Web Supplement:

Sputum eosinophils, but not blood eosinophils, associate with COPD exacerbations in SPIROMICS cohort.

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METHODS-Additional Details:

Subjects:

Subjects with a current or former history of tobacco use (≥20 pack-year) and nonsmokers, age 40-80 (N=2737), were enrolled in the Subpopulations and Intermediate Outcome Measures in COPD Study (SPIROMICS) at six clinical sites and additional subsites (Columbia University [with Johns Hopkins University and University of Iowa]; University of Michigan [with Temple University]; University of California-Los Angeles; University of California-San Francisco [with National Jewish Health]; University of Utah [with University of Illinois]; Wake Forest University [with University of Alabama]). The overall study design has been reported.[S1] Briefly, subjects underwent extensive phenotypic characterization at baseline including lung function assessment pre- and post-bronchodilator (4 puffs of both albuterol and ipratropium), total lung capacity and residual volume (TLC and RV) using QCT indicators for emphysema (%voxels<-950HU) and air-trapping (%voxels<-856HU) and parametric response mapping (PRM) for functional small airways disease (fSAD,),[S2,S3] collection of blood (for DNA, RNA, plasma, sera, and complete blood cell counts [CBC]), urine, 6 minute walk distance, BODE index, COPD Assessment Score (CAT), St. Georges Respiratory Questionnaire (SGRQ), and administration of questionnaires covering medical history, exacerbations, hospitalizations, respiratory exposures, symptoms (self-reported wheeze, cough, shortness of breath) and medications. COPD was defined as post-bronchodilator FEV1/FVC ratio<0·7. In the N=2499 enrolled ever smokers (analyses excluded nonsmokers, Consort Diagram, Figure S1) mean blood eosinophil count was 200±240/μL (median=190/μL; range 0-8300/μL), the mean was used to stratify the entire ever-

smoker cohort. A higher cutpoint of blood eosinophils ≥300/μL was also examined; these cutpoints coincide with those reported in observational or clinical studies (181·6/μL,[S4]; 210/μL,[S5]; 150 and 300/μL, biomarker negative and positive groups, respectively,[S6]) and as obtained in the classification tree modeling exacerbations (Figure S2). Higher blood eosinophil stratification did not alter associations with many phenotypic characteristics or significant differences between high and low eosinophil groups (See results comparing Tables 1-5 in main manuscript with Supplement Tables S2-S6, respectively). There were some site differences for eosinophil blood cell differentials as determined at each clinical site, but post-hoc analysis did not find any pairwise differences. The two sites which appeared to have outlying median levels for blood eosinophils were ones having among the fewest number of subjects analyzed (6 and 48) and their interquartile ranges were equivalent to all other sites. Thus, it was determined that these would not greatly impact results and these subjects were not excluded from analyses based on blood eosinophils.

A separate repeatability substudy conducted 2-6 weeks after the initial baseline study (N=98) (Abstract A3515 presented at ATS 2016: Short-term stability of pulmonary function and clinical measures in COPD using a cohort from SPIROMICS. W. Anderson et al) collected blood and sputum. The regression slope for the model of baseline and repeat blood eosinophils was 0·95 ($p<0·0001$) indicating very good reproducibility.

Subjects with a current asthma diagnosis were excluded, but all subjects were asked if they had ever had a health care professional say they had asthma ("prior asthma label").

Additional information regarding subjects who experienced bronchoconstriction during the sputum induction process was collected and is presented here with emphasis that this

group was stratified by their FEV1%predicted post bronchodilator into those >35% but <50%, and those >50% and for a large portion, no sputum sample was collected. Thus, this information does not directly match with those subjects having acceptable sputum slide counts. After removing the normal, non-smoking subjects (N=199), there were 1794 subjects whose FEV1%predicted was >50% and 258 subjects whose FEV1%predicted was >35% but < 50% prior to induction. An additional 486 subjects did not have sputum induction forms. Of the 1794, 1122 did not stop early due to bronchoconstriction (although 53 required albuterol post induction). There were 322 (18%) who stopped early due to bronchoconstriction; 332 stopped for other reasons, and for the remaining 18 subjects this information was missing. Of the 322 who dropped >20%, 196 (11% of total in the >50% group) required albuterol administration; of the 332 who stopped for other reasons, only 30 (1·7%) required albuterol administration.

For the more restricted lung function group (258 subjects whose FEV1%predicted was between 35-50% prior to induction), there were 180 who stopped early; 133 of these were due to FEV1%predicted dropping >20% (52% of the total group with FEV1% predicted between 35-50%); the remaining 47 stopped for other reasons. Of the 133 subjects stopping early for FEV1%predicted dropping>20%; 79 (59%) required albuterol post-induction. There were 73 subjects who did not stop early and of these 8 (3%) required albuterol post-induction. A remaining 5 in the >35% but <50% group did not have this information available. As may be expected, the greater proportion of this group with poorer lung function prior to induction, experienced bronchoconstriction during the induction process and required albuterol afterwards.

Sputum Induction and Processing:

All staff were centrally trained and certified for sputum induction and processing. SPIROMICS subjects whose post-bronchodilator FEV_1%predicted was $\geq$35% but <50% were eligible for sputum induction with normal saline followed by 3% saline if their FEV_1 did not drop >10%; those with FEV_1%predicted $\geq$50% were induced with sequential 3%, 4% and 5% saline solutions, nebulized for 7min intervals each with spirometry performed 2 min after the start of each higher saline concentration. If the subject's FEV_1 dropped more than 20% of predicted at any step or the subject complained of breathing discomfort, the induction process was stopped. Sputum samples were expectorated after each 7 min interval, or at the end of a procedure terminated before completion. Sputum samples were immediately processed. Initially samples had aliquots removed for mucin, microbiome, and viscoelasticity, but this procedure was later modified to remove these from only those samples weighing $\geq$2·5g because a large number of samples below this amount had insufficient sample remaining for preparation of cytospin slides (see Consort diagram, Figure S1). The remaining sample was weighed and diluted with a 1:4 ratio (weight:volume) of 10% sputolysin solution, and rocked at room temperature for 15 minutes. The sample was further diluted with an equal 4-fold volume of 1mM EDTA and rocked for an additional 5 minutes. The sample was next filtered through a 48-52 μm nylon mesh filter. The filtrate was centrifuged at 500xg for 10 minutes to pellet cells. Supernatant fluid was dispensed into 1-4 aliquots for nucleotide and for cytokine examination. The cell pellet was resuspended in 1 or 2 ml of HBSS and a cell count performed. This count was employed to determine cytospin slide preparation with approximately 60,000 cells per slide. Cytospin slides were stained, dried, coverslips applied and slides shipped to the central sputum slide reading center

(University of NC, Chapel Hill, NC). Any remaining cells were pelleted, and resuspended in 1 ml of Trizol reagent with 10 µl of GGD. Supernatant aliquots and cell pellet were stored at -80°C until shipped to the central biospecimen repository at University of North Carolina, Chapel Hill, NC.

