Systemic Markers of Adaptive and Innate Immunity Are Associated with COPD Severity and Spirometric Disease Progression

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ABSTRACT

The progression of chronic obstructive pulmonary disease (COPD) is associated with marked alterations in circulating immune cell populations, but no studies have characterized alterations in these cell types across the full spectrum of lung function impairment in current and former smokers. In 6,299 subjects from the COPDGene and ECLIPSE studies, we related Coulter blood counts and proportions to cross-sectional FEV_1 adjusting for current smoking status. We also related cell count measures to three-year change in FEV_1 in ECLIPSE subjects. In a subset of subjects with blood gene expression data, we used cell type deconvolution methods to infer the proportions of immune cell subpopulations, and we related these to COPD clinical status. We observed that FEV_1 levels are positively correlated with lymphocytes and negatively correlated with myeloid populations such as neutrophils and monocytes. In multivariate models, absolute cell counts and proportions were associated with cross-sectional FEV_1, and lymphocytes, monocytes, and eosinophil counts were predictive of three year change in lung function. Using cell type deconvolution to study immune cell subpopulations, we observed that subjects with COPD had a lower proportion of CD4+ resting memory cells and naive B cells compared to non-COPD smokers. Alterations in circulating immune cells in COPD support a mixed pattern of lymphocyte suppression and an enhanced myeloid cell immune response. Cell counts and proportions contribute independent information to models predicting lung function suggesting a critical role for immune response in long-term COPD outcomes. Cell type deconvolution is a promising method for immunophenotyping in large cohorts.

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Background

Chronic obstructive pulmonary disease (COPD) is associated with profound alterations in immune cells within the lung and in the systemic circulation. The systemic inflammation may reflect "spill-over" of inflammatory processes within the lung, primary alterations in the extra-pulmonary immune response, or a combination of both processes(1). These alterations affect cell types involved in both the innate(2-6) and adaptive(7-15) immune response. In population studies(16, 17) and a systematic review(18), total counts of peripheral leucocytes were associated with cross-sectional and prospective changes in lung function, but few studies have been performed in large cohorts with detailed cell count data to observe relationships across the full spectrum of lung function. In addition, current smoking has an independent effect on immune cells(8, 19) and often serves as an important potential confounder of immunologic studies of current and former smokers with COPD.

Cell type quantification by flow cytometry is rarely available from large, population-based studies of COPD. However, novel, cell-type "deconvolution" approaches have been shown to infer accurately the relative proportions of immune cell types from genome-wide blood gene expression data(20, 21). Thus, cell-type deconvolution is a potentially powerful approach to enable the simultaneous study of many different cell types in large cohorts of subjects with available blood gene expression, but it has not yet been applied to cohorts of subjects with COPD.

We hypothesized that 1) peripheral immune cell types quantified through Coulter complete blood counts (CBC) have significant associations to cross-sectional FEV_1 and prospective FEV_1 decline; and that 2) cell-type deconvolution methods can enable the simultaneous study of multiple immune cell subpopulations in cohorts of smokers with COPD and blood gene expression data. We explored the first hypothesis in two large cohorts of smokers enriched for COPD, the COPDGene and ECLIPSE studies, which enabled the characterization of immune cell profiles across the full spectrum of lung function impairment while accounting for current smoking effects. The large number of study subjects allowed for detailed modeling of the relationship between

25 multiple cell types, current smoking status, and lung function. To explore the second hypothesis, we used two
26 cell type deconvolution methods to infer immune cell subpopulation proportions in a subset of smokers with
27 blood gene expression data in the ECLIPSE Study, and we validated these inferred cell type proportions against
28 measured CBC data. We then compared levels of inferred circulating immune cell subpopulations by COPD
29 status, confirming that inferred estimates of circulating immune cell types such as monocytes, naive B cells, and
30 resting T memory cells are altered in the COPD state.
31
32 **Methods**

Study Populations

33 Recruitment criteria and study protocols for the ECLIPSE and COPDGene studies have been previously
34 reported. COPDGene enrolled 10,192 subjects across the entire GOLD spectrum between the ages of 45 and 80
35 with at least a 10 pack-year smoking history(22). These subjects completed their Phase 1 study visit between
36 2007-2011. As of September 24, 2016, 5000 subjects had completed their Phase 2 five-year follow-up visit
37 which included all of the data items collected in Phase 1 as well as complete blood count data, which was not
38 collected at the Phase 1 visit.
39 The Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study was a
40 multicenter study that enrolled subjects aged 40-75 with COPD and at least a 10-year smoking history (COPD
41 defined by FEV_1< 80% of predicted and FEV_1/FVC <= 0.7) or who were smokers without COPD (FEV_1>85% and
42 FEV_1/FVC > 0.7). Details of this study have been previously published(23). Gene expression analyses were
43 performed in a subset of subjects in this study from whom genome-wide gene expression data were generated
44 on the Affymetrix Human U133 Plus2 chip as previously reported(24). For both COPDGene and ECLIPSE, the
45 institutional review boards of all participating centers approved these studies, and written informed consent
46 was obtained from all subjects.
47

