (30)

In summary, we conclude that many asthmatics treated with ICS have increased type 2 inflammation in their airways and that these steroid treated type 2-high patients are older and have more severe asthma. This asthma subgroup is eligible for adjunctive treatment with specific inhibitors of type 2 inflammation, but care needs to be exercised in identifying eligible patients using blood- or breath-based biomarkers, because age and body mass index modify the performance of these biomarkers as indicators of refractory airway type 2 inflammation.

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Characteristic	Healthy (N = 30)	Asthma		p-value srT2 High vs. stT2 Low
		stT2-Low (N = 129)	srT2-high (N= 130)	
Age (years)	38.0 (13.1)	46.2 (13.7)*	50.3 (14.0)* [†]	0.02
Female sex - no. (%)	18 (60)	81 (63)	96 (74)	0.06
Race - no. (%) [†]				0.01
American Indian and Alaska Native	0 (0)	1 (1)	0 (0)	0.01
Asian	2 (7)	1 (1)	11 (9)	
African American	4 (13)	25 (19)	34 (26)	
Caucasian	20 (67)	94 (73)	76 (59)	
Native Hawaiian or other Pacific Islander	0 (0)	0 (0)	0 (0)	
Mixed race	4 (13)	8 (6)	9 (7)	
BMI (kg/m ²)	26.7 (4.9)	33.0 (8.8)*	32.4 (8.3)*	0.53
% with BMI > 30	6 (21)	76 (59)*	70 (54)*	0.41
Spirometry				
FEV1 (% predicted)	97.5 (12.2)	76.1 (19.2)*	69.5 (17.9)* [†]	0.005
FVC (% predicted)	98.0 (14.6)	85.9 (16.5)*	84.1 (16.2)*	0.36
FEV1/FVC	99.3 (4.6)	87.5 (12.1)*	81.7 (11.0)* [†]	<0.001
FEV1% <80 n(%)	0 (0)	70 (54)	93 (72)	0.004
Blood cell counts (X 10 ⁶ /L)				
Total white blood cells	6.0 (1.5)	7.5 (2.7)	7.9 (2.6)	0.21
Neutrophils	3422 (1526)	4585 (2269)*	4632 (2050)	0.86
Eosinophils	126 (62)	199 (157)*	399 (319)* [†]	<0.001

Serum IgE (IU/L)	55 (59)	265 (395)*	468 (608)* [†]	<0.001
FeNO (ppm)	19 (14)	20 (16)	35 (23)* [†]	<0.001
Number of Specific IgE, out of 15 [‡]	0 (0-3)	3 (1-6)*	4 (1-8)*	0.15
Sputum cell counts (%)				
Eosinophils	0.25 (0.1-0.7)	0.4 (0.1-0.7)	1.9 (0.6-7.2) [†]	<0.001
Neutrophils	64 (45-79)	51 (34-73)	53 (37-75)	0.43
Sputum <i>IL17</i> gene expression [§]	17.9 (2.4)	17.9 (2.3)	18.3 (2.4)	0.14
Sputum <i>IFNγ</i> gene expression [§]	18.4 (2.0)	17.7 (2.5)	18.5 (2.4)	0.01
Score on ACT [¶]		18 (14, 21)	17 (14,21)	0.43
Severity ATS/ERS Criteria				0.02
Mild		26 (20)	11 (9)	
Moderate		25 (19)	23 (18)	
Severe		78 (61)	96 (74)	
On High Dose ICS no. (%)		80 (62)	98 (75)	<0.001
On daily oral corticosteroids no. (%)		13 (10)	19 (15)	0.27
Asthma exacerbations – no. (%)				
No exacerbation in past year		63 (49)	41 (39)	0.02
1-2 exacerbations in the past year		39 (31)	51 (40)	
>2 exacerbations in the past year		26 (20)	36 (28)	
Necessitating hospitalization past year no. (%)		16 (12)	13 (10)	0.54
Necessitating ER visit past year no. (%)		33 (26)	30 (23)	0.64
Age of Asthma Onset (years)		15.8 (13.7)	18.1 (16.1)	0.22
Maximal BD Response Test (FEV1%)		9.7 (7.1)	14.0 (8.6)	<0.001
Systemic Corticosteroid Response Test (FEV1%)		-0.06 (7.1)	5.3 (8.9)	<0.001

Data reported as mean (SD) unless otherwise indicated. FEV₁ = forced expiratory volume in 1 second. BMI = body mass index. FVC = forced vital capacity, ACT = asthma control test, ATS= American Thoracic Society, ER = emergency room. MBRT = Maximal Bronchodilator Response Test, SCRT= Systemic Corticosteroid Response Test.

* Indicates statistically different from Healthy ($p < 0.05$). [†] Indicates statistically different between rT2 High vs sT2 Low Asthma ($p < 0.05$). [‡] Total number of positive specific blood IgE tests (ImmunoCap), out of the 15 allergens tested. [§] Gene expression data is in log2 scale normalized to 4 housekeeping genes. [¶] Scores on ACT range from 25 to 5 with lower scores indicating worse asthma control. ^{||} Definition of high dose ICS is $\geq$ to the equivalent of 1000ug fluticasone daily.

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Figure Legends:

Figure 1. Sputum gene expression measures in 30 healthy control subjects, 259
 asthma subjects on inhaled corticosteroids before intramuscular injection of
 triamcinolone (Pre-CS), and 283 asthma subjects on inhaled corticosteroids after 40mg
 intramuscular injection of triamcinolone (Post-CS). **A-C)** Measures of IL-4, IL-5, and IL-
 13 are higher in asthma subjects on inhaled corticosteroids compared to healthy control
 subjects, and remain elevated following triamcinolone injection. **D)** The T2GM (a
 summary metric of IL-4, IL-5, and IL-13 sputum gene expression) is significantly higher
 in asthma subjects on inhaled corticosteroids than in healthy subjects at baseline and
 remains significantly higher after the triamcinolone injection. Asthma subjects above the
 median T2GM value (pre-CS) were classified as having steroid resistant T2-high (srT2-
 high) asthma, and subjects below this median value were classified as having stT2-low
 (stT2-low) asthma. **E,F:** Measures of IFN γ and IL17 are no different in asthma subjects
 compared to healthy control subjects. **G:** Kernel density plot demonstrating the T2GM is
 lower in health control subjects (black line) compared to non-severe asthma subjects
 (blue line) or severe asthma subjects (red line) ($p < 0.01$). T2GM measures are
 significantly higher in severe asthma subjects compared to non-severe asthma subjects
 ($p < 0.01$). *Indicates significantly different to healthy control, $p < 0.05$

Figure 2. A) T2GM measures gradually increase in older subjects. Age Quintiles: 1 (18-
 33.7), 2 (33.8-45.1), 3 (45.3-52.9), 4 (52.9-61.6), 5 (62.0-84.4). **B)** T2GM measures do
 not change in more obese subjects.

Figure 3. A) Receiver operating characteristic curves for the type-2 biomarkers for the outcome of steroid resistant type-2 high (srT2-high) asthma. **B)** Performance characteristics of blood eosinophil cell counts for predicting srT2-high asthma. * Indicates that blood eosinophil cell count of ≥ 189 cells/uL had the highest specificity and sensitivity for differentiating stT2-high asthma from stT2-low asthma, LR= Likelihood Ratio.

Figure 4. A) The relationship between serum IgE and the T2GM is modified by age (Wald test for interaction, $p=0.004$). Five linear regression lines demonstrate the relationship between log transformed serum IgE levels and the T2GM sub-grouped by age quintiles. Asthma subjects in the youngest quintile have serum IgE levels that strongly correlate to airway T2GM measures (red line) whereas this relationship decreases in the older quintiles (green, blue, maroon, and orange lines). **B)** The relationship between blood eosinophil and T2GM is modified by BMI (Wald test for interaction, $p=0.02$). Five linear regression lines demonstrate the relationship between log transformed blood eosinophils and the T2GM sub-grouped by WHO BMI categories. Whereas asthma subjects in BMI categories <40 have blood eosinophil levels that correlate to airway T2GM measures (green, blue, maroon, and orange lines), this relationship decreases in subjects with BMI ≥ 40 (red line); dy/dx is the marginal effect of serum IgE on the T2GM stratified by age quintiles or the marginal effect of blood eosinophils on the T2GM stratified by BMI WHO classification. The p-values are for: (i) the comparison of the average marginal effects of the four oldest age quintiles compared to the youngest (reference) quintile; (ii) the comparison of the average

marginal effects of the four lowest BMI WHO categories compared to the highest (reference) BMI WHO category.

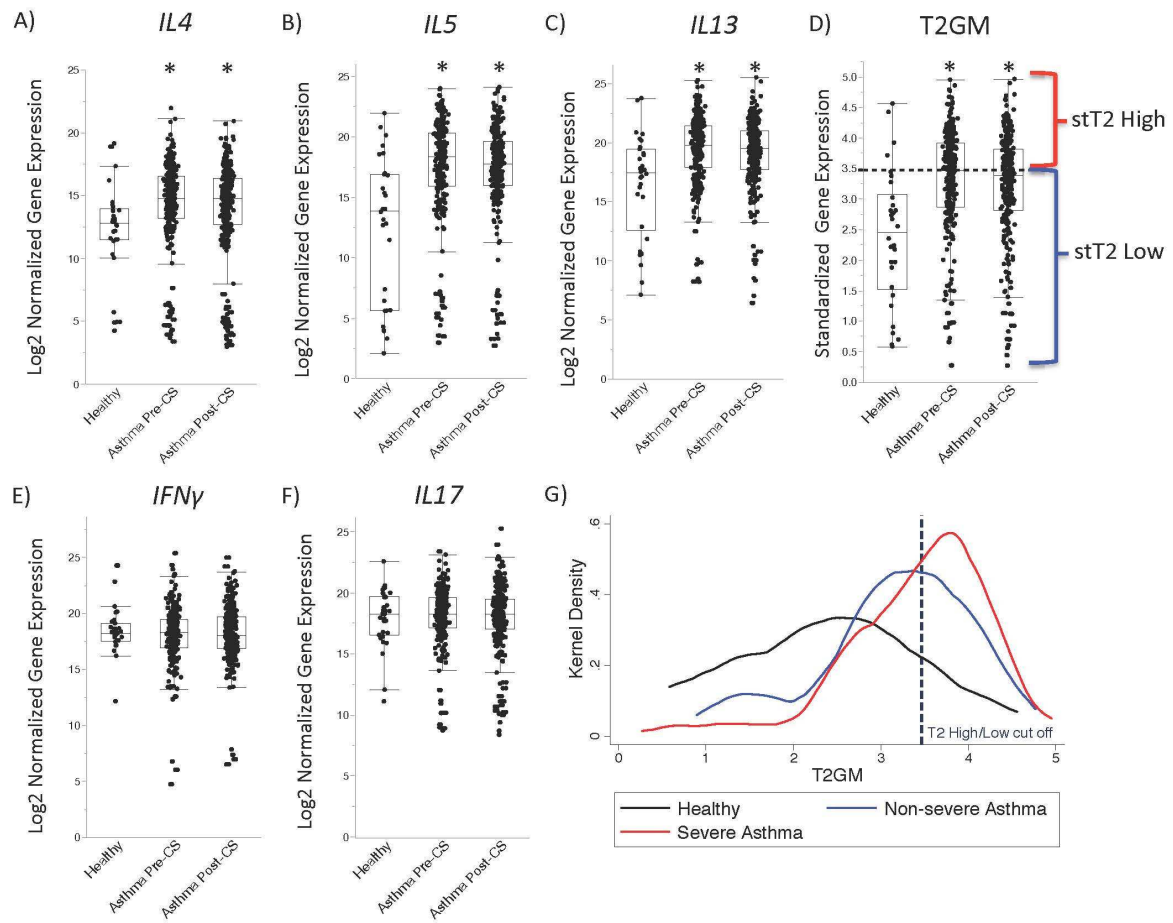
Figure 5: A) Maximal response to bronchodilators (BD-response) is higher in srT2-high asthma compared to stT2-low asthma, B) Absolute Change in FEV1% 2 weeks after a triamcinolone injection is higher in stT2-high compared to stT2-low asthma, C) Receiver operating characteristic curves demonstrating the T2GM as a superior predictor of a 5% absolute change in FEV1% two weeks after a triamcinolone injection compared to other biomarkers of airway type-2 inflammation. AUC= Area Under the Curve, * Indicates p-value <0.05 for the T2GM AUC compared to other biomarkers. AUC = Area Under the Curve, 95% CI = 95% confidence interval of AUC.