Slides were available for 1001 subjects; other subjects were either ineligible due to safety restricting induction for an FEV_1%predicted<35%, or did not produce sputum upon induction, or had aliquots of sputum removed from small samples which precluded sufficient sample for slide preparation. Differential cell counts were performed on 500-600 total cells by two readers. If more than 10% variation in counts was observed between the two readings, a third read was performed and the average of the two closest counts recorded. Differential counts which had <100 leukocytes total (N=179) or ≥80% squamous (N=11) were excluded as unacceptable samples; normal subjects without a smoking history (N=199) were also excluded. The remaining subjects in the sputum cohort (N=827) were used for analyses; thus, 77% were considered acceptable slides which was comparable to the 81% success rate reported for a randomized controlled trial in COPD.[S7] The mean sputum eosinophil percentage for the SPIROMICS sputum cohort was 1·25±4·25%. The mean sputum eosinophil % was used for subject stratification; a higher eosinophil cutpoint of 2% was also examined and results reported (supplement tables S3-S7). The median for sputum eosinophils was 0·3% (interquartile range, 0·00-0·97%) which was considered too low to use as a cutpoint for stratifying subjects into "low" and "high" sputum eosinophil groups. The mean sputum eosinophil % for the SPIROMICS cohort was comparable to other reports (1·2% at study entry;[S5] 0·7% and 1·1% for biomarker groups, blood eosinophils < or >2%;[S6] 1·3%;[S8] 1·0%[S9]). The

stratification by < or ≥2% sputum eosinophils did not greatly alter phenotypic characteristics with significant differences associated with high eosinophils compared to low eosinophils (compare Tables 1-5 in main manuscript with Supplement Tables S3-S7, respectively). Protocol exclusion for safety reasons limited GOLD Stage 4 subjects in the sputum subgroup due to avoiding sputum induction in subjects with post-bronchodilator FEV_1%predicted <35%. Thus, lung function was higher in the subgroup with sputum, but still showed greater spread between baseline and post-bronchodilator FEV_1%predicted, and FEV_1/FVC in the sputum eosinophil < and ≥1·25% groups, and in < or ≥2% groups.

Sputum was also induced in a repeat subset of the subjects in the repeatability substudy (N=36). However, acceptable sputum samples between the baseline and repeat group were limited to N=23. The linear regression model for sputum eosinophils in these two samples had a slope of 0·78 (p=0·02), which indicates reasonable reproducibility in this limited subset.

Quantitative Computed Tomography:

Measures for emphysema (TLC %voxels less than -950Hounsfield Units [HU] in both lungs, Left Lower Lobe, Left Upper Lobe, Right Lower Lobe and Right Upper Lobe: each %<-950HU), for hyperinflation or air-trapping (RV both lungs %voxels less than -856HU), for average airway wall thickness at the 50% point for RB1 (prespecified pathway in apical segment of right upper lobe) and RB10 (prespecified pathway in the posterior basal segment of the right lower lobe), and taper ratio for RB1 and RB10, as an indicator of bronchiectasis, in both the entire cohort and the sputum subgroup were

examined. Functional small airways disease as assessed by parametric response mapping (PRM fSAD) was examined.[S2,S3]

Statistical Analyses:

Subjects were stratified by blood or sputum mean counts or % for eosinophils (cutpoints of 200 or 300 cells/μL for blood Eos, and of 1·25% or 2% for sputum Eos, respectively). For ROC analysis of blood Eos to predict sputum Eos, higher cutpoints up to 500 eosinophils/μL were examined to determine maximum sensitivity and specificity. Demographic and biomarker data are presented as means $\pm$ standard deviations, or medians (25%-75% interquartile range) for continuous variables, and as % positive for categorical variables. Measures not meeting Kolmogorov-Smirnov test for normal distribution were transformed to log, or square root values. Continuous variables were tested by parametric (t-test for 2 groups, one-way or two-way ANOVA for greater than 2 groups with one or two independent variables, respectively); or non-parametric tests (Mann-Whitney Rank Sum Test for 2 groups, or Kruskal-Wallis One-way Analysis of Variance on ranks for more than 2 groups) (SAS 9·2, or Sigmastat 12·5). Analyses with a significant difference were further explored by post-hoc pairwise tests (Tukey or Dunn's). Categorical variables were analyzed using Chi-square tests or Fisher Exact tests. Correlations were examined by Pearson Correlation test or linear regression. Receiver Operating Curve (ROC) analysis was performed for blood eosinophil prediction of sputum eosinophils. The False Discovery Rate (= False Positives / False Positives+True Positives) was examined. Classification tree analysis examining sputum and blood eosinophils to model exacerbations was performed using Rpart routines in R software package. Variables with a p value $<0{\cdot}05$ were accepted as significant.[S10]

Additional Results:

Medications:

Medications, including theophylline, oral corticosteroid, inhaled bronchodilator, nebulized bronchodilator, and leukotriene receptor antagonist, did not differ among the groups stratified by blood eosinophils. For subjects stratified by sputum eosinophils, the group with eosinophils ≥1·25% reported significantly increased use of inhaled or nebulized bronchodilators in the last 3 months, but not of theophylline, oral corticosteroids, or leukotriene receptor antagonist (see Table S1). Inhaled corticosteroids were reported in Table 1 of the main manuscript and were increased for both high blood and high sputum eosinophil groups.

In addition, subjects with self-reported chronic bronchitis or emphysema diagnoses were small subgroups of the ever-smoker cohort. When stratified by blood eosinophils, there was no significant difference for chronic bronchitis: 20% of <200/μL eosinophil group, compared to 23% of ≥200/μL eosinophil group, p=0·31; or for emphysema, 35% of <200/μL eosinophil group compared to 36% of ≥200/μL eosinophil group, p=0·20. For the sputum cohort stratified by sputum eosinophils, there was a significant difference only for self-reported emphysema, 25% of <1·25% eosinophil group compared to 37% of ≥1·25% eosinophil group, p=0·002; but not for chronic bronchitis, 18% of <1·25% eosinophil group, compared to 24% of ≥1·25% eosinophil group, p=0·22. The increased report for emphysema in the high sputum eosinophil group raised the concern that this difference might be due to higher sputum neutrophils, however this was found not to be true (Table S2).