48 *Phenotype and Covariate Definitions*

49 In COPDGene and ECLIPSE, spirometry was performed before and after administration of 180 mcg of
50 albuterol according to international guidelines(25). COPD cases and GOLD stages were defined according to
51 GOLD spirometric criteria (FEV1 % of predicted < 80% and FEV1/FVC < 0.7) (26). Subjects with preserved ratio
52 impaired spirometry (PrISM) were defined by post-bronchodilator FEV_1 % of predicted <80% and FEV_1/FVC >
53 0.7(27). COPD blood gene expression subtypes were previously defined by Chang et al. using network-based
54 stratification (NBS)(24). Of the four NBS subtypes identified in the original publication, the two most prevalent
55 subtypes were analyzed. These subtypes are referred to as the less impaired lung function (LI-NBS) and the
56 more impaired lung function (MI-NBS) subtypes. Current smoking status, inhaled corticosteroid use, and oral
57 corticosteroid use were ascertained by questionnaire. In ECLIPSE, only 8 subjects reported using oral steroids at
58 baseline, and these were removed from subsequent analyses.

59

60 *Association of CBC Cell Types with COPD GOLD Stage, Cross-Sectional FEV_1, and Prospective Change in FEV_1*

61 Using 4,558 subjects with complete CBC and spirometric data from the COPDGene Phase 2 visit, we
62 plotted the distribution of neutrophil and lymphocyte counts and proportions against GOLD Stage after
63 removing outlying cell count observations greater than +/- 4 SD from the mean. We tested for univariate
64 associations between cell counts and proportions with post-bronchodilator FEV_1 % of predicted using Wald
65 tests from linear regression models, and we constructed multivariate regression models relating cell counts and
66 proportions to FEV_1 adjusting for current smoking status and oral and inhaled steroid use reported at baseline.

67 In 1,741 smokers from the ECLIPSE Study with complete covariate, CBC, baseline and prospective FEV_1
68 measurements, we related absolute cell counts and proportions to post-bronchodilator FEV_1 % of predicted
69 levels as described above. For the analysis of three-year change in FEV_1 % of predicted levels, we calculated the
70 difference between the first and last available post-bronchodilator FEV_1 % of predicted measurement in all
71 study subjects (calculated as last measurement - first measurement, i.e. negative values represent decline in

72 lung function). To determine the association between CBC measurements and change in FEV_1, we used
73 multivariate linear regression models with change in FEV_1 as the response variable adjusting for baseline FEV_1,
74 days of follow up, and smoking status at the first and last study visit. Smoking status was represented in four
75 groups, i.e. current smokers at first and last visit, former smokers at first and last visit, current smoker at first
76 visit and former smoker at last visit, and former smoker at first visit and current smoker at last visit. Subjects
77 with less than 1000 days between their first and last spirometric measurements were excluded from analysis.
78
79 *Gene Expression*
80 Sample preparation and quality control procedures for genome-wide gene expression data in ECLIPSE
81 have been previously described(28). Standard quality control and quantile normalization were performed. Gene
82 expression data are accessible via GEO [ECLIPSE GSE76705].
83
84 *Cell Type Deconvolution and Association of Inferred Cell Types with COPD Status*
85 Cell type deconvolution was performed in 221 ECLIPSE subjects with complete genome-wide gene
86 expression and covariate data. Cell type proportions were inferred using two methods - CIBERSORT(21) and the
87 method of Abbas et al. using linear least squares regression (LR)(20). Cell type reference expression profiles
88 were used from the LM22 pure-cell dataset obtained on 12/21/2015 from the CIBERSORT website
89 (https://cibersort.stanford.edu). Detailed description is provided in the Online Supplement.
90 After obtaining cell type estimates of the 22 cell types from both methods, we organized groups of
91 similar cell types into broader categories to create estimates of an additional 9 aggregated groups: CD4+ cells, T
92 cells, B cells, lymphocytes, and monocytes/macrophages. We performed this aggregation by summing
93 individual cell type values for cell types within each category. The 22 inferred cell type proportions and
94 aggregated cell type estimates were tested for association with COPD status and COPD molecular subtypes

using the Wilcoxon-Mann-Whitney test. Significant cell type associations were considered to be those with a Wilcoxon-Mann-Whitney test p-val < 0.05 for both CIBERSORT and LR.

Prediction Models for COPD Status and COPD Molecular Subtypes

Classification of subjects according to COPD status or NBS molecular subtype using estimated cell-type quantities, CBC quantities, and clinical covariates was performed in 221 subjects from ECLIPSE using the support vector machine implementation in the e1071 package(29). Validation within ECLIPSE involved performing one round of partitioning in which half of the subjects were used in the training set and the other half were used in the validation set. Probabilities were returned from the SVM and used with the R package ROCR to generate ROC plots and calculate AUCs(30).

Additional details regarding study cohorts and statistical methods are included in the Supplemental Materials.

Results

Relating Circulating Immune Cells to Cross-sectional FEV_1 and Current Smoking

We examined complete blood count (CBC) data from 4,558 smokers from the COPDGene phase 2 visit and an additional 1,741 smokers with >1000 days of spirometric follow-up data in the ECLIPSE Study. The clinical characteristics and cell type distributions of analyzed subjects in both studies are shown in Table 1. The CONSORT diagram for the analyses of cross-sectional and longitudinal data in ECLIPSE is shown in Supplemental Figure 1, and comparison of characteristics of ECLIPSE analyzed and excluded subjects is shown in Table E1.

Linear regression relating the absolute amount and percentage of five cell types to FEV_1 % of predicted indicated that 1) neutrophils, lymphocytes, monocytes, and eosinophils are strongly correlated with FEV_1, 2) the absolute counts of eosinophils and monocytes are more strongly correlated with FEV_1 than the relative percentages of these cell types, and 3) there are differences in the patterns of association between absolute

19 cell counts and cell count proportions with COPD severity (Tables 2 and E2). Boxplots showing the amount of
20 each cell type by GOLD stage for COPDGene and ECLIPSE are shown in Supplemental Figures 2 and 3.
21 Given the predominance of neutrophils and lymphocytes in blood, we examined the absolute counts
22 and percentages of these cell types across GOLD stages, and we observed two phenomena. First, with
23 increasing COPD severity, the proportion of neutrophils increases and lymphocytes decreases. However, in
24 terms of absolute cell counts the number of neutrophils increases while the total number of lymphocytes
25 remains relatively stable (Figure 1, Panels A and C), suggesting that the observed changes in neutrophil and
26 lymphocyte proportions associated with COPD severity are primarily driven by an increase in the number of
27 circulating neutrophils. The same pattern is present in ECLIPSE subjects (Figure 1, Panels B and D).
28 We evaluated these relationships in a series of models in COPDGene relating cell count, cell proportion,
29 and current smoking (CS) to FEV_1 % of predicted while adjusting for inhaled and oral steroid use (Table 3). The
30 models explaining the largest proportion of variance in FEV_1, after adjusting for model complexity, included
31 both cell counts and proportions demonstrating that both measures have independent association to FEV_1.
32 Both lymphocyte and neutrophil absolute counts and percentages were significantly associated with FEV_1
33 across most models.
34
35 *Relating Circulating Immune Cells to Prospective, Three-Year Change in Lung Function*
36 Since the CBC data in COPDGene were obtained at visit 2, longitudinal FEV_1 analysis measures were not
37 available. We performed longitudinal analysis in 1,741 smokers from the ECLIPSE Study with CBC data and
38 three-year change in FEV_1 who were not taking oral steroids at baseline, relating baseline peripheral blood
39 counts and proportions to change in lung function. In an analysis of single cell type measures, monocyte and
40 eosinophil counts and proportions were significantly associated with change in FEV_1 (Table 4). Higher monocyte
41 levels at baseline were associated with greater FEV_1 decline, and the opposite pattern was observed for
42 eosinophils. Neutrophil and lymphocyte proportions, but not counts, were significantly associated with lung

43 function decline. Larger neutrophil proportions were associated with more lung function decline, with the
44 opposite relationship observed for lymphocyte proportion.
45 Table 5 demonstrates that for multivariate models including counts and proportions of these four cell
46 types, cell counts, but not proportions, showed significant associations to change in FEV_1. Absolute counts of
47 monocytes, eosinophils, and lymphocytes were significantly associated with FEV1 decline (p=0.0003, 0.0004,
48 and p=0.02, respectively). Higher levels of monocytes were associated with larger amounts of FEV_1 decline, and
49 the opposite pattern was present for lymphocytes and eosinophils.
50
51 *Association of Inferred Lymphocyte Subpopulations to COPD and COPD Subtypes*
52 In a subset of 221 subjects from ECLIPSE with complete genome-wide blood gene expression and
53 covariate data (subject characteristics shown in Table E3), we used cell type deconvolution to estimate the
54 proportion of immune cell subpopulations in each study subject, and we related these proportions to COPD
55 case-control status.
56 To first assess the performance of cell type deconvolution in blood gene expression from smokers, we
57 examined results from applying two methods that have been previously validated for the detection of immune
58 cell types, CIBERSORT and the linear regression (LR) method of Abbas et al.(20, 21) To benchmark these
59 algorithms against known cell type quantifications, we compared their neutrophil, aggregated lymphocyte,
60 aggregated monocyte, eosinophil, and basophil quantifications against concurrently drawn CBC counts (Figure
61 2). Both methods showed high correlation to neutrophils and lymphocytes (Spearman *r* ranges from 0.7-0.8,
62 p<0.001), with weaker correlations for eosinophils and monocytes. Correlation with basophils was low for both
63 methods. For the inferred proportions of neutrophils and lymphocytes, the correlation between methods was
64 high (0.86 and 0.83, respectively).

We compared the inferred cell type proportions by COPD case/control status and observed that, relative to smoker controls, subjects with COPD had significantly lower levels of aggregated lymphocytes, aggregated T-cells, CD4+ resting memory cells, naive B-cells, and increased levels of monocytes (Table 6).

Inferred Cell Type Proportions Predict COPD Blood Gene Expression Subtypes

In a previous publication, the ECLIPSE blood gene expression data had been used to define COPD molecular subtypes that differed in clinical characteristics and blood gene expression patterns, and we previously demonstrated that these subtypes could not be recovered using CBC data alone(24). To determine whether these molecular subgroups could be accurately predicted from inferred cell count proportions, we trained support vector machine classifiers to predict NBS subtype and COPD case/control status using CBC data, clinical covariates, or inferred cell type proportions. Figure 3 demonstrates that predictive models for NBS subtypes using inferred cell type proportions classified subjects by COPD molecular subtype with high accuracy (AUC = 0.95) and demonstrated better performance than models using only CBC cell-type quantities (AUC = 0.53) or clinical covariates (AUC = 0.65). Predictive models for COPD case/control status using inferred cell type proportions also showed statistically significant, but less powerful, predictive performance (AUC = 0.71), with the cell type subpopulation models still outperforming the models using CBC data (Table E4).

We compared levels of the inferred cell type proportions between the NBS-MI and NBS-LI COPD molecular subtypes and observed that the list of specific immune cell types that were significantly different between COPD molecular subtypes was more extensive than between COPD cases and controls. This list included T-regulatory cells, CD4 resting and activated memory T-cells, memory B-cells, aggregated T-cells, aggregated B-cells, and monocytes. (Table E5, Figures E4 and E5).

Discussion

88 Using two large cohorts enriched for subjects with COPD, we characterized alterations in circulating
89 immune cell types associated with cross-sectional FEV_1 and longitudinal FEV_1 decline. The main findings are: 1)
90 the predominant peripheral immune cell type alteration associated with increasing COPD severity is an increase
91 in the absolute count of neutrophils, 2) monocytes and eosinophils have strong multivariate associations to
92 prospective change in FEV_1, and 3) cell-type estimates from gene expression deconvolution methods show good
93 accuracy for some, but not all, cell types.
94 Prior immunologic studies of COPD have identified important associations with increased innate
95 immune activation and progression of COPD lung disease including neutrophil stimulation(31, 32), alvelolar
96 macrophage immune surveillance(33), protease/matrikine activation(34), and activation of the dendritic
97 cell/macrophage axis(35). Most of these studies have been performed in murine models or small to moderate
98 sized study samples with limited ability to control for the effects of current smoking. Our findings complement
99 and extend previous results by demonstrating that 1) the decrease in overall lymphocyte proportion in COPD is
00 primarily driven by an increase in absolute neutrophil counts, 2) absolute counts and proportions of immune
01 cell types have independent, statistically significant associations to lung function, and 3) absolute lymphocyte,
02 monocyte, and eosinophil counts are predictive of COPD disease progression. This point extends previous
03 observations relating total peripheral leucocyte count to cross-sectional and longitudinal lung function(16-18)
04 by implicating specific immune cell types.
05 To study immune cell subpopulations not quantified by CBC, we explored the use of cell type
06 deconvolution approaches to quantify 22 distinct cell subpopulations in a subset of ECLIPSE subjects with blood
07 genome-wide gene expression data. Both methods we employed have been previously validated against flow
08 cytometry quantifications(20, 21), and these methods are a promising alternative for estimating cell type
09 proportions in large study samples with available expression data. However, these approaches have not been
10 widely applied in smokers with COPD. Our findings demonstrate that in smokers enriched for COPD, the
11 deconvolution approaches studied yielded consistent and reasonably reliable estimates of neutrophil and

lymphocyte cell proportions with mixed performance for other cell types. Inferred cell type proportions enabled significantly better prediction of externally defined COPD molecular subtypes than CBC and clinical data alone, providing indirect evidence that these inferred proportions capture meaningful information on the cell type composition of bulk blood expression samples. These data provide proof-of-concept of the feasibility of using cell type deconvolution to study immune cell subpopulations in large cohorts of smokers with available blood gene expression data.

Analysis of the cell type deconvolution data supports previous observations of an overall decrease in lymphocytes and T-cells in the COPD state, coupled with an increase in monocytes. When we studied deconvolved cell types in previously defined COPD molecular subtypes, differences in cell type proportions were more pronounced, with the more severely affected subtype characterized by increased monocytes, T-regulatory cells, memory T-cells, and memory B-cells as well as decreased total lymphocytes. This pattern is consistent with the expected behavior of T-regulatory cells, which play a critical immunomodulatory role by suppressing other lymphocyte populations in part through IL-10 and TGF-β signalling(36). Overall, these findings provide additional support for the model that the circulating immune response to CS and COPD is characterized by distinct aspects of suppression of the adaptive immune response(7) and a chronic increase in myeloid cell types, and it suggests that within smokers there are patterns of coordinated immune response that can be used to identify clinically distinct subgroups of subjects.

The main strength of this study is that peripheral cell type quantifications were available from a large number of smokers with a broad range of lung function from two independent studies. The study design also enabled the study of immune cell alterations adjusting for CS, a major confounder of the relationship between immune cell alterations and COPD severity. The use of cell type deconvolution to study the immune response in COPD is novel and enabled the simultaneous study of a large number of lymphocyte subpopulations. Because CBC quantifications and blood gene expression were available in the same ECLIPSE subjects from the same time point, we could benchmark our deconvolution approaches against a known standard.

This study also has important limitations. We did not have access to immune cells in the lung or specific lung compartments in our study subjects, thus we could not relate the blood observations to the lung compartment. We also were not able to characterize immune cell functional states through cytokine profiling or quantification of response to antigenic stimulation. While we observed significant associations between circulating immune cell subpopulations and COPD, further study is required to determine the pathophysiological significance of these observations. We did not have flow cytometry values against which we could benchmark our cell type deconvolution estimates, but we did have Coulter counter data available, and our deconvolution results were generated using methods that has been previously validated against flow cytometry for immune cell populations(20, 21).

In conclusion, analysis of CBC counts and proportions in >6,000 subjects from the COPDGene and ECLIPSE studies demonstrated that cross-sectional FEV_1 is associated with alterations in multiple circulating immune cell types, including total neutrophil count. COPD disease progression, as quantified by decline in FEV_1, is associated with increased absolute monocyte counts and decreased lymphocytes and eosinophil counts at baseline. Cell type deconvolution is a viable approach to simultaneously study multiple immune cell populations in smokers with COPD, and our findings support a model of suppressed adaptive immune responses coupled with enhanced innate immunity. Future studies to characterize COPD-related alterations in more fine-grained immune cell types will benefit from quantification of both cell type proportions and absolute counts.

Abbreviations

AUC – area under the curve

CBC – complete blood count

COPD – chronic obstructive pulmonary disease

CT – computed tomography

FEV_1 – forced expiratory volume in one second

GOLD – Global Initiative for Obstructive Lung Disease

LAA950 – low attenuation area less than 950 Hounsfield units (computed tomorgraphy based emphysema measure)

LR – linear regression

NBS – network-based stratification

ROC – receiver operating characteristic

SVM – support vector machine

Declarations

Ethics approval and consent to participate

For both COPDGene and ECLIPSE, the institutional review boards of all participating centers approved these studies, and written informed consent was obtained from all subjects.

Consent for publication

Not applicable.

Availability of data and material

Gene expression data used in this study are accessible via GEO [ECLIPSE GSE76705, COPDGene GSE42057].

Competing interests

PJC receives research support and has served on an Advisory Board for GSK. All other authors have no conflicts to disclose.

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Authors' contributions

Study concept and design: Stromberg, Castaldi, Bowler

Acquisition, analysis, or interpretation of data: Stromberg, Castaldi, Bowler

Drafting of the manuscript: Stromberg, Castaldi, Bowler

Critical revision of the manuscript for important intellectual content: All authors

Statistical analysis: Stromberg, Castaldi, Bowler

Obtained funding: Castaldi and Bowler

Table 1. Characteristics of Analyzed Subjects in COPDGene and ECLIPSE

	COPDGene	ECLIPSE
N	4,568	1741
Age	65.44 (8.71)	61.89 (7.85)
Gender, % Female	50	36
Race, % African-American	27	0
FEV1 % of predicted	78.32 (24.93)	55.0 (26.13)
FEV1/FVC	0.67 (0.15)	0.66 (0.21)
COPD, % GOLD 2-4	57	84
Pack Years	43.96 (23.95)	45.40 (26.48)
Current Smoking	37	39
BMI	28.91 (6.28)	26.59 (5.34)
Oral Steroids, %	2	0
neutrophil , %	59.38 (10.01)	63.88 (8.17)
neutrophil , 1000 cells/ul	4.29 (1.62)	4.95 (1.64)
lymphocyte , %	29.37 (9.36)	26.84 (7.62)
lymphocyte , 1000 cells/ul	2.02 (0.73)	2.00 (0.64)
monocyte , %	8.07 (2.37)	6.15 (2.13)
monocyte , 1000 cells/ul	0.56 (0.19)	0.46 (0.18)
eosinophil , %	2.57 (1.65)	2.79 (1.73)
eosinophil , 1000 cells/ul	0.18 (0.12)	0.21 (0.14)
basophil , %	0.6 (0.53)	0.34 (0.2)
basophil , 1000 cells/ul	0.03 (0.04)	0.03 (0.02)
Data are mean (SE) unless otherwise indicated.		

Table 2. Relationship of Cell Type Counts and Proportions to FEV_1 % of Predicted in 4,558 Smokers in COPDGene

	Cell Type Count (1000 cells/uL)		Cell Type Proportion	
	Beta	P value	Beta	P value
Neutrophils	-3.53 (0.22)	<0.001	-0.48 (0.04)	<0.001
Lymphocytes	2.34 (0.51)	<0.001	0.58 (0.04)	<0.001
Monocytes	-21.84 (1.88)	<0.001	-0.29 (0.16)	0.06
Eosinophils	-21.25 (3.06)	<0.001	-0.54 (0.22)	0.02
Basophils	-33.28 (9.81)	0.001	0.09 (0.7)	0.89
Beta - change in FEV_1 per unit change in cell count or proportion. Each row corresponds to a separate univariate model.				

Table 3. Multivariate Models of the Relationship between FEV_1 % of Predicted and Neutrophil and Lymphocyte Quantifications

	Neutrophil Count	Neutrophil %	Lymphocyte Count	Lymphocyte %	Current Smoking	Neutrophil Count x Current Smoking	Neutrophil % x Current Smoking	Lymphocyte Count x Current Smoking	Lymphocyte % x Current Smoking	Adjusted R2
Cell Proportions	---	0.39 (0.10) *	---	0.75 (0.11) *	---	---	---	---	---	0.274
Cell Counts	-2.19 (0.20) *	---	1.72 (0.44) *	---	---	---	---	---	---	0.275
Cell Counts + Current Smoking	-2.73 (0.27) *	---	1.79 (0.58) *	---	-5.74 (2.48) *	1.21 (0.40) *	---	`-0.06 (0.90)	---	0.276
Cell Proportions + Counts + Current Smoking	-2.47 (0.62) *	0.74 (0.14) *	-0.41 (1.32)	0.94 (0.16) *	-10.99 (18.91)	0.24 (1.03)	0.13 (0.23)	1.85 (2.07)	-0.1 (0.27)	0.285

* $p<0.05$

Adjusted R2 - variance explained adjusted for model complexity. Cell counts are in units of 1000 cells/ul.

Each row corresponds to a separate multivariate model. Each model is also adjusted for oral and inhaled steroid use.

Table 4. Relationship of Cell Type Counts and Proportions at Baseline to Three-Year Change in FEV_1 % of Predicted in 1,741 Smokers in ECLIPSE

	Cell Type Count (1000 cells/uL)		Cell Type Proportion	
	Beta	P value	Beta	P value
Neutrophils	-0.11 (0.12)	0.36	-0.06 (0.02)	0.02
Lymphocytes	0.59 (0.32)	0.07	0.08 (0.03)	0.005
Monocytes	-3.35 (1.09)	0.002	-0.3 (0.09)	0.001
Eosinophils	4.26 (1.44)	0.003	0.37 (0.11)	0.001
Basophils	-5.41 (12.91)	0.68	-0.12 (1.01)	0.91

Beta - change in FEV_1 over three years per unit change in cell count or proportion.
Change in FEV_1 calculated as (last visit value - first visit value), i.e. negative values indicate decline in FEV_1
Models analyze one cell type at a time. All models are adjusted for FEV1 % of predicted at baseline, number of days of follow-up, and smoking status at baseline and at last study visit.

Table 5. Multivariate Models Relating Cell Type Counts and Proportions at Baseline to Three-Year Change in FEV_1 % of Predicted in 1,741 Smokers in ECLIPSE

	Neutrophil Count	Neutrophil %	Lymphocyte Count	Lymphocyte %	Monocyte Count	Monocyte %	Eosinophil Count	Eosinophil %
Cell Proportions	---	1.03 (1.03)	---	1.11 (1.03)	---	0.69 (1.04)	---	1.43 (1.05)
Cell Counts	0 (0.13)	---	0.76 (0.32) *	---	-4.64 (1.18) *	---	4.98 (1.46) *	---

* $p<0.05$

Cell counts are in units of 1000 cells/ul.

Each row corresponds to a separate multivariate regression model.

Models adjusted for baseline FEV1 % of predicted, days of follow-up, inhaled corticosteroid use at baseline, and current smoking status at baseline and last measurement.

Table 6. Inferred Immune Cell Types Significantly Associated with COPD Status

	Linear Regression			CIBERSORT		
	Controls	Cases	pvalue	Controls	Cases	pvalue
Aggregated monocytes	-0.41 (0.93)	0.25 (0.96)	5.4E-07	-0.35 (0.98)	0.20 (0.94)	6.3E-05
Monocytes	-0.33 (0.99)	0.21 (0.95)	4.9E-05	-0.32 (0.97)	0.19 (0.94)	2.1E-04
Naïve B cells	0.21 (0.89)	-0.13 (1.06)	2.4E-03	0.15 (0.92)	-0.11 (1.04)	1.5E-03
Aggregated T cells	0.24 (0.99)	-0.15 (0.99)	5.1E-03	0.4 (0.98)	-0.22 (0.94)	3.7E-05
CD4 memory resting T cells	0.25 (1.05)	-0.16 (0.95)	6.4E-03	0.23 (1.25)	-0.15 (0.79)	0.01
Aggregated lymphocytes	0.24 (0.94)	-0.13 (1.01)	0.02	0.25 (0.97)	-0.13 (0.98)	0.02

Cell count values normalized to mean 0 and standard error of 1. See Figures E4 and E5 for boxplot distributions.
Values are mean (SD) unless otherwise indicated.
P-values calculated using Wilcoxon rank sum test.
The following cell types were not analyzed because they were detected in less than 10% of samples: M1 Macrophages, activated dendritic cells, activated Mast cells, gamma-delta T-cells, and T follicular helper cells.

Figure 1: Neutrophil and Lymphocyte Counts and Proportions Stratified by GOLD Spirometric Stage. As GOLD Stage increases, the relative proportions of peripheral neutrophils and lymphocytes increase and decrease, respectively (Panel A, COPDGene and Panel C, ECLIPSE). This phenomenon is driven by an increase in absolute neutrophil count, while the absolute amount of lymphocytes remains relatively stable across GOLD Stages (Panel B, COPDGene and Panel D, ECLIPSE). NS = non-smoker. PRISm = Preserved ratio impaired spirometry, i.e. subjects with $FEV_1/FVC > 0.7$ but FEV_1 % predicted < 80%.

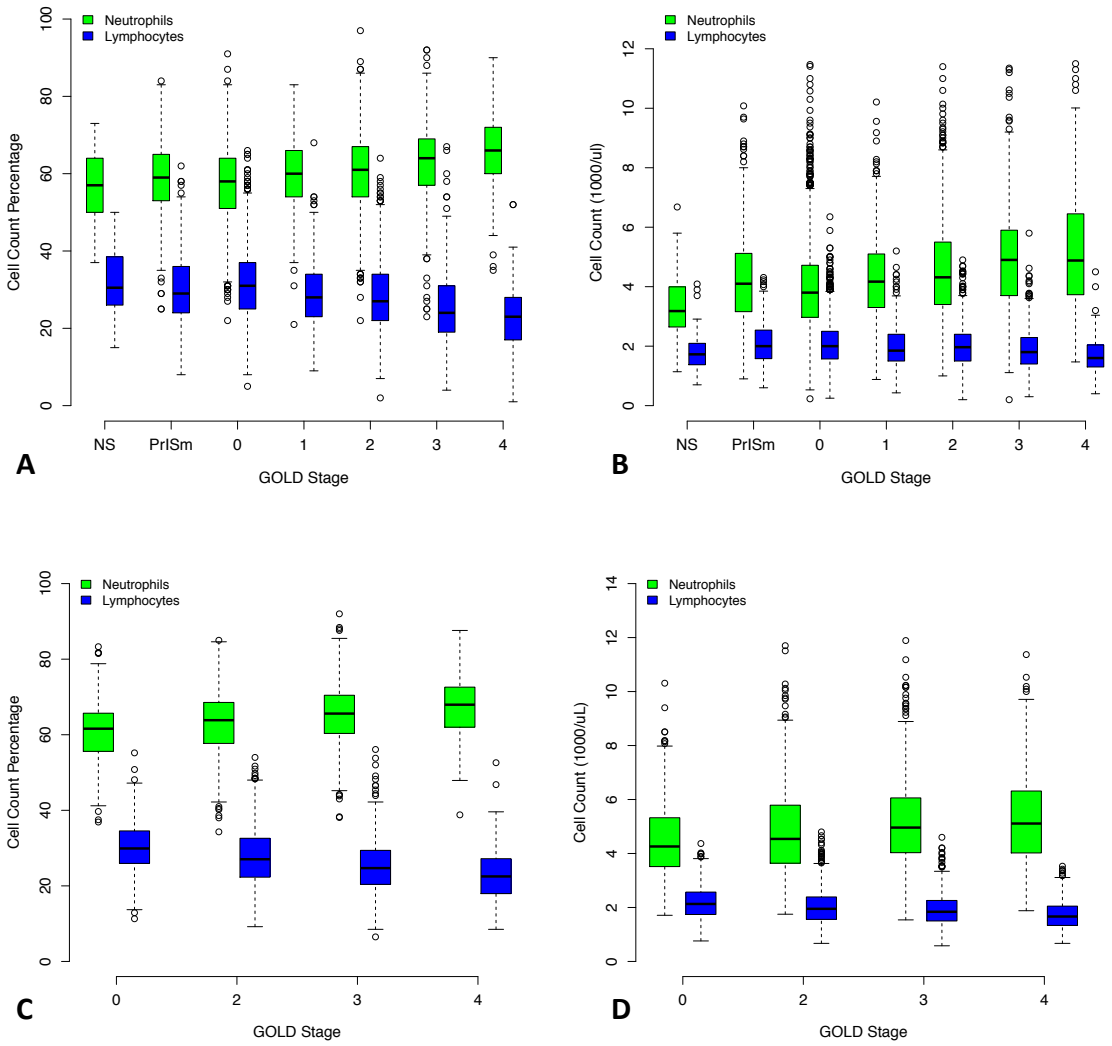


Figure 2. Correlation Between Inferred Cell Subpopulation Proportions and Complete Blood Count Cell Type Proportions. Spearman correlation between estimated cell subpopulations proportions from three deconvolution methods and CBC proportions. Abbreviations: LR- Linear Regression. CI- CIBERSORT. CBC- Complete Blood Count. Neuts- Neutrophils. Lymph- Lymphocytes. Eos- Eosinophils. Mons- Monocytes. Basos- Basophils.

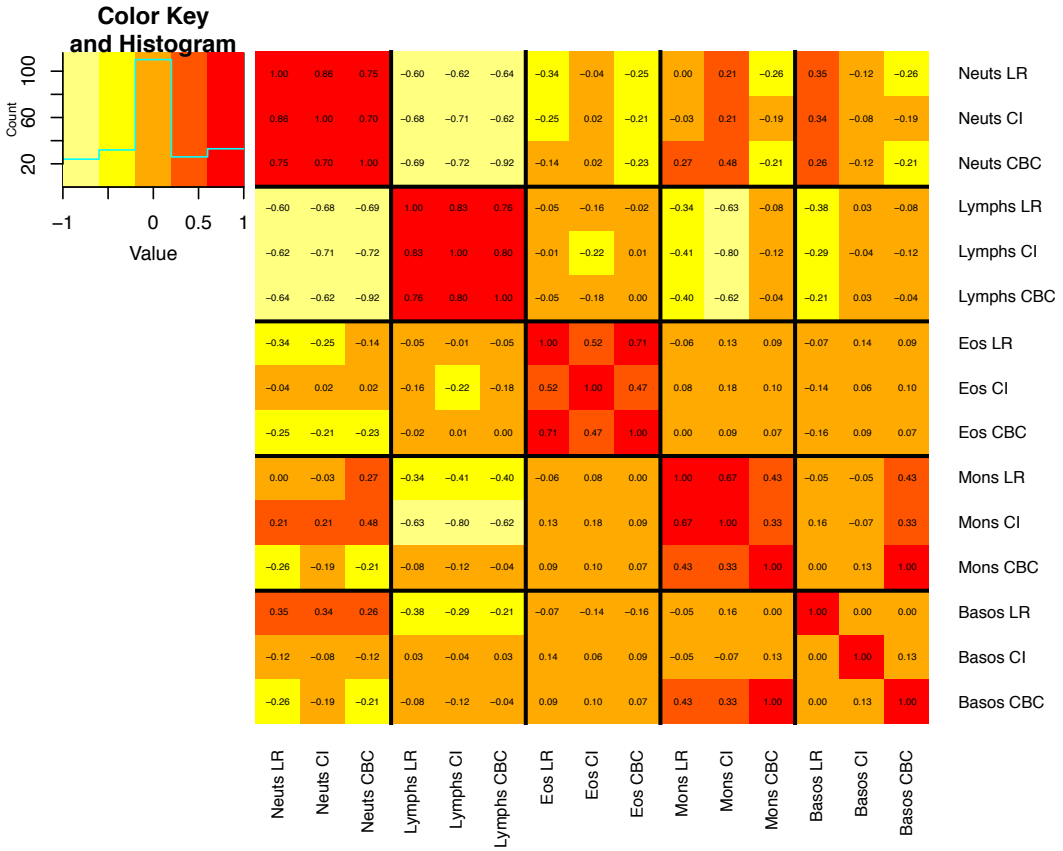
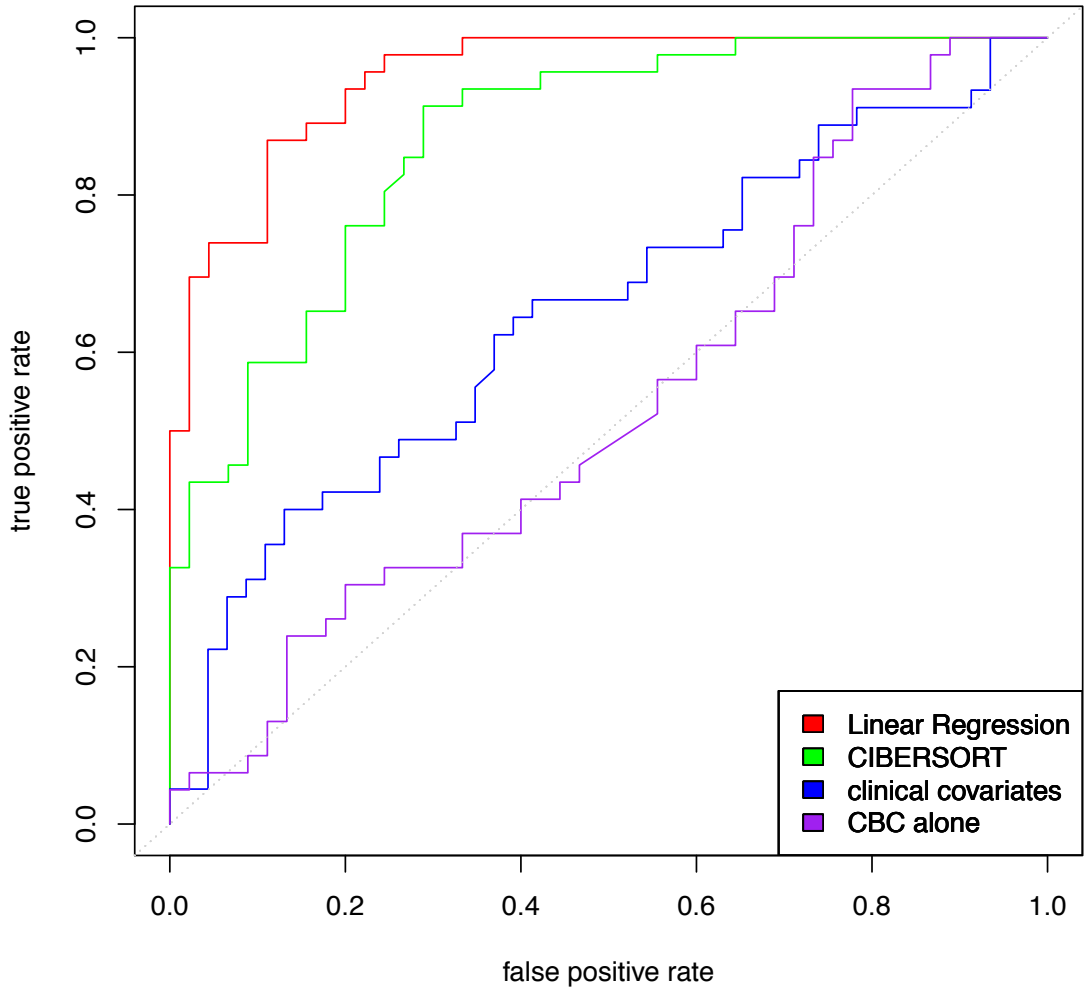


Figure 3. Performance of Predictive Models for COPD Molecular Subtypes Using Complete Blood Counts, Inferred cell Subpopulation Proportions, and Clinical Covariates. Receiver operating characteristic curves demonstrative the predictive performance of SVM classifiers using complete blood count data, inferred cell subpopulation data, and clinical covariates for COPD molecular subtypes in 221 ECLIPSE subjects. Clinical covariates are age, sex, and pack-years.



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