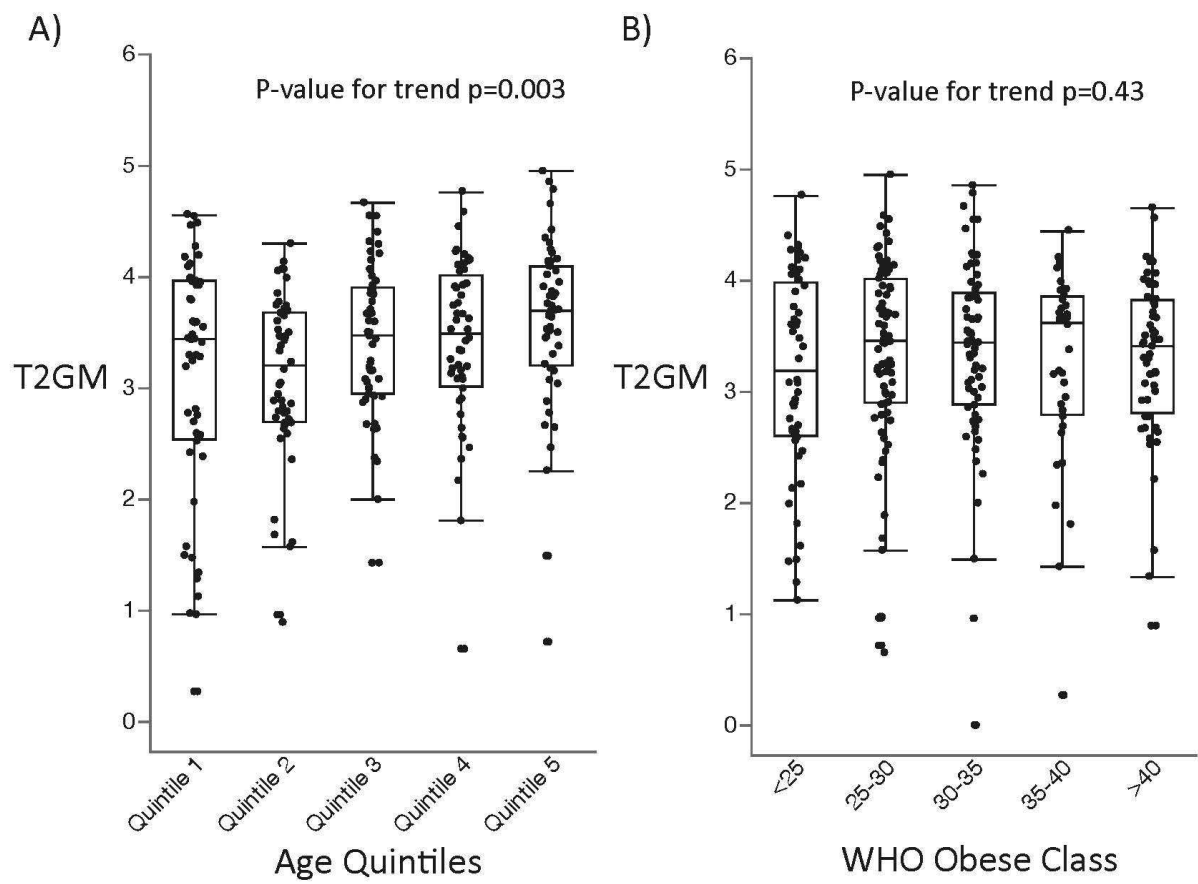
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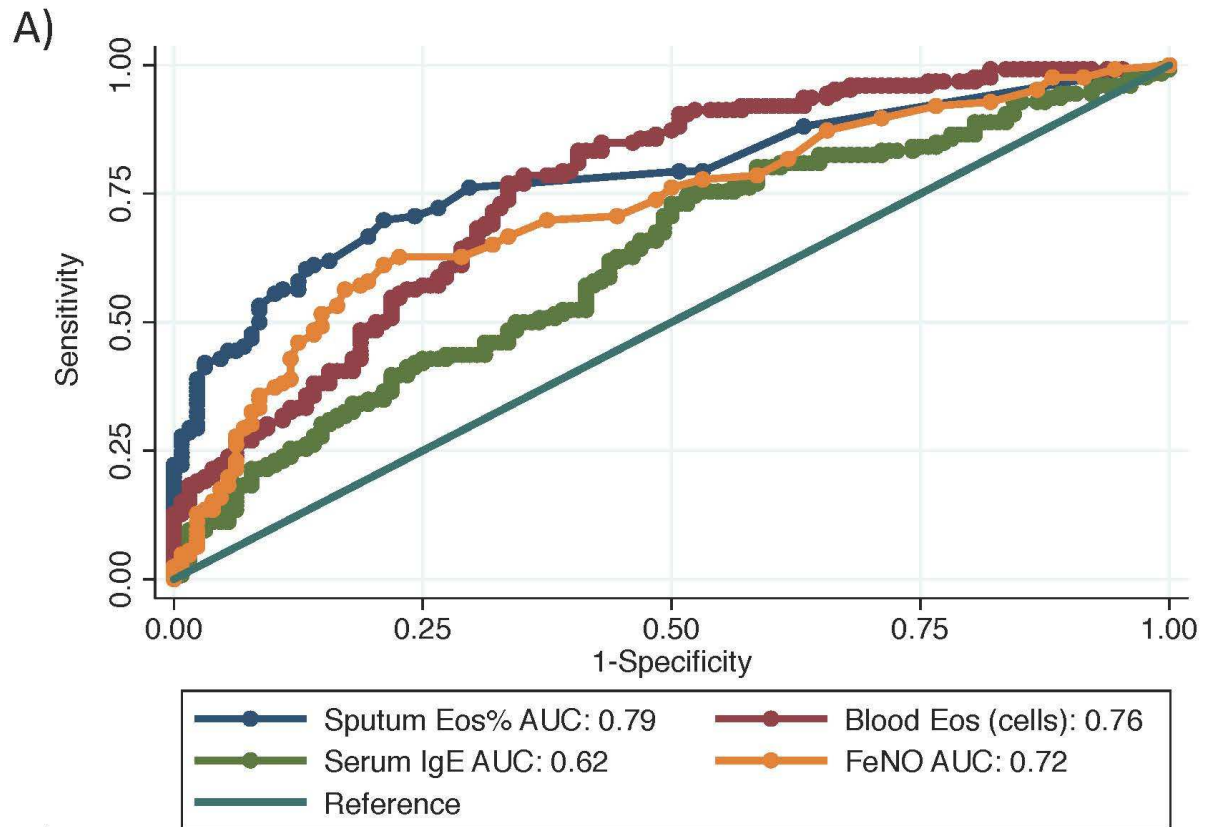
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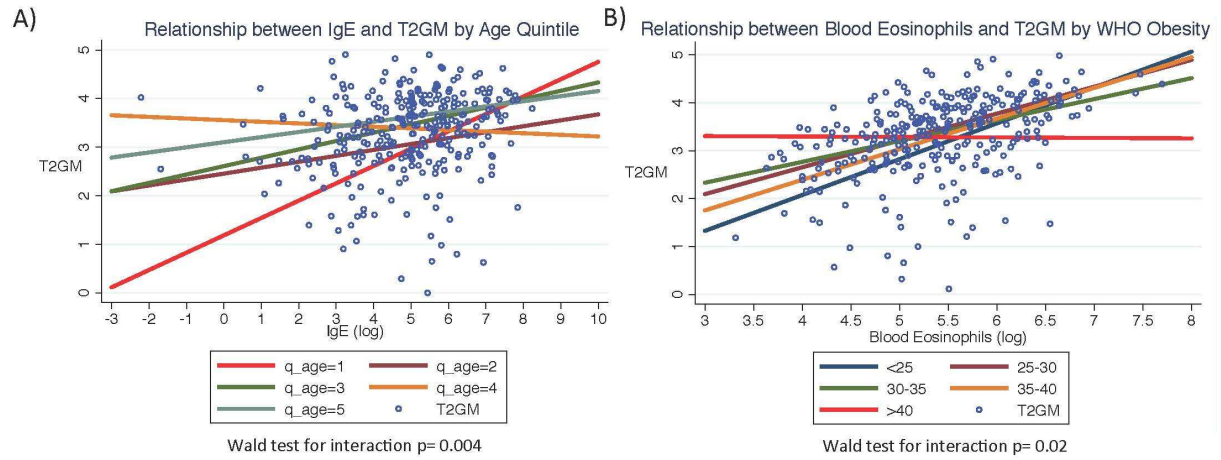






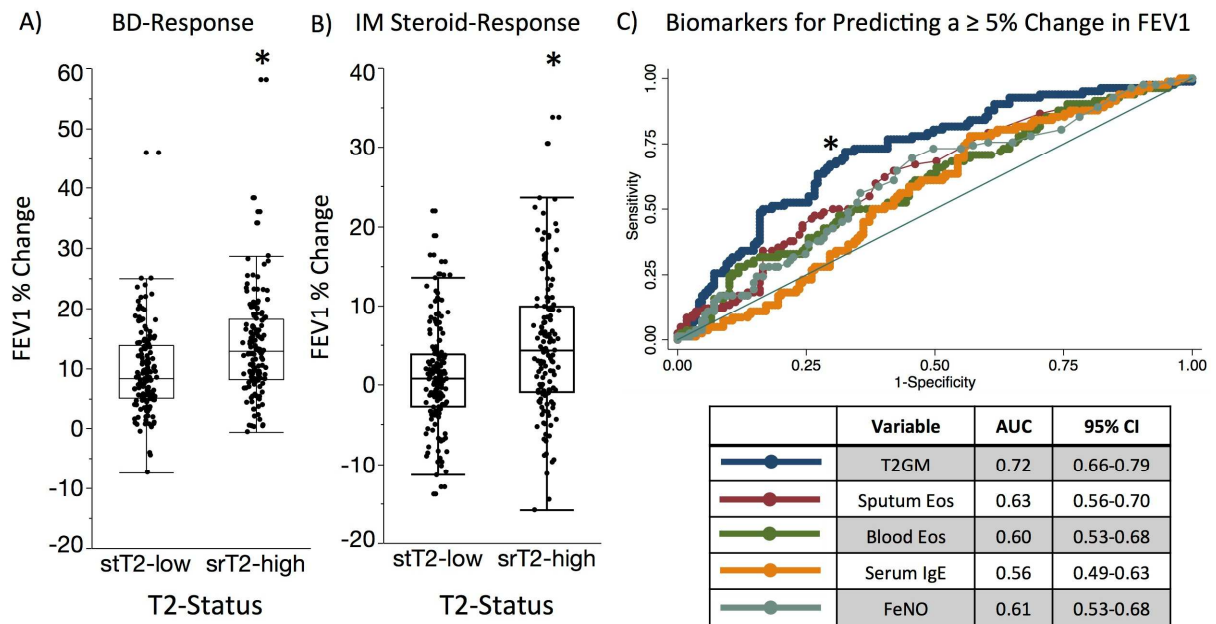
B)

Blood Eosinophil	Sensitivity	Specificity	LR+	LR-
$\geq 189^*$	77.7	66.7	2.34	0.33
≥ 200	74.6	66.7	2.24	0.38
≥ 250	60.7	71.3	2.11	0.55
≥ 300	50.8	78.3	2.34	0.63
≥ 350	40.8	83.0	3.29	0.71



Age Quintile (year range)	N (%)	dy/dx	P-value
1 (18.0-33.7)	52 (20)	0.36	ref
2 (33.8-45.1)	52 (20)	0.12	0.0212
3 (45.3-52.9)	52 (20)	0.17	0.0893
4 (52.9-61.6)	52 (20)	-0.03	0.0001
5 (62.0-84.4)	51 (20)	0.11	0.0194

WHO Obese Category (BMI range)	N (%)	dy/dx	P-value
1 (18-24.9)	42 (16)	0.74	0.0008
2 (25-29.9)	71 (27)	0.56	0.0049
3 (30-34.9)	62 (24)	0.44	0.0333
4 (35-39.9)	34 (13)	0.64	0.0231
5 (40-69.8)	50 (19)	-0.01	ref



**Title: Refractory Airway Type-2 Inflammation in a Large Subgroup of Asthmatics
treated with Inhaled Corticosteroids**

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Supplementary Materials and Methods

SARP Patients

Adult asthma patients were recruited to the SARP from November 1, 2012 to October 1, 2015 by seven clinical research centers (including UCSF) across the United States. Recruitment techniques varied by study site and included the 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 email to students/faculty, and the use of recruitment databases. The SARP protocol included 2-3 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 meet the American Thoracic Society/European Respiratory Society (ATS/ERS) definition for severe asthma. The medication requirements for this definition included treatment with either continuous or near continuous systemic corticosteroids or high-dose ICS (>880 mcg fluticasone daily or equivalent) for at least half of the past year and continuously for the prior 3 months, plus a second controller medication (i.e. long-acting beta agonist [LABA], leukotriene modifier). In addition to the medication requirement, patients with severe asthma had to meet at least one minor criterion that indicated persistence of significant asthma impairment or report that their symptoms immediately deteriorated after a medication taper. The remaining 40% of patients with asthma did not meet the criteria for severe asthma and were classified as having non-severe asthma. All patients were non-smokers (<10 pack-years of tobacco use if >30y of age; <5 pack-years if <30y of age) and were required to have evidence of BHR (defined as a PC20 methacholine < 16mg/mL) or reversible airflow obstruction, as evidenced by an increase in FEV1 of $\geq 12\%$ following albuterol inhalation (up to 720 mcg) and/or ipratropium bromide inhalation (136 mcg). Patients were excluded if they were pregnant or breastfeeding 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 measurement. In addition, patients completed extensive questionnaires that characterized asthma symptoms, quality of life, medication use, and health care utilization. The SARP protocol included two baseline visits in which patients underwent detailed characterization studies and provide samples of venous blood and induced sputum. The data reported here is from these two baseline visits. All patients signed an informed consent form approved by their local institutional review board.

Procedures for withholding asthma and allergy medications

SARP asthma patients were also asked to hold their bronchodilator medications prior to spirometry testing. The medication holds for SARP were as follows; short-acting beta agonists - 4 hours; short-acting anticholinergics - 6 hours; LABA - 12 hours; LAMA - 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 mcg) were administered. Spirometry was then repeated after 15 minutes. An additional 2 puffs of albuterol (180 mcg) was then administered and spirometry was repeated again after 15 minutes. If the change in FEV1 from the spirometry maneuver performed after 4 puffs and after 6 puffs was greater than 5%, an additional 2 puffs of albuterol was 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.

Table S1. Sequences of Primers and Probes used for sputum cell gene expression

Gene Primers	Sequence
PPIA-outer forward	ATGAGAACTTCATCCTAAAGCATACG
PPIA-outer reverse	TTGGCAGTGCAGATGAAAACT
PPIA-inner forward	ACGGGTCCTGGCATCTTGT
PPIA-probe	ATGGCAAATGCTGGACCCAACACA
PPIA-inner reverse	GCAGATGAAAACTGGGAACCA
GAPDH-outer forward	CAATGACCCCTTCATTGACCTC
GAPDH-outer reverse	CTCGCTCCTGGAAGATGGTGAT
GAPDH-inner forward	GATTCCACCCATGGCAAATTC
GAPDH-probe	CGTTCTCAGCCTTGACGGTGCCA
GAPDH-inner reverse	GGGATTTCCATTGATGACAAGC
YWHAZ-outer forward	CTTCTGTCTTGTCAACCAACCATTC
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	GTAAGTGAATGTCCAACGC
IFNG-outer reverse	GACAACCATTACTGGGATGCTC
IFNG-inner forward	CCAACGCAAAGCAATACATGA
IFNG-probe	TCCAAGTGATGGCTGAACTGTCGCC
IFNG- inner reverse	TTTCGCTTCCCTGTTTTAGCT

Table S2: Comparison of Asthma Subjects on ICS with sputum samples at Visit 2 compared to those without sputum samples at Visit 2.

Characteristic	No sputum (N=214)	Sputum (N=259)	P-value
Age (years)	48.7 (12.9)	50.3 (14.0)	0.76
Female sex - no. (%)	141 (66)	177 (68)	0.57
Race - no. (%)			0.59
American Indian and Alaska Native	1 (0)	1 (0)	
Asian	6 (3)	12 (5)	
African American	61 (29)	59 (23)	
Caucasian	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)	0.90
ATS/ERS Criteria Severe Asthma	138 (65)	174 (67)	0.54

Table S3: Comparison of Asthma Subjects on ICS with sputum samples at Visit 3 compared to those without sputum samples at Visit 3.

Characteristic	No sputum (N=190)	Sputum (N= 283)	P-value
Age (years)	48.01 (13.5)	48.7 (13.5)	0.65
Female sex - no. (%)	121 (64)	197 (69)	0.25
Race - no. (%)			0.69
American Indian and Alaska Native	0 (0)	2 (1)	
Asian	6 (3)	12 (4)	
African American	52 (27)	68 (24)	
Caucasian	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)	0.08
ATS/ERS Criteria Severe Asthma	130 (68)	182 (64)	0.36

Table S4: Quantile regression models evaluating the effect of a one unit change in each characteristic on the T2GM*.

Characteristic	Beta Coefficient	95% CI	p-value
Age (years)	0.01	(-0.00 to 0.19)	0.05
Male sex	-0.28	(-0.54 to -0.03)	0.03
BMI (kg/m ²)	0.00	(-0.12 to 0.01)	0.44
Spirometry			
FEV1 (% predicted)	-0.01	(-0.02 to -0.00)	0.001
FVC (% predicted)	-0.01	(-0.01 to 0.00)	0.15
FEV1/FVC	-0.02	(-0.03 to -0.01)	<0.001
Blood cell counts (X 10 ⁶ /L)			
Neutrophils (100 cell change)	0.00	(-0.00 to 0.01)	0.65
Eosinophils (100 cell change)	0.10	(0.06 to 0.14)	<0.001
Serum IgE (IU/L, 100 IU change)	0.02	(0.00 to 0.04)	0.03
FeNO (ppm)	0.02	(0.01 to 0.02)	<0.001
Sputum cell counts (%)			
Eosinophils	0.04	(0.03 to 0.05)	<0.001
Neutrophils	0.00	(0.00 to 0.01)	0.34
Sputum <i>IL17</i> gene expression ^s	0.05	(0.00 to 0.10)	0.05
Sputum <i>IFNγ</i> gene expression ^s	0.05	(0.00 to 0.10)	0.04
Score on ACT [¶]	-0.01	(-0.03 to 0.02)	0.55
Severity ATS/ERS Criteria			
Mild	Ref	Ref	Ref
Moderate	0.10	(-0.34 to 0.55)	0.65
Severe	0.37	(0.00 to 0.75)	0.05

Asthma exacerbations – no. (%)			
No exacerbation in past year	Ref	Ref	Ref
1-2 exacerbations in the past year	0.27	(-0.03 to 0.57)	0.08
>2 exacerbations in the past year	0.41	(0.12 to 0.70)	0.006
Age of Asthma Onset (years)	0.01	(0.00 to 0.02)	0.12
Maximal BD Response Test (FEV1%)	0.03	(0.02 to 0.04)	<0.001
Systemic Corticosteroid Response Test (FEV1%)	0.03	(0.02 to 0.04)	<0.001

Beta coefficient is reported as a one unit change in the T2GM for each one unit change in the predictor unless otherwise indicated. ATS= American Thoracic Society

* T2GM is a scaled measure of airway type-2 inflammation on a log2 scale.

FEV₁ = forced expiratory volume in 1 second. BMI = body mass index. FVC = forced vital capacity, ACT = asthma control test,

[§]Gene expression data is in log2 scale normalized to 4 housekeeping genes. [¶] Scores on ACT range from 25 to 5 with lower scores indicating worse asthma control.

Table S5: Interaction between Age Quintiles on the relationship between biomarkers of type-2 inflammation and the T2GM.

Type-2 Biomarkers	P-value Wald Test for Interaction
Blood IgE (IU)	0.004
Blood Eosinophils ($\times 10^6/L$)	0.59
Sputum Eosinophils (%)	0.09
FeNO (PPM)	0.20

Table S6: Interaction between BMI WHO Categories on the relationship between biomarkers of type-2 inflammation and the T2GM.

Type-2 Biomarkers	P-value Wald Test for Interaction
Blood IgE (IU)	0.79
Blood Eosinophils ($\times 10^6/L$)	0.02
Sputum Eosinophils (%)	0.35
FeNO (PPM)	0.27

Table S7: Clinical characteristics of subjects with persistent or fluctuating T2GM measure before and after triamcinolone injection.					
Characteristic	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 (years)	44.7 (13.1)	45.8 (12.6)	48.4 (13.7)	52.5 (14.6)	0.01
Female sex - no. (%)	47 (66)	15 (60)	34 (83)	44 (75)	0.14
Race - no. (%)					0.06
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)	
Caucasian	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)	0.10
Spirometry (baseline)					
FEV1 (% predicted)	79.1 (14.6)	72.6 (19.5)	76.6 (17.1)	68.5 (17.0)	0.003
FVC (% predicted)	88.1 (13.5)	84.2 (13.8)	90.0 (16.1)	83.3 (15.1)	0.09
Spirometry (post-steroid)					
FEV1 (% predicted)	78.1 (16.4)	73.2 (20.2)	81.5 (15.3)	72.9 (19.3)	0.07
FVC (% predicted)	87.1 (14.7)	84.9 (15.7)	93.3 (15.5)	85.8 (15.3)	0.06

Severity ATS/ERS Criteria no. (%)					0.034
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 following SCRT					
T2GM	-0.11 (0.89)	1.04 (0.59)	-0.86 (0.54)	-0.13 (0.41)	<0.001
Blood Eosinophils ($\times 10^6/L$)	-30 (146)	-7 (106)	-230 (335)	-232 (341)	0.001
Sputum Eosinophils (%)	-0.2 (1.2)	0.4 (2.5)	-2.7 (4.6)	-4.6 (10.0)	0.01
FeNO (ppm)	-3 (16)	2 (20)	-12 (20)	-6 (17)	<0.001
FEV1% predicted	-1.0 (6.8)	0.6 (9.6)	4.9 (8.1)	4.4 (9.1)	<0.001
Data reported as mean (SD) unless otherwise indicated. FEV₁ = forced expiratory volume in 1 second. BMI = body mass index. FVC = forced vital capacity, ATS= American Thoracic Society, ERS = European Respiratory Society, SCRT = Systemic Corticosteroid Response Test, FeNO= Fractional Exhaled Nitric Oxide. * p-value comparisons use ANOVA for continuous variables and Pearson's chi-squared for categorical variables. ‡ Blood Eosinophil data was available on 101 asthma subjects.					

Supplemental Figure Legends:

Figure S1: *Schematic of the SARP protocol (6 visits over 3 years).* **V1:** Characterization of asthma severity (lung function measures and asthma questionnaires); **V2,3,4,5,6:** Disease Characterization, lung function (pre/post bronchodilation), blood and sputum collection, asthma disease questionnaires; **V3a,4a,5a:** Phone interview, asthma disease questionnaires. **Black arrow:** Indicates a visit with an intramuscular injection of triamcinolone acetonide (40mg) **Red arrows:** Indicates visits that include sputum induction and blood draws; **Blue arrows:** Indicate visits that measure FEV1 before and after bronchodilation with albuterol; **Purple arrows:** Indicate visits that document exacerbation history;

Figure S2: *Sample Attrition:* Numbers of samples excluded from our study. * 4 healthy subjects were excluded for having evidence of significant atopy (>5 positive tests for common aeroallergens on ImmunoCAP® immunologic testing).

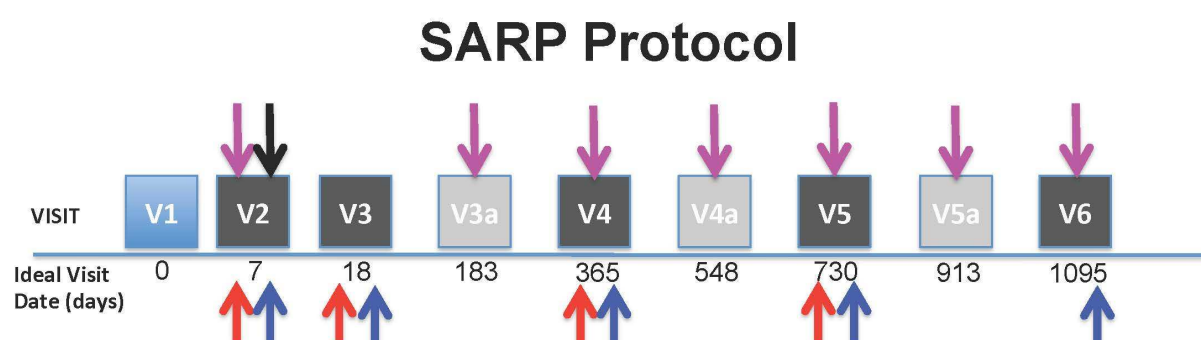
Figure S3: **A)** Receiver operating characteristic (ROC) curves for **A)** blood eosinophil cell counts as a predictor of refractory T2-high asthma in asthma subjects taking ICS with BMI<40 (n=210) and BMI≥40 (n=50) and **B)** blood IgE levels as a predictor of refractory T2-high asthma in asthma subjects taking ICS with age<34 (n=52) and age≥34 (n=208).

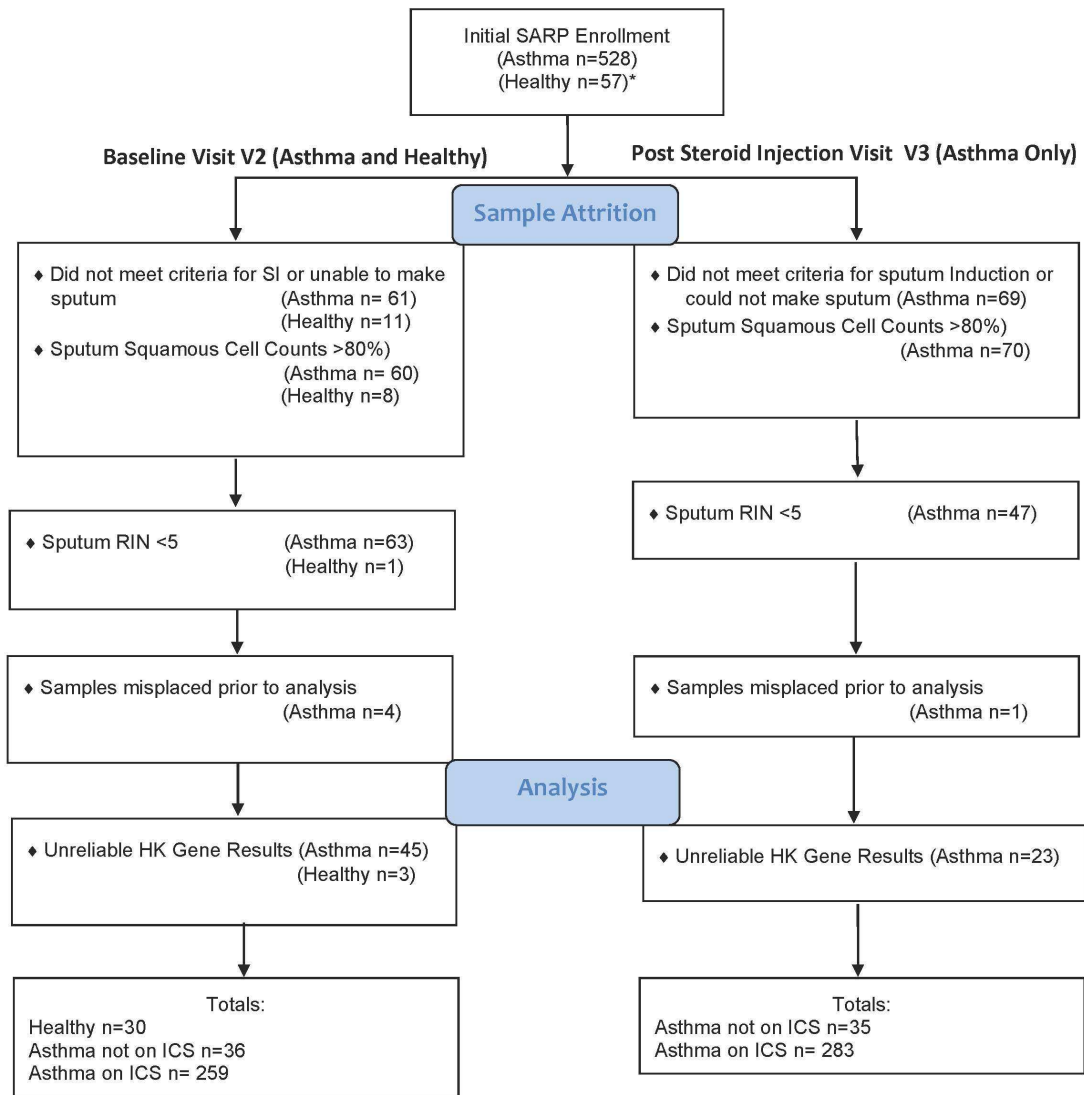
Figure S4: A sensitivity analysis using quantile regression models as opposed to OLS models. **A)** The relationship between serum IgE and the T2GM is modified by age (Wald test for interaction, $p=0.01$). Five quantile regression lines demonstrate the relationship between log transformed serum IgE levels and the T2GM sub-grouped by age quintiles. Asthma subjects in the youngest quintile have serum IgE levels that strongly correlate to airway T2GM measures (red line) whereas this relationship decreases in the older quintiles (green, blue, maroon, and orange lines). **B)** The relationship between blood eosinophil and T2GM is modified by BMI (Wald test for interaction, $p=0.05$). Five quantile regression lines demonstrate the relationship between log transformed blood eosinophils and the T2GM sub-grouped by WHO BMI categories. Whereas asthma subjects in BMI categories <40 have blood eosinophil levels that correlate to airway T2GM measures (green, blue, maroon, and orange lines), this relationship decreases in subjects with BMI≥40 (red line); dy/dx is the marginal effect of serum IgE on the T2GM stratified by age quintiles or the marginal effect of blood eosinophils on the T2GM stratified by BMI WHO classification. The p-values are for: (i) the comparison of the average marginal effects of the four oldest age quintiles compared to the youngest (reference) quintile; (ii) the comparison of the average

marginal effects of the four lowest BMI WHO categories compared to the highest (reference) BMI WHO category.

Figure S5: Receiver operating characteristic curves demonstrating the T2GM as a superior predictor of a 7.5% absolute change in FEV1% **A)** and 10% absolute change in FEV1% **B)** two weeks after a triamcinolone injection compared to other biomarkers of airway type-2 inflammation. AUC= Area Under the Curve

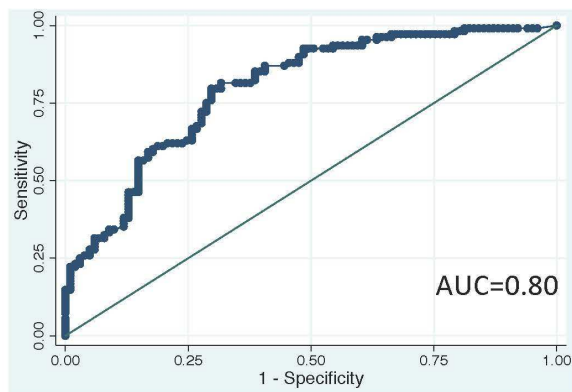
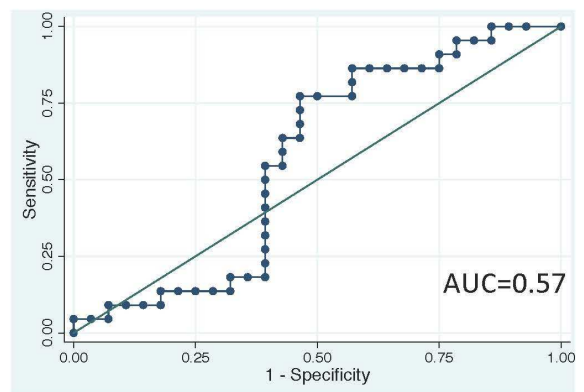
Figure S6: Asthma patients with sputum eosinophil % between 2-5% (n=31) or >5% (n=51) have higher sputum T2GM measurements compared to subjects with sputum eosinophil % <2% (n=213).





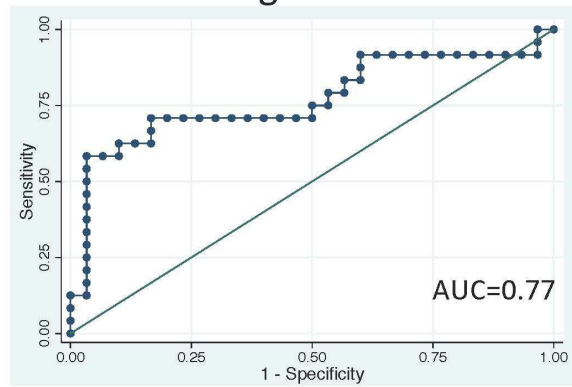
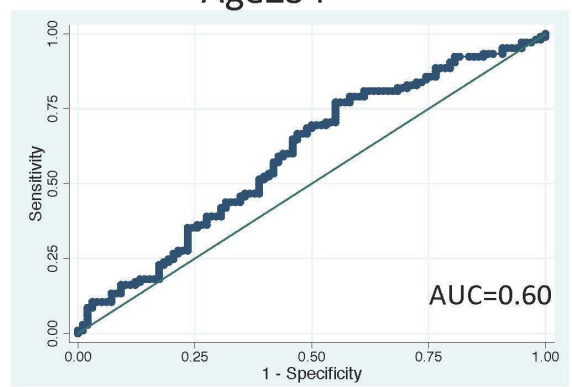
A)

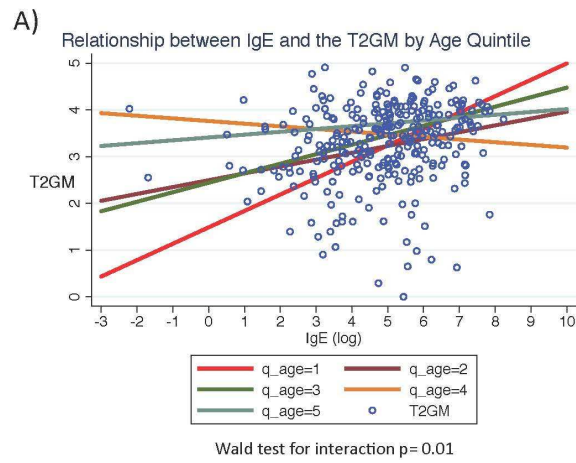
BMI<40

BMI $\geq$ 40

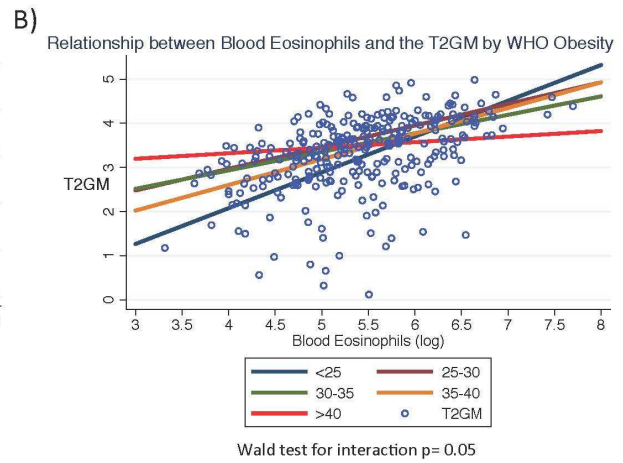
B)

Age<34

Age $\geq$ 34



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