Demographic, Spirometry, Imaging, Clinical Characteristics and Exacerbations for Subjects Stratified by higher blood eosinophils (Eos < or ≥300/μL), or by higher sputum eosinophils (Eos < or ≥2%):

For both the entire cohort and the subgroup with induced sputum, similar results were obtained if these cohorts were stratified by higher eosinophil levels: < or ≥300/μL for blood, and < or ≥2% for sputum eosinophils. These results are presented in Tables S3 through S7; the few characteristics which became significant or lost significance at these higher eosinophil cutpoints were noted as the following:

Demographics (Table S3): The blood subgroup with eosinophils≥300/μL reported significantly fewer cigarettes/day, and lost significant difference for % current smokers. The sputum subgroup with eosinophils≥2% had a significant increase in the % subjects reporting childhood asthma.

Spirometry (Table S4): The sputum subgroup with eosinophils ≥2% had a significant decrease in the baseline FVC% predicted.

Imaging (Table S5): Blood eosinophils ≥300/μL had less significance for RB1 average wall thickness. The sputum subgroup with eosinophils ≥2% had less significance for emphysema indicated in the TLC Right Upper Lobe %<-950HU.

Clinical Characteristics (Table S6): The blood subgroup with eosinophils ≥300/μL showed a significant shift for proportions in GOLD Stages 0-4 distribution. The sputum subgroup with eosinophils ≥2% had significant increases in the BODE Index, and % positive for wheeze.

Exacerbations (Table S7): The sputum subgroup with eosinophils ≥2% gained significance for increased % reported exacerbations in those defined as Total, those requiring healthcare utilization, and those requiring treatment with antibiotics.

Blood Eosinophil Prediction of Sputum Eosinophils:

Receiver Operating Curve analyses demonstrated relatively weak, although significant relationship of blood eosinophils to predict sputum eosinophils either ≥ 1·25% or ≥2% (Figure 1 main manuscript shows ROC for ≥1·25% sputum eosinophils, AUC=0·63, p<0·0001; ROC for ≥2% sputum eosinophils, supplement Figure S3, had nearly identical AUC=0·64, p<0·0001). The highest sensitivity and specificity for predicting sputum eosinophils ≥1·25% and ≥2% were found at 150/μL and 250/μL blood eosinophils, respectively; but similar or identical, significant AUCs were observed for adjacent cutpoints (supplement Table S8). The prediction of sputum eosinophils, however, had a very large false discovery rate of 72% and a false negative rate of 22% for 150/μL blood eosinophil prediction of sputum eosinophils ≥1·25%, and a false discovery rate of 74%, and higher false negative rate of 50% for 250/μL blood eosinophil prediction of sputum eosinophils ≥2%.

Additional Stratifications of Blood and Sputum Eos Subgroups:

Clinical characteristics such as "prior asthma label" and ICS use which may be associated with higher blood (≥200/mL) or sputum eosinophils (≥1·25%) effects on lung function were examined by two-factor ANOVA (Tables S9, and S10, for blood or sputum, respectively). In these analyses, subjects positive for a prior asthma label or ICS use had significantly decreased lung function, while blood eosinophils had little

significant effect other than decreased FVC% predicted (pre- and post-bronchodilator) for those responding positive for ICS use. Blood eosinophils had no interaction with prior asthma label or ICS (supplement Table S9). By Chi-square analyses, both positive response for prior asthma label and for ICS use had approximately 2-fold higher exacerbation rates, but without any difference due to blood eosinophil level.

Similar analyses in the sputum subgroup found no effect of a "prior asthma label" on lung function except for reversibility, whereas sputum eosinophils $\geq$1·25% had significantly lower pre-bronchodilator FEV_1%predicted, and no interactions with prior asthma label. Sputum eosinophils $\geq$1·25% increased exacerbation frequencies by more than 3-fold compared to only 2-fold increased rate for <1·25% sputum eosinophils and a prior asthma label (supplement Table S10). ICS use showed significant effect on all lung function parameters; sputum eosinophils $\geq$1·25% showed significant effects on pre-bronchodilator FEV_1%predicted, pre- and post-bronchodilator FEV_1/FVC ratios, and reversibility, but no significant interactions with ICS use (Table S10). Although increased exacerbation rates were associated with ICS use, the fold increase was greater for sputum eosinophils <1·25% than for sputum eosinophils $\geq$1·25%.

Subjects who did not have acceptable sputum slides for various reasons (N=1498) were stratified by blood eosinophil counts to determine whether these subjects represented a phenotype with different characteristics (supplement Table S11). There was slightly higher proportion of subjects using ICS, lower lung function and increased proportions of GOLD Stage 3 and 4 subjects as would be expected in these groups which contained subjects ineligible for sputum induction, but otherwise resembled the larger cohort of smokers.

Another stratification examined whether blood eosinophil groups < or ≥200/μL showed differences when restricted to just those subjects in the sputum cohort (supplement Table S12). The sputum cohort divided into blood eosinophil subgroups had slightly greater proportion of current smokers, less ICS use and somewhat better lung function, but did not show the same radiologic, clinical or exacerbation associations observed for sputum eosinophil stratification.

To examine whether some of the associations in the high sputum eosinophil group were due to elevated sputum neutrophils in addition to the high eosinophils, we stratified the sputum cohort into 4 groups based on < or ≥1·25% eosinophils + < or > 68% neutrophils (mean + std deviation: 68% ±21%) (Table S13). Although lung function measures for the high eosinophil+ high neutrophil subgroup were slightly lower than the high eosinophil+low neutrophil subgroups, post-hoc analyses (Dunn's Method) did not find any pairwise significant difference between these groups. Similarly, slightly higher measures for emphysema (%voxels in left or right upper lobes < -950 Hounsfield Units) did not differ between the high eosinophil + high neutrophil and the high eosinophil + low neutrophil subgroups in posthoc pairwise analyses. These observations combined with the lack of significant difference in sputum neutrophil % between high and low eosinophil groups, indicates that significant associations observed for the high sputum eosinophil group compared to the low eosinophil group are not being driven by a more neutrophilic constitution.

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Table S1. Medication use for subjects stratified by blood Eos (< or ≥200/mL) or sputum Eos (< or ≥ 1.25%).

Variable	Blood Eos<200/μL	Blood Eos≥200/μL	P Value*	Sputum Eos<1.25%	Sputum Eos≥1.25%	P Value*
Number	**1262**	**1237**		**656**	**171**	
Theophylline, N (% positive)	25 (2)	25 (2)	0·94	5 (0·77)	1 (0·59)	1
Oral Corticosteroids, N (% positive)	37 (3)	24 (2)	0·16	7 (1)	2 (1)	1
Inhaled Bronchodilator last 3 mon, N (%positive)	628 (50)	648 (53)	0·25	275 (42)	99 (58)	**<0.001**
Nebulized Bronchodilator last 3 mon, N (%positive)	160 (13)	161 (13)	0·90	45 (7)	25 (15)	**0·002**
Leukotriene Receptor Antagonist last 3 mon, N (%positive)	60 (5)	50 (4)	0·42	20 (3)	9 (5)	0·17

* Chi-square, results as %positive response.

Table S2. Sputum differential counts for all cell types assessed, stratified by sputum eosinophils < or ≥ 1·25%. As expected sputum eosinophils differed and also lymphocytes, but not macrophages, or neutrophils between the high and low sputum eosinophil subgroups.

Variable – Sputum cell differential	**Sputum Eos<1.25% N=656**	**Sputum Eos≥1.25% N=171**	P value
Macrophage/Monocyte %	26 (14-44)	23 (13-39)	0.15
Lymphocyte %	0.00 (0.00-0.11)	0.00 (0.00-0.20)	**<0.001**
Eosinophil %	0.16 (0.00-0.47)	2.7 (1.8-4.5)	**<0.001**
Neutrophil %	73 (55-86)	71 (57-82)	0.12

* Mann-Whitney rank sum test for continuous variables, results as median (25-75% interquartile range).

Table S3. Demographics for entire cohort or sputum subgroup stratified either by blood or sputum eosinophils (Eos; < or ≥300/μL, or < or ≥2%, respectively).

Variable	**Blood Eos<300/μL**	**Blood Eos≥300/μL**	**P Value***	**Sputum Eos<2%**	**Sputum Eos≥2%**	**P Value***
Number	**1949**	**550**		**715**	**112**	
Age (yr)	65 (57 - 70)	66 (59 - 71)	**0·009**	65 (57 - 71)	65 (57 - 71)	0·98
Male Gender, N (%)	994 (51)	352 (64)	**<0·001**	414 (58)	62 (55)	0·69
RACE N(%)Cau/N(%)AA/N(%) other)	1494 (77)/ 380 (19)/ 75 (4)	444 (81)/ 78 (14)/ 28 (5)	**<0·001**	558 (78)/ 119 (17)/ 38 (5)	85 (76)/ 20 (18)/ 7 (6)	0·96
BMI	27·4 (23·9 - 31·3)	28·4 (24·5 - 32·4)	**<0.001**	28·2 (24·6 - 32·2)	28·5 (25·05 - 31·95)	0·94
Smoking packyears	42 (31 - 60)	45 (35 - 60)	**0·008**	43 (32 - 60)	44 (31 - 56)	0·51
Cigarettes/day	15 (10 - 20)	13 (6 - 20)	0·05	15 (10 - 20)	13 (4 - 20)	0·11
Current Smoker, N (%positive)	767 (39)	204 (37)	0·15	320 (45)	43 (39)	0·38
Inhaled corticosteroid, N (%positive)	656 (34)	222 (41)	**0·004**	188 (27)	48 (43)	**<0.001**
IgE (geometric)	35 (14-98)	77 (26-213)	**<0·001**	42·7 +4·4	55·6 +5	0·30
Sputum Eos %	0·21 (0-0·75)	0·63 (0·11-2·21)	**<0·001**			
Blood Eos count/μL				170 (100 - 210)	250 (180 - 400)	**<0·001**
"Prior asthma label", N (% positive)	382 (20)	113 (21)	0·46	136 (19)	37 (33)	**<0·001**
Childhood asthma, N (% positive)	159 (8.3)	53 (9.8)	0·34	58 (8)	14 (12·5)	**0·005**

* Mann-Whitney rank sum test for continuous variables, results as median (25-75% interquartile range); Chi-square for categorical variables, results as %positive response.

Table S4. Lung function parameters for stratification either by blood or sputum eosinophils (Eos; < or ≥300/μL, or < or ≥2%, respectively).

Variable	Blood Eos<300/μL (N=1949)	Blood Eos≥300/μL (N=550)	P Value	Sputum Eos<2% (N=715)	Sputum Eos≥2% (N=112)	P value
Pre-bronchodilator:						
FEV_1 (L)	1.84 (1.19-2.54)	1.82 (1.17-2.54)	0·52	2.15 (1.57-2.75)	1.69 (1.29-2.21)	**<0·001**
FEV_1% predicted	70 (45·2 - 87·6)	64 (40·4 - 85·3)	**0·015**	75·3 (59·2 - 89·9)	61 (48·4 - 77·4)	**<0·001**
FVC% predicted	86.3 (73-98)	84.1 (69-97)	**0·011**	90.6 (79-100)	85.5 (75-94)	**0·007**
FEV1/FVC	0·63 (0·49 - 0·73)	0·6 (0·48 - 0·71)	**0·008**	0·66 (0·57 - 0·73)	0·59 (0·47 - 0·68)	**<0·001**
Post-bronchodilator:						
FEV_1 (L)	2.05 (1.41-2.73)	1.99 (1.40-2.73)	0·74	2.34 (1.80-2.95)	1.99 (1.54-2.50)	**<0·001**
FEV_1% predicted	77.3 (53-93)	72.2 (50-90)	**0·015**	82.9 (68-96)	72.1 (61-86)	**<0·001**
FVC% predicted	92 (80·5 - 103·3)	90 (77·75 - 101·25)	**0·026**	94·4 (85·3 - 105·2)	94·5 (85·4 - 103)	0·94
FEV1/FVC	0·65 (0·5 - 0·76)	0·62 (0·49 - 0·73)	**0·005**	0·68 (0·59 - 0·76)	0·63 (0·53 - 0·7)	**<0·001**
%FEV_1 reversible	9·5 (4·4 - 18)	9·9 (4·6 - 19·4)	0·47	8·2 (3·7 - 15·5)	14·8 (7·2 - 23·4)	**<0·001**

* Mann-Whitney rank sum test for continuous variables, results as median (25-75% interquartile range).

Table S5. Imaging parameters for stratification either by blood or sputum eosinophils (Eos; < or ≥300/μL, or < or ≥2%, respectively).

Variable	Blood Eos<300/μL (N=1949)	Blood Eos≥300/μL (N=550)	P value*	Sputum Eos<2% (N=715)	Sputum Eos≥2% (N=112)	P value*
DENSITY MEASURES						
TLC Left Upper Lobe %<-950 HU	3·44 (1·15 -12·15)	3·52 (1·16 -10·64)	0·66	2·23 (0·92 – 5·79)	3·21 (1·19 – 7·73)	**0·031**
TLC Right Upper Lobe %<-950HU	2·86 (0·71 -13·13)	2·68 (0·76 -10·66)	0·83	1·8 (0·61 – 5·81)	2·3 (0·97 – 7·15)	**0·044**
TLC Left Lower Lobe %<-950 HU	2·29 (0·83-7·50)	2·26 (0·85-6·86)	0·92	1·60 (0·71 – 3·87)	2·10 (0·90 – 5·30)	**0·030**
RV Both Lungs %<-856	18·0 (6·72-40)	19·0 (8·09-39·6)	0·41	12·5 (5·3-25·3)	17·8 (10·2-34·5)	**<0·001**
PRM fsad	15 (4-33)	15 (5-33)	0.62	9 (3-22)	14 (7-31)	**<0·001**
AIRWAY MEASURES						
RB1_01 AVG WALLTHICK_50	1·27 (1·15-1·38)	1·28 (1·16-1·40)	**0·044**	1·28 (1·18-1·38)	1·28 (1·17-1·43)	0·28
RB1_01_Taper Ratio	0·038 (-0·01-0·086)	0·041 (-0·01-0·09)	0·35	0·031 (-0·02-0·08)	0·033 (-0·01-0·09)	0·41

* Mann-Whitney rank sum test for continuous variables, results as median (25-75% interquartile range).

Table S6. Additional clinical characteristics for stratification either by blood or sputum eosinophils (Eos; < or ≥300/μL, or < or ≥2%, respectively).

Variable	Blood Eos<300/μL (N=1949)	Blood Eos≥300/μL (N=550)	P Value*	Sputum Eos<2% (N=715)	Sputum Eos≥2% (N=112)	P Value*
GOLD Stage 0 N(%)/1 N(%)/2 N(%)/3 N(%)/4 N(%)	762 (39)/ 232 (12)/ 510 (26)/ 294 (15)/ 128 (7)	168 (31)/ 71 (13)/ 172 (31)/ 96 (17)/ 37 (7)	**0·013**	317 (44)/ 120 (17)/ 222 (31)/ 48 (7)/ 0 (0)	29 (26)/ 17 (15)/ 54 (48)/ 10 (9)/ 1 (1)	**<0·001**
6 Minute Walk Distance	414·76 (347 - 480)	411·74 (344 - 462)	0·42	426·72 (372 - 484)	411·48 (354 - 470)	0·24
BODE Index	1 (0 - 2)	1 (0 - 2)	0·50	0 (0 - 1)	1 (0 - 2)	0·05
COPD Assessment Score	13 (7-20)	13.5 (8-19)	0·21	12 (7-19)	13·5 (8-20)	0·14
St. George Respiratory Quest. (Total)	31·14 (15 - 47·4)	31·93 (17·96 - 48·02)	0·10	26·36 (14·32 - 43·65)	32·58 (17·23 - 49·81)	**0·038**
SGRQ (Symptoms)	45·85 (23·785 - 65·82)	50·83 (30·37 - 67·66)	**0·002**	45·86 (24·23 - 65·11)	54·76 (34·83 - 71·16)	**0·005**
Symptoms:						
Wheezing, N (% positive)	1167 (60)	362 (66)	**0·012**	425 (60)	81 (72)	**0·012**

* Mann-Whitney rank sum test for continuous variables, results as median (25-75% interquartile range); Chi-square for categorical variables, results as %positive response.

Table S7. Comparison of exacerbations ≥1 (in the previous year) for subjects stratified by blood or sputum eosinophils (Eos; either 300/mL, or 2% cutoff values, respectively). All values are percentage positive.

Variable	BLOOD EOS<300	BLOOD EOS≥300	P value	SPUTUM EOS<2%	SPUTUM EOS≥2%	P value
Number	**1949**	**550**		**715**	**112**	
Definition of exacerbation:						
Total, N (%)	470 (24)	150 (27)	0·20	138 (19)	33 (29)	**0·019**
Healthcare Utilization, N (%)	444 (23)	141 (26)	0·23	133 (19)	32 (29)	**0·020**
Antibiotic treatment, N (%)	354 (18)	118 (21)	0·14	102 (14)	25 (22)	**0·040**
Corticosteroid treatment, N (%)	303 (16)	105 (19)	0·09	71 (10)	26 (23)	**<0·001**
Any drug treatment, N (%)	406 (21)	132 (24)	0·17	112 (16)	29 (26)	**0·011**
Severe, N (%)	231 (12)	68 (12)	0·60	55 (8)	17 (15)	**0·015**

* Chi-square, results as N (%positive response).

Table S8. Sensitivity, Specificity, Youden Index, and AUC for adjacent blood eosinophil cutpoints for predicting either ≥1.25% or ≥2% sputum eosinophils. The maximum Youden Index, greatest sensitivity and specificity for blood eosinophils, was observed at 150/μL to predict ≥1.25% sputum eosinophils, and at 250/μL to predict ≥2% sputum eosinophils (bold highlight for each).

Blood Eos Cutpoint	**To predict sputum eosinophils ≥1.25%**					**To predict sputum eosinophils ≥2%**				
	Sensitivity	Specificity	Youden Index	AUC		Sensitivity	Specificity	Youden Index	AUC	
150	0·78	0·49	**0·27**	0·63		0·47	0·80	0·27	0·63	
200	0·70	0·55	0·25	0·63		0·54	0·73	0·27	0·64	
250	0·47	0·79	0·26	0·63		0·78	0·51	**0·28**	0·64	

Table S9. Lung function and exacerbations within the subgroup with blood eosinophils <200/µL(Eos; left-hand columns), or blood Eos≥200/µL (right-hand columns) respectively, and additional subgroup stratification for subjects with "prior asthma label" or ICS use (no or yes).

	Eos<200/µL			Eos≥200/µL			P Value Eos	P Value asthma
Baseline_Asthma_label:	**NO**	**YES**		**NO**	**YES**			
Number	**941**	**249**		**907**	**255**			
Baseline FEV1%pred	69·6	60·9		66·5	59·2		0·23	**<0·001**
Baseline FVC%predicted	87·3	82·9		84·4	81		0·22	**<0·001**
Baseline FEV1/FVC	0·62	0·58		0·60	0·57		0·25	**<0·001**
postBD FEV1%predicted	75·9	68·7		72·7	68·2		0·42	**<0·001**
postBD FVC%predicted	92·7	90·1		90·4	89·7		0·34	0·13
postBD FEV1/FVC	0·63	0·60		0·62	0·59		0·28	**<0·001**
% Reversible	11·9	15·3		12·3	18·1		0·07	**<0·001**
Exacerbation Definition:			Fold change Yes/No			Fold change Yes/No		
% total	19·4	41	**2·1**	20·1	41·6	**2·1**		**<0·001**
% with HCU	18·7	37·8	**2·0**	19	38·8	**2·0**		**<0·001**
% with antibiotics	14·7	29·7	**2·0**	15·8	31·8	**2·0**		**<0·001**
% with steroids	11·6	29·3	**2·5**	12·5	31·8	**2·5**		**<0·001**
% with drugs	16·4	35·7	**2·2**	17·6	36·9	**2·1**		**<0·001**
% severe	9·6	23·7	**2·5**	8·8	18·8	**2·1**		**<0·001**
Inhaled Corticosteroids:	**No**	**Yes**		**No**	**Yes**		**P Value Eos**	**P Value ICS**
number	845	404		753	470			
Baseline FEV1%pred	76	49·8		75·1	47·7		0·13	**<0·001**
Baseline FVC%predicted	90·4	77·9		88·7	75		**0·004**	**<0·001**
Baseline FEV1/FVC	0·66	0·50		0·65	0·50		0·41	**<0·001**
postBD FEV1%predicted	82·4	56·6		81·7	55		0·24	**<0·001**
postBD FVC%predicted	95·2	85·7		93·9	83·4		**0·017**	**<0·001**
postBD FEV1/FVC	0·68	0·52		0·67	0·51		0·27	**<0·001**
% Reversible	10·9	16·1		11	17·9		0·09	**<0·001**
Exacerbation Definition:			Fold change Yes/No			Fold change Yes/No		
% total	15·3	44·8	**2·9**	13·4	43	**3·2**		**<0·001**
% with HCU	14·8	41·6	**2·8**	12·7	40·2	**3·2**		**<0·001**
% with antibiotics	9·8	36·9	**3·8**	10·2	33·6	**3·3**		**<0·001**
% with steroids	7·2	34·2	**4·8**	7·6	31·7	**4·2**		**<0·001**
% with drugs	11·6	41·3	**3·6**	11·3	38·7	**3·4**		**<0·001**
% severe	7·0	25·2	**3·6**	6	18·7	**3·1**		**<0·001**

Table S10. Lung function and exacerbations within the subgroup with sputum eosinophils <1.25% (Eos; left-hand columns), or sputum Eos≥1.25% (right-hand columns) respectively, and additional subgroup stratification for subjects with "prior asthma label" or ICS use (no or yes).

	Eos<1.25%			Eos≥1.25%			P Value Eos	P Value asthma
Baseline_Asthma_label:	**NO**	**YES**		**NO**	**YES**			
Number	**503**	**123**		**108**	**50**			
Baseline FEV1%pred	75·8	71·2		68·8	65·8		**0·017**	0·18
Baseline FVC%predicted	90·6	87·5		89·5	85·9		0·06	0·16
Baseline FEV1/FVC	0·65	0·64		0·60	0·60		0·15	0·73
postBD FEV1%predicted	82·1	80·2		76·9	77·2		0·06	0·53
postBD FVC%predicted	95·2	95·2		96·5	95·0		0·13	0·27
postBD FEV1/FVC	0·67	0·67		0·62	0·64		0·19	0·45
% Reversible	9·9	15		13·5	19·5		0·07	**<0·001**
Exacerbation Definition:			Fold change Yes/No			Fold change Yes/No		
% total	15·5	33·3	**2·1**	13	46	**3·5**		**<0·001**
% with HCU	15·1	31·7	**2·1**	12	46	**3·8**		**<0·001**
% with antibiotics	12·1	21·1	**1·7**	10·2	32	**3·1**		**<0·001**
% with steroids	7	20·3	**2·9**	9·3	36	**3·9**		**<0·001**
% with drugs	12·7	26	**2·0**	11·1	40	**3·6**		**<0·001**
% severe	5·4	16·3	**3·0**	6·5	24	**3·7**		**<0·001**
Inhaled Corticosteroids:	**No**	**Yes**		**No**	**Yes**		**P Value Eos**	**P Value ICS**
number	480	171		104	65			
Baseline FEV1%pred	79·1	62·4		71·9	59·9		**0·009**	**<0·001**
Baseline FVC%predicted	91·6	85·4		90·5	83·9		0·39	**<0·001**
Baseline FEV1/FVC	0·67	0·58		0·62	0·57		**0·009**	**<0·001**
postBD FEV1%predicted	85·4	70·9		80·3	70·1		0·08	**<0·001**
postBD FVC%predicted	96·1	92·9		97·3	92·7		0·78	**0·006**
postBD FEV1/FVC	0·69	0·60		0·65	0·60		**0·021**	**<0·001**
% Reversible	9·6	15·2		13·2	19·6		**<0·001**	**<0·001**
Exacerbation Definition:			Fold change Yes/No			Fold change Yes/No		
% total	12·3	37·4	**3·0**	17·3	41·5	**2·4**		**<0·001**
% with HCU	11·9	36·3	**3·1**	17·3	38·5	**2·2**		**<0·001**
% with antibiotics	8·1	30·4	**3·8**	12·5	32·3	**2·6**		**<0·001**
% with steroids	4·2	25·7	**6·1**	11·5	30·8	**2·7**		**<0·001**
% with drugs	9	33·3	**3·7**	14·4	36·9	**2·6**		**<0·001**
% severe	3·3	18·7	**5·7**	9·6	18·5	**1·9**		**<0·001**

Table S11: Demographic, lung function, clinical, radiologic and exacerbation characteristics for subjects in ever-smoker cohort without available sputum data, stratified by blood eosinophils < or ≥ 200/mL

Variable	Eos<200/μL	Eos≥200/μL	P Value
Number	761	736	
Age (yr)	64 (56 - 70)	65 (58 - 70)	**0·034**
Male Gender, N (%)	358 (47)	397 (54)	**0·003**
Smoking packyears	43 (31 - 60)	45 (33·8 - 60)	0·13
Cigarettes/day	15 (7 - 20)	15 (10 - 20)	0·59
Current Smoker, N (%positive)	300 (40)	247 (34)	**0·046**
Inhaled corticosteroid, N (%positive)	278 (37)	327 (45)	**0·003**
"Prior asthma label", N (% positive)	148 (20)	152 (21)	0·31
Pre BD FEV_1% predicted	62·7 (37 - 86)	58·3 (33 - 84)	**0·034**
Pre BD FVC% predicted	83·5 + 20·3	80·3 + 21·2	**0·004**
Pre BD FEV1/FVC	0·6 (0·4 - 0·7)	0·6 (0·4 - 0·7)	0·11
Post BD FEV_1% predicted	69·0 + 28·5	65·9 + 28·4	**0·035**
Post BD FVC% predicted	90·7 (78·5 - 102·8)	88·1 (73·5 - 99·3)	**0·006**
Post BD FEV1/FVC	0·6 (0·4 - 0·8)	0·6 (0·4 - 0·7)	**0·035**
TLC Left Upper Lobe %<-950 HU	3·1 (0·4 - 16·2)	3·7 (0·7 - 14·2)	0·34
TLC Right Upper Lobe %<-950HU	2·7 (0·3 - 16·4)	2·8 (0·5 - 16·7)	0·31
RV Both Lungs%<-856HU	22·8 (8-50)	24·6 (8-50)	0·80
GOLD Stage N (%) 0/1/2/3/4	278 (37)/ 70 (9)/ 181 (24)/ 155 (20)/ 77 (10)	232 (31)/ 70 (10)/ 185 (25)/ 161 (22)/ 88 (12)	0·33
6 Minute Walk Distance	409·5 (332·2 - 476·7)	401·6 (320 - 462)	0·11
BODE Index	1 (0 - 3)	1 (0 - 3)	0·28
COPD Assessment Score	14 (7 - 20)	14 (8 - 20)	0·30
Exacerbation, Corticosteroid treatment, N (%)	149 (20)	152 (21)	0·31
Exacerbation, Severe, N (%)	118 (16)	100 (14)	0·21

Table S12: Demographic, lung function, clinical, radiologic and exacerbation characteristics for subjects in sputum cohort, stratified by blood eosinophils < or ≥ 200/mL

Variable	Eos<200/μL	Eos≥200/μL	P Value
Number	405	406	
Age (yr)	65 (55 - 71)	66 (59 - 71)	**0·018**
Male Gender, N (%)	219 (54)	248 (61)	**0·039**
Smoking packyears	40 (30 - 57)	45 (35 - 60)	**0·016**
Cigarettes/day	15 (10 - 20)	15 (7 - 20)	0·52
Current Smoker, N (%positive)	190 (47)	171 (42)	0·14
Inhaled corticosteroid, N (%positive)	106 (26)	125 (31)	0·17
"Prior asthma label", N (% positive)	79 (19·5)	90 (22)	0·79
Pre BD FEV_1% predicted	75·7 (59·1 - 90·3)	71·6 (54·4 - 87·7)	**0·040**
Pre BD FVC% predicted	90·89 + 16·34	88·33 + 17·19	**0·012**
Pre BD FEV1/FVC	0·66 (0·57 - 0·74)	0·64 (0·55 - 0·72)	0·07
Post BD FEV_1% predicted	81·75 + 19·72	79·35 + 20·14	0·07
Post BD FVC% predicted	96 (85·9 - 105·8)	93 (84·7 - 104·1)	**0·050**
Post BD FEV1/FVC	0·68 (0·59 - 0·76)	0·66 (0·59 - 0·74)	**0·050**
TLC Left Upper Lobe %<-950 HU	1·64 (0·4 - 5·07)	1·75 (0·31 - 5·27)	0·78
TLC Right Upper Lobe %<-950HU	1·2 (0·27 - 5·04)	1·2 (0·23 - 4·91)	0·87
RV Both Lungs %<-856	12·5 (5-25)	14·2 (7-27)	0·08
GOLD Stage N (%) 0/1/2/3/4	185 (46)/ 66 (16)/ 126 (31)/ 27 (7)/ 1 (0.2)	155 (38)/ 71 (17)/ 150 (37)/ 30 (7)/ 0 (0)	0·19
6 Minute Walk Distance	426·72 (369·57 - 487·68)	425·75 (368 - 479)	0·83
BODE Index	0 (0 - 1)	0 (0 - 1)	0·94
COPD Assessment Score	12 (7-20)	13 (7-19)	0·71
Exacerbation, Corticosteroid treatment, N (%)	43 (10·6)	49 (12·1)	0·59
Exacerbation, Severe, N (%)	38 (9·4)	32 (7·9)	0·53

Table S13. Demographic, lung function, clinical, radiologic and exacerbation characteristics for sputum cohort, stratified into 4 groups by eosinophils < or ≥1·25% and neutrophils < or ≥ 68%.

Variable	Sputum Eos<1.25% + Neu <68%	Sputum Eos<1.25% + Neu ≥68%	Sputum Eos≥1.25% + Neu <68%	Sputum Eos≥1.25% + Neu ≥68%	P Value*
Number	**269**	**387**	**74**	**97**	
Age (yr)	64 (56-70)	66 (58-71)	63 (57-71)	65 (59-72)	**0·013**
Male Gender, N (%)	154 (57)	223 (58)	45 (61)	54 (56)	0·93
Smoking packyears	41 (30-57)	45 (33-60)	40 (30-61)	44 (35-55)	0·23
Cigarettes/day	15 (10-20)	15 (10-20)	15 (5.5-20)	15 (7.5-20)	0·75
Current Smoker, N (%positive)	124 (46)	169 (44)	33 (45)	37 (38)	0·78
Inhaled corticosteroid, N (%positive)	52 (20)	119 (31)	25 (34)	40 (42)	**<0·001**
"Prior asthma label", N (% positive)	49 (18·2)	74 (19·1)	22 (29·7)	28 (28·9)	**0.013**
Pre BD FEV_1% predicted	84 (69-95)	71 (53-86)	66 (54-88)	64 (50-79)	**<0·001**
Pre BD FVC% predicted	94 (82-104)	88 (77-99)	89 (77-99)	86 (76-96)	**<0·001**
Pre BD FEV1/FVC	0·70 (0·63-0·76)	0·64 (0·54-0·72)	0·61 (0·54-0·70)	0·60 (0·50-0·69)	**<0·001**
Post BD FEV_1% predicted	89 (75-100)	79 (63-91)	80 (67-93)	77 (59-87)	**<0·001**
Post BD FVC% predicted	97 (86-106)	93 (83-104)	95 (89-105)	93 (84-104)	**0·033**
Post BD FEV1/FVC	0·72 (0·64-0·78)	0·65 (0·56-0·75)	0·65 (0·59-0·73)	0·63 (0·52-0·70)	**<0·001**
TLC Left Upper Lobe %<-950 HU	1·61 (0·7-4·7)	3·1 (1·3-7·6)	2·1 (1·1-5·2)	3·1 (1·2-9·1)	**<0·001**
TLC Right Upper Lobe %<-950HU	1·3 (0·4-3·9)	2·7 (0·8-8·9)	1·6 (0·7-4·9)	3·1 (1·0-9·7)	**<0·001**
RV Both Lungs %<-856	9·1 (3.5-18.1)	15 (7.5-30.4)	15 (8.4-24.5)	18 (9.5-35.4)	**<0.001**
GOLD Stage N (%) 0/1/2/3/4	146 (54)/ 52 (19)/ 65 (24)/ 6 (2.2)/ 0 (0)	152 (39)/ 56 (14)/ 138 (36)/ 41 (11)/ 0 (0)	24 (32)/ 15 (20)/ 31 (42)/ 4 (5)/ 0 (0)	27 (28)/ 17 (18)/ 45 (46)/ 7 (7)/ 1 (1)	**<0·001**
6 Minute Walk Distance	436 (384-491)	417 (364-478)	438 (366-495)	405 (360-454)	**0·003**
BODE Index	0 (0-1)	1 (0-2)	1 (0-1)	1 (0-2)	**<0·001**
COPD Assessment Score	10 (6-17)	13 (8-20)	12 (7-19)	14 (9-21)	**<0.001**
Exacerbation, Corticosteroid treated, N (%)	19 (7)	46 (12)	13 (18)	19 (20)	**0·003**
Exacerbation, Severe, N (%)	16 (6)	34 (9)	10 (14)	12 (12)	0·10

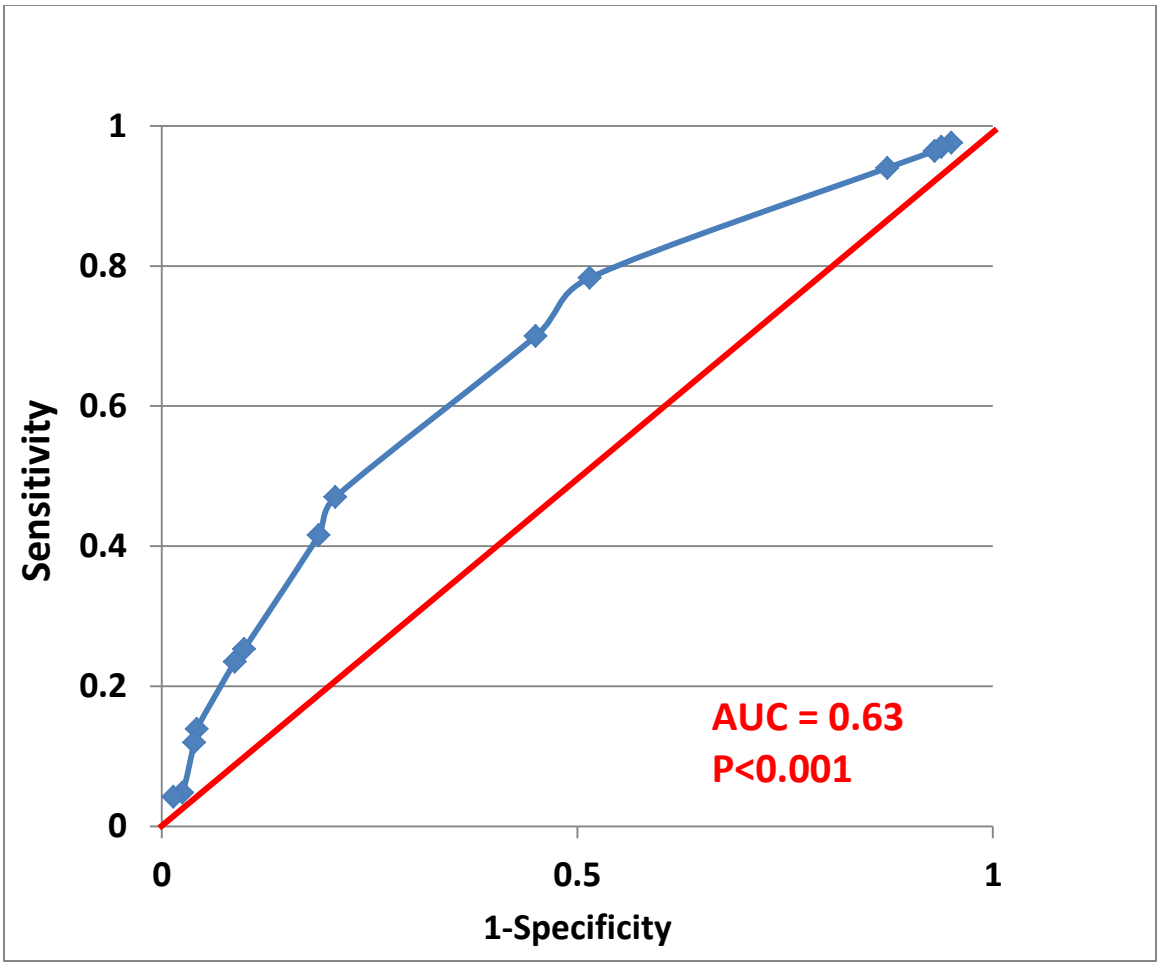
Figure Legend:

Supplement Figure S1. Consort Diagram for subjects recruited and reason for removal from analysis. All normal, neversmokers were removed. Although 90% of subjects (including normal neversmokers) may have been eligible for sputum induction, there were several reasons that reduced the actual number of sputum slide samples available for analysis: no sputum produced upon completion of induction, no sputum processing form entered, removal of aliquots for mucus analysis and microbiome prior to processing leaving too little remaining sample for cytospin slide preparation, slides not sent to central slide reading center, and finally slide counts that were deemed unacceptable (leukocyte cell count<100 or too high, >80% squamous epithelial cells). There were 16 subjects with acceptable sputum differential count but without blood counts who were added to those with both acceptable sputum counts and blood counts (N=811).

Supplement Figure S2. Classification tree diagram for model of exacerbations by sputum and blood eosinophils. The Root has 811 subjects with 92 exacerbations in previous year. The first number in each node is the number of subjects without exacerbations; the second number is the number with exacerbations. The model first divides the subjects based on sputum eosinophils < and $\geq$1.9% and secondly divides the subjects by both sputum and blood eosinophils (< or >176/μL).

Supplement Figure S3. ROC analysis for blood Eos prediction of sputum Eos. Blood Eos at cutpoints from 50/μL (highest sensitivity) to 500/μL (lowest sensitivity) were examined for correct prediction of sputum Eos < or $\geq$2%. Although significant ($p<0{\cdot}001$), the area under the curve (AUC) was only 0·64, demonstrating a lack of strength for the prediction. Maximum

sensitivity and specificity were observed at a blood Eos cutpoint of 250/μL, but with very large false discovery rate (74%) and false negative rate (50%).


1
0.8
0.6
0.4
0.2
0
Sensitivity
0
0.5
1
1-Specificity
AUC = 0.63
P<0.001