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Blood Biomarkers as Predictors of Long-Term Mortality in COPD

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Abstract

Background—Blood biomarkers are easily accessible and might reflect chronic obstructive pulmonary disease (COPD) activity.

Aim—To determine whether a panel of blood biomarkers (C-reactive protein [CRP], neutrophils, eosinophils, albumin and vitamin D) could predict mortality in COPD.

Methods—we analyzed data from 431 COPD participants to the 2007–2010 National Health and Nutrition Examination Surveys who were followed for a median time of 36 months. COPD was defined as post-bronchodilator forced expiratory volume in 1 second (FEV1) and forced vital capacity ratio <0.70. Weibull survival analysis adjusted for covariates was performed to calculate the risk of mortality associated with the biomarkers and C-statistics was used to assess their added predictive value.

Results—During follow-up, 38 of the 431 participants died. Participants with high CRP, eosinophil count <2%, hypoalbuminemia, and hypovitaminosis D had worse baseline FEV1 and subsequently higher mortality compared to controls. In adjusted analysis, increasing CRP (hazard ratio [HR]: 4.45, 95% CI: 1.91–10.37) and neutrophil count (HR: 1.07, 95% CI: 1.03–1.11) as well as decreasing eosinophil count (HR: 7.03, 95% CI: 2.05–24.01) were associated with an increased risk of mortality. The addition of CRP with eosinophil and/or neutrophil count significantly improved a base model for the prediction of mortality which included age, gender, race/ethnicity, body mass index, smoking, poverty income ratio, asthma, diabetes, hypertension, and history of stroke or myocardial infarction.

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Conclusion—high CRP and neutrophils as well as low eosinophils are predictive of poor COPD prognosis. They also add significant value to prediction models of mortality in COPD.

Keywords

Biologic markers; Chronic Obstructive Pulmonary Disease; COPD; prognosis

INTRODUCTION

Chronic obstructive pulmonary disease (COPD) affects 65 million people globally, including 15 million Americans. Over the past two decades, the disease has progressed from the fourth to the third leading cause of death in the U.S. and worldwide (1–3). COPD classically consists of emphysema characterized by alveolar wall destruction with a loss of elastic recoil and of chronic bronchitis due to airway inflammation and remodeling (4, 5). Beyond emphysema and chronic bronchitis, COPD is a complex and heterogeneous condition with several phenotypes resulting from different mechanisms and pathophysiology (6). It involves not only airflow limitation, but it can also lead to systemic manifestations (e.g. cardiovascular, musculoskeletal, hematologic, and metabolic) (7).

At first, the forced expiratory volume in the first second of expiration (FEV1) was the sole marker of COPD severity (6–8). However, given FEV1 limitations, other biomarkers have recently been of great interest as a way to better phenotype COPD, predict its prognosis, and evaluate the efficacy of treatment (7, 8). Some markers of inflammation such as fibrinogen have been found to be predictors of COPD exacerbation or death (8), whereas other ones involved in inflammation (such as C-reactive protein [CRP] neutrophils, and eosinophils) and nutrition (albumin and vitamin D) are still under investigation (9–13). Blood biomarkers are easily measured and can be systemic markers of COPD activity or they can be reflective of substances released from lung into the bloodstream (14). Yet, little is still known on their performance to predict COPD prognosis. Therefore, we aimed to study these biomarkers as potential predictors of long-term mortality COPD in a nationally representative sample of participants with COPD.

MATERIALS AND METHODS

Data source and study design

We used data from the National Health and Nutrition Examination Survey (NHANES) conducted from 2007 to 2010 and the corresponding Mortality-Linked File. The NHANES is a continuous cross-sectional survey of the US non-institutionalized civilian population conducted by the National Center for Health Statistics (NCHS) of the Centers for Disease Control and Prevention (CDC). It uses a complex multistage sampling design to derive a representative sample of the US population. Of the 12,153 adult participants to the NHANES between 2007 and 2010, 590 were identified as having COPD. Among them, 447 had data on mortality after follow-up through December 31, 2011. Data on the biomarkers of interest was available on 431 participants who were included in our study. NHANES participants were matched to the National Death Index (NDI) records and death certificates were used for additional confirmation. The cause of death was determined based on the

underlying cause listed on the death certificates, coded using the International Classification of Diseases, Tenth Revision (ICD-10).

NHANES protocols were approved by the Institutional Review Boards of the NCHS and CDC and informed consent was obtained from all participants (<http://www.cdc.gov/nchs/nhanes/irba98.htm>).

COPD Definition and Analysis of Biomarkers

Eligible NHANES participants aged 20–79 years underwent baseline spirometry, including Forced Vital Capacity (FVC) and FEV1. Examinees with baseline FEV1/FVC <70% or <Lower Limit of Normal (LLN) for their age, sex, weight, height, and race/ethnicity were considered for post-bronchodilator (albuterol) spirometry. COPD was defined as post-bronchodilator FEV1/forced vital capacity (FVC) ratio <70%. Because participants receiving daytime oxygen therapy were excluded from spirometry testing for safety reasons, we classified those who reported COPD diagnosis (chronic bronchitis or emphysema) and were currently taking supplemental oxygen during the day as having COPD.

CRP was quantified in NHANES by latex-enhanced nephelometry. Eosinophils and neutrophils were part of the complete blood count parameters assessed with the Beckman Coulter method and white blood cell differential used VCS technology. The percentages of eosinophils and neutrophils were derived from the white blood cells scatterplot. Serum albumin was measured using the DcX800 method as a bichromatic digital endpoint method. 25-hydroxyvitamin D3 (25OHD3) was measured using an ultra-high-performance liquid chromatography-tandem mass spectrometric method. Detailed description of all these methods are reported on the NHANES website (<https://wwwn.cdc.gov/nchs/nhanes/continuousnhanes/default.aspx?BeginYear=2009>). Blood biomarkers were dichotomized using cut-offs reported in the literature. We categorized CRP as normal if ≤ 1 mg/dL and high if >1 mg/dL. The CRP cut-off of 1 mg/dL is also used to discriminate between low and intermediate to high cardiovascular risk (15). We classified neutrophil count as normal ($\leq 70\%$) or high ($>70\%$) (16). Eosinophil count was divided into levels $\leq 2\%$ or $>2\%$, the cut-off used in previous studies (17). Serum albumin was considered as low if <30 g/dL or normal if ≥ 30 g/dL (18), and serum vitamin D was defined as low if <50 nmol/L and normal if ≥ 50 nmol/L (19).

Demographics and comorbidities

The NHANES collected data on age, gender, race/ethnicity, poverty income ratio (PIR), cigarette smoking, body mass index (BMI), and medical conditions. Diabetes was defined by self-reported physician diagnosis, fasting plasma glucose ≥ 126 mg/dL, two-hour oral glucose tolerance test ≥ 200 mg/dL, or glycohemoglobin $\geq 6.5\%$ (20). Hypertension was defined by self-reported physician diagnosis or mean systolic blood pressure (of up to four measurements on 2 separate occasions) ≥ 140 mm Hg. Other comorbid conditions included reported diagnosis of asthma and history of stroke or myocardial infarction (MI).

Statistical analysis

Descriptive analysis was performed and because blood eosinophils and CRP were significantly skewed, they were log-transformed to improve the normality of their distribution. We explored the correlation between the biomarkers by means of the Pearson method to calculate correlation coefficients (r). Since the assumption of proportionality was violated, the traditional Cox proportional hazards regression modeling could not be used, instead the parametric Weibull survival analysis was used to estimate the hazard ratio (HR) and corresponding 95% confidence interval (CI) for mortality associated with each biomarker. It allows to examine the effect of biomarkers on mortality in term of HR and whether it is increasing, decreasing, or constant over time. The models were adjusted for age, gender, race/ethnicity, smoking, PIR, BMI, and comorbidities (asthma, diabetes, hypertension, history of stroke or MI). We reported the risks of mortality for each biomarker used both as a continuous and dichotomized variable. Although the dichotomization reduces statistical power, it was done for clinical relevance, using clinically relevant cut-offs reported in the literature. We also assessed whether biomarkers might interact to affect mortality by including a product term in the models. The discriminative power or ability of the model to predict mortality was evaluated using the C-statistic and the added predictive value of the biomarkers was assessed. NHANES sample weights were used in all analyses to obtain unbiased national estimates. The analyses were performed in SAS (Version 9.4) and STATA (Version 14). P-values < 0.05 were considered statistically significant in all analyses.

RESULTS

Descriptive analysis

Our sample consisted of 431 participants who were followed for a median time of 36 months with a maximum duration of 60 months. During follow-up, 38 participants (8.6%) died. There were weak ($-0.3 < r \leq -0.1$ or $0.1 < r \leq 0.3$) to moderate ($-0.5 < r \leq -0.3$ or $0.3 < r \leq 0.5$) correlations between some of the biomarkers. Log-CRP and neutrophils were weakly related, while vitamin D weakly correlated with log-eosinophils, neutrophils, and albumin. Moderate associations occurred between log-CRP and albumin and between log-eosinophils and neutrophils (data not shown).

The characteristics of participants are showed in Table 1. In summary, participants with high CRP were 8.6% of the study sample. Compared to those with normal CRP, they were older, had a higher prevalence of hypertension and a history of MI/stroke. They also had a significantly lower FEV1 and a higher mortality. The prevalence of participants with high neutrophil count was 12.4%. Compared to those with normal neutrophil count, they were more likely to be smokers and to have a higher mortality. About 30.7% of participants had eosinophil count $< 2\%$. Compared to participants with eosinophil count $\geq 2\%$, they were mostly women, non-Hispanic Blacks, smokers. They had lower FEV1 and higher mortality. Low albumin was present in 1.5% of participants who, compared to those with normal albumin, were mainly women, or participants with diabetes, hypertension, history of stroke or MI, low FEV1, and higher mortality. The prevalence of low vitamin D was 17.9%. compared to participants with normal vitamin D, they were mostly women, non-Hispanic

Black, participants with obesity, asthma, diabetes, history of MI/stroke and lower FEV1 (Table 1).

Biomarkers and risk of mortality

In regression analysis adjusted for covariates and using biomarkers as continuous variables, Increasing CRP (HR 4.45, 95% CI: 1.91–10.37) and neutrophil count (HR 1.07, 95% CI: 1.03–1.11) was associated with higher mortality. However, decreasing eosinophil count correlated with a higher risk of death (HR: 7.03, 95% CI: 2.05–24.01). In addition to neutrophil count, neutrophil-to-lymphocyte ratio (NLR) was also associated with a higher mortality (HR: 1.48, 95% CI: 1.28–1.72) (Table 2).

When the biomarkers were dichotomized, higher mortality in COPD was predicted by high CRP (HR: 3.95, 95% CI: 1.27–12.31 [Figure 1a]), high neutrophil count (HR: 2.53, 95% CI: 1.07–6.00 [Figure 1b]), and eosinophil count <2% (HR: 2.54, 95% CI: 1.06–6.08 [figure 1c]). Serum Albumin (Figure 1d) and vitamin D (Figure 1e) had no effect COPD prognostic (Table 2). The number of participants and deaths in each category of the biomarkers associated with mortality (high CRP, high neutrophils count and eosinophil count <2%) is reported in Figure 2.

CRP and eosinophils significantly interacted to affect mortality (interaction P-value <0.001). In stratified analysis by CRP levels, decreasing eosinophil was associated with higher mortality in participants with normal CRP (HR 10.05, 95% CI: 2.28–44.30), but not in those with high CRP. Because of the small number of COPD-specific mortality (only eight COPD deaths), we could not examine the association of the biomarkers with this outcome. Still, participants who subsequently died of COPD had significantly higher baseline levels of CRP (P=0.0087) and neutrophil count (P=0.010).

Prediction of COPD prognosis

Table 3 reports the predictive value (C-statistic) of different models. The initial model, which included age, gender, race/ethnicity, PIR, smoking, BMI, and comorbidities (diabetes, hypertension, history of MI/stroke) had a C-statistic of 0.79 (95% CI: 0.71–0.86). Its predictive value did not significantly improve with the addition of any of the individual biomarkers. However, the addition of CRP with eosinophils and/or neutrophil significantly improve the predictive value of the initial model (Table 3). Figure 3 displays the improvement in receiver operating characteristic (ROC) area (similar to the C-statistic) of the model with the highest predictive value. It included CRP, eosinophils, and NLR added to the initial model.

DISCUSSION

The current study uses a sample from the US population to examine the prediction of mortality in COPD by blood biomarkers such as CRP, eosinophils, neutrophils, albumin, and vitamin D. The results suggest that high CRP and neutrophil count as well as low eosinophil levels were associated with death in COPD. low eosinophil count was only associated with increased mortality risk in patients with low CRP. The addition of CRP and eosinophils and/or neutrophilia to the initial model significantly improved the prediction of mortality.

Consistent with our results, several studies found CRP to be a strong predictor of mortality in COPD (21–23). This was confirmed by a recent meta-analysis of 15 studies and 11,180 participants, which showed a solid association of CRP with late (after 24 months) mortality in COPD (24). However, the study failed to find a significant association with early mortality (<24 months of follow-up) (24). Other investigations have also reported that elevated CRP in COPD may be associated with decreased lung function (25), lower quality of life (26), more frequent exacerbation and hospital admission (27–29). Moreover, COPD patients with high CRP hospitalized for exacerbations seem more likely to experience treatment failure than those with lower CRP concentration (30). CRP is a known risk factor for cardiovascular mortality in both COPD and non-COPD subjects (31). Although our analysis did not include COPD death as an outcome because of the small number of outcome events, baseline CRP was higher in participants who later died of COPD than in those who survived or died of other causes during follow-up. At the contrary of CRP, the literature on blood neutrophil count and COPD prognosis is scarce and has yielded conflicting results. In the ECLIPSE (Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints) cohort, 1,843 COPD participants were followed for 3 years and blood neutrophil count was found to be associated with higher mortality (23). Other authors have advocated for the use of the blood NLR as a promising biomarker of inflammation. Consistent with our results, NLR has been shown to be associated with both mortality and COPD severity (21, 34, 35). Although NLR had strong association with mortality in our analysis, it did not have a predictive value significantly different from that of blood neutrophil count.

We found that low eosinophil count was a strong predictor of poor prognosis in COPD. Consistent with our findings, a study conducted in a respiratory intensive care unit reported that COPD participants admitted for exacerbations with acute respiratory failure had longer length of hospital stay and poorer survival if their initial blood eosinophil count was $\geq 2\%$ (36). Bafadhel et al. also observed that, among patients admitted for COPD exacerbation, those with blood eosinophils $\geq 2\%$ or ≥ 200 cells/uL had a shorter length of hospital stay (37). Similar results were found in a study of 137 patients with COPD hospitalized in Italy (38). Studying the effect of asthma-like features on the clinical course of COPD in Japan, Suzuki et al. also found that blood eosinophils <300 cells/uL was associated with a significantly faster decline in post-bronchodilator FEV1 (39). With regards to pulmonary infections, it has been suggested that in COPD, a low eosinophil count correlated with more frequent bacterial infections and a higher bacterial load of *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pneumoniae*. Furthermore, there are several reports that high blood eosinophil count in COPD is associated with better response to corticosteroid therapy (40–42). Abnormally low eosinophil count (eosinopenia) could also be an indicator of bacterial infection and might therefore be associated with poor survival in COPD patients (43). The authors speculated that osteopenia in bacterial infections might be the consequence of circulating eosinophil chemotaxis and sequestration in tissues (43). It is worth noting that in our study, only one participant had eosinopenia as defined in previous studies by eosinophil count $<0.40/\text{mm}^3$ (43). Conflicting with these findings, other research suggests that blood eosinophils is associated with increased risk of COPD exacerbation and hospital admissions (44, 45). The role of eosinophils in COPD and the reason why low eosinophil counts may be

associated with poor prognosis in COPD is unclear. Eosinophils originate from the bone marrow and migrate to the digestive and thymus organs. Under pathologic conditions, they travel to the lungs and stimulate the secretion of cytokines and cytotoxic granules, leading to eosinophilic inflammation (46). At the contrary of circulating eosinophils involved in inflammatory processes, tissue-resident eosinophils seem to be beneficial to the respiratory system by regulating the homeostasis of immune processes and protecting the lungs against inflammation (47). Interestingly, we found that the association between lower eosinophil count and increased mortality in COPD was observed only in the absence of inflammation.

Our study was one of a very few to evaluate the predictive value of blood biomarkers on COPD prognosis. Using the C-statistic Celli et al. assessed the addition of inflammatory biomarkers in their initial model which included age, the BODE index (BMI, FEV1, dyspnea, 6-minute walk distance), and previous COPD hospitalizations (23). They found that the addition of interleukin (IL)-6 significantly improved the predictive value of the initial model and that the addition of all the studied inflammatory biomarkers (IL-6, neutrophils, leucocytes, CRP, fibrinogen, IL-8, surfactant protein D) had a greater improvement of the prediction of mortality (23). In a sample selected from the 2001–2003 Copenhagen City Heart Study and the 2003–2008 Copenhagen General Population Study, the addition of baseline CRP, fibrinogen, and leucocyte count significantly improved a base model in predicting future COPD exacerbations. The base model included age, gender, FEV1, cigarette smoking, inhaled medication use, BMI, and previous COPD exacerbations (29). The mechanism by which inflammation could predict COPD prognosis might be due to the role of lung and airway inflammation in COPD progression and severity (24). This also supported by our finding of lower lung function in participants with higher CRP, and neutrophils and the fact that participants who died of specifically of COPD had higher baseline CRP and neutrophil count. It is also possible that inflammation could contribute to atherosclerosis and cardiovascular diseases, leading to higher death in COPD patients. However, unlike previous reports, our initial prediction model included important comorbidities such asthma, diabetes, hypertension, history of stroke or MI, all associated with mortality. In the absence of inflammation, we found lower eosinophil count to be predictive of mortality in COPD and more importantly, the combination of both CRP and eosinophil count had the higher improvement in the prediction of mortality in COPD.

Our study had limitations. Serum biomarkers were only measured once at baseline and their temporal stability is still unclear. We did not have data on COPD exacerbation to analyze it as an outcome. Other predictors of mortality in COPD such as exercise tolerance (6MWD), dyspnea (mMRC), fat-free mass, and exacerbations were not measured. Because of the small number of COPD-specific death, it could not be examined as an outcome in the survival analysis. Nonetheless, our study had major strengths. It used a prospective design and its sample was selected from the general U.S. population, which contributes to the generalizability of the findings. An additional strength is that we examined the predictive value of inflammatory biomarkers, taking into account important comorbidities, some of which were missing in previous studies. These were rigorously defined using both self-reports of doctor diagnosis and complementary tests to capture unreported cases.

In conclusion, high levels of inflammatory markers such as CRP and neutrophils as well as low eosinophil count (especially in the absence of inflammation) predict poor mortality in COPD. The combination of CRP and eosinophil and/or neutrophil count significantly improves the mortality prediction. Future studies could evaluate whether interventions aimed at modifying the levels of these biomarkers can have an effect on COPD prognosis.

References

1. Heron M. Deaths: leading causes for 2010. *Natl Vital Stat Rep.* 2013; 62(6):1–96.
2. Burney PG, Patel J, Newson R, Minelli C, Naghavi M. Global and regional trends in COPD mortality, 1990–2010. *Eur Respir J.* 2015; 45(5):1239–47. [PubMed: 25837037]
3. Diaz-Guzman E, Mannino DM. Epidemiology and prevalence of chronic obstructive pulmonary disease. *Clin Chest Med.* 2014; 35(1):7–16. [PubMed: 24507833]
4. Sciurba FC. Physiologic similarities and differences between COPD and asthma. *Chest.* 2004; 126(2_suppl_1):117S–24S. [PubMed: 15302772]
5. Barnes PJ. Mechanisms in COPD: differences from asthma. *Chest.* 2000; 117(2_suppl):10S–4S. [PubMed: 10673467]
6. Rosenberg SR, Kalhan R. Biomarkers in chronic obstructive pulmonary disease. *Transl Res.* 2012; 159(4):228–37. [PubMed: 22424427]
7. Barnes PJ, Celli BR. Systemic manifestations and comorbidities of COPD. *Eur Respir J.* 2009; 33(5):1165–85. [PubMed: 19407051]
8. Horita, N., Kaneko, T. *Chronic Obstructive Pulmonary Disease.* Springer; 2017. Assessment of Inflammation in COPD: Are There any Biomarkers that Can be Used to Assess Pulmonary and Systemic Inflammation?; p. 135–58.
9. Pavord ID, Lettis S, Locantore N, Pascoe S, Jones PW, Wedzicha JA, et al. Blood eosinophils and inhaled corticosteroid/long-acting beta-2 agonist efficacy in COPD. *Thorax.* 2016; 71(2):118–25. [PubMed: 26585525]
10. Bafadhel M, Davies L, Calverley PM, Aaron SD, Brightling CE, Pavord ID. Blood eosinophil guided prednisolone therapy for exacerbations of COPD: a further analysis. *Eur Respir J.* 2014; 44(3):789–91. [PubMed: 24925917]
11. Agustí A, Edwards LD, Rennard SI, MacNee W, Tal-Singer R, Miller BE, et al. Persistent systemic inflammation is associated with poor clinical outcomes in COPD: a novel phenotype. *PloS one.* 2012; 7(5):e37483. [PubMed: 22624038]
12. de Torres JP, Cordoba-Lanus E, Lopez-Aguilar C, Muros de Fuentes M, Montejo de Garcini A, Aguirre-Jaime A, et al. C-reactive protein levels and clinically important predictive outcomes in stable COPD patients. *Eur Respir J.* 2006; 27(5):902–7. [PubMed: 16455829]
13. Lee H, Um S, Kim YS, Kim DK, Jang AS, Choi HS, et al. Association of the Neutrophil-to-Lymphocyte Ratio with Lung Function and Exacerbations in Patients with Chronic Obstructive Pulmonary Disease. *PloS one.* 2016; 11(6):e0156511. [PubMed: 27258044]
14. Shaw JG, Vaughan A, Dent AG, O'Hare PE, Goh F, Bowman RV, et al. Biomarkers of progression of chronic obstructive pulmonary disease (COPD). *J Thorac Dis.* 2014; 6(11):1532–47. [PubMed: 25478195]
15. Dev D, Wallace E, Sankaran R, Cunniffe J, Govan J, Wathen C, et al. Value of C-reactive protein measurements in exacerbations of chronic obstructive pulmonary disease. *Respir Med.* 1998; 92(4):664–7. [PubMed: 9659534]
16. Rodak, BF., Carr, JH. *Clinical Hematology Atlas-E-Book.* Elsevier Health Sciences; 2015.
17. Serafino-Agrusa L, Scichilone N, Spatafora M, Battaglia S. Blood eosinophils and treatment response in hospitalized exacerbations of chronic obstructive pulmonary disease: A case-control study. *Pulm Pharmacol Ther.* 2016; 37:89–94. [PubMed: 26996272]
18. Luk JKH, Chiu PKC, Tam S, Chu LW. Relationship between admission albumin levels and rehabilitation outcomes in older patients. *Arch Gerontol Geriatr.* 2011; 53(1):84–9. [PubMed: 20678814]

19. Alemzadeh R, Kichler J, Babar G, Calhoun M. Hypovitaminosis D in obese children and adolescents: relationship with adiposity, insulin sensitivity, ethnicity, and season. *Metab Clin Exp*. 2008; 57(2):183–91. [PubMed: 18191047]
20. Khot UN, Khot MB, Bajzer CT, Sapp SK, Ohman EM, Brener SJ, et al. Prevalence of conventional risk factors in patients with coronary heart disease. *JAMA*. 2003; 290(7):898–904. [PubMed: 12928466]
21. Xiong W, Xu M, Zhao Y, Wu X, Pudasaini B, Liu J. Can we predict the prognosis of COPD with a routine blood test? *Int J Chron Obstruct Pulmon Dis*. 2017; 12:615. [PubMed: 28243079]
22. Man SF, Connett JE, Anthonisen NR, Wise RA, Tashkin DP, Sin DD. C-reactive protein and mortality in mild to moderate chronic obstructive pulmonary disease. *Thorax*. 2006; 61(10):849–53. [PubMed: 16738034]
23. Celli BR, Locantore N, Yates J, Tal-Singer R, Miller BE, Bakke P, et al. Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med*. 2012; 185(10):1065–72. [PubMed: 22427534]
24. Leuzzi G, Galeone C, Taverna F, Suatoni P, Morelli D, Pastorino U. C-reactive protein level predicts mortality in COPD: a systematic review and meta-analysis. *Eur Respir Rev*. 2017 Jan 31;26(143)
25. Engström G, Lindberg C, de Verdier MG, Nihlén U, Anderson M, Svartengren M, et al. Blood biomarkers and measures of pulmonary function—a study from the Swedish twin registry. *Respir Med*. 2012; 106(9):1250–7. [PubMed: 22687639]
26. Kang HK, Kim K, Lee H, Jeong B, Koh W, Park HY. COPD assessment test score and serum C-reactive protein levels in stable COPD patients. *Int J Chron Obstruct Pulmon Dis*. 2016; 11:3137. [PubMed: 27994452]
27. Gallego M, Pomares X, Capilla S, Marcos MA, Suárez D, Monsó E, et al. C-reactive protein in outpatients with acute exacerbation of COPD: its relationship with microbial etiology and severity. *Int J Chron Obstruct Pulmon Dis*. 2016; 11:2633. [PubMed: 27799762]
28. Duran L, Unsal M, Yardan T, Kati C, Bedir A, Turkeli S, et al. The Evaluation of Serum Pentraxin-3 and High-Sensitivity C-Reactive Protein Levels in Patients with Acute Attack of COPD. *Clin Lab*. 2015; 61(12):1911–6. [PubMed: 26882815]
29. Thomsen M, Ingebrigtsen TS, Marott JL, Dahl M, Lange P, Vestbo J, et al. Inflammatory biomarkers and exacerbations in chronic obstructive pulmonary disease. *JAMA*. 2013; 309(22):2353–61. [PubMed: 23757083]
30. Crisafulli E, Torres A, Huerta A, Guerrero M, Gabarrús A, Gimeno A, et al. Predicting in-hospital treatment failure (< 7 days) in patients with COPD exacerbation using antibiotics and systemic steroids. *COPD*. 2016; 13(1):82–92. [PubMed: 26451913]
31. Sin DD, Man SF. Why are patients with chronic obstructive pulmonary disease at increased risk of cardiovascular diseases? The potential role of systemic inflammation in chronic obstructive pulmonary disease. *Circulation*. 2003; 107(11):1514–9. [PubMed: 12654609]
32. Singh D, Edwards L, Tal-Singer R, Rennard S. Sputum neutrophils as a biomarker in COPD: findings from the ECLIPSE study. *Respir Res*. 2010; 11(1):77. [PubMed: 20550701]
33. Bhowmik A, Seemungal TA, Sapsford RJ, Wedzicha JA. Relation of sputum inflammatory markers to symptoms and lung function changes in COPD exacerbations. *Thorax*. 2000; 55(2):114–20. [PubMed: 10639527]
34. Sørensen AK, Holmgaard DB, Mygind LH, Johansen J. Neutrophil-to-lymphocyte ratio, calprotectin and YKL-40 in patients with chronic obstructive pulmonary disease: correlations and 5-year mortality—a cohort study. *J Inflamm*. 2015; 12(1):20.
35. Lee H, Um S, Kim YS, Kim DK, Jang AS, Choi HS, et al. Association of the neutrophil-to-lymphocyte ratio with lung function and exacerbations in patients with chronic obstructive pulmonary disease. *PloS one*. 2016; 11(6):e0156511. [PubMed: 27258044]
36. Salturk C, Karakurt Z, Adiguzel N, Kargin F, Sari R, Celik ME, et al. Does eosinophilic COPD exacerbation have a better patient outcome than non-eosinophilic in the intensive care unit? *Int J Chron Obstruct Pulmon Dis*. 2015; 10:1837–46. [PubMed: 26392758]

37. Bafadhel M, Greening NJ, Harvey-Dunstan TC, Williams JE, Morgan MD, Brightling CE, et al. Blood eosinophils and outcomes in severe hospitalized exacerbations of COPD. *Chest*. 2016; 150(2):320–8. [PubMed: 26851799]
38. Serafino-Agrusa L, Scichilone N, Spatafora M, Battaglia S. Blood eosinophils and treatment response in hospitalized exacerbations of chronic obstructive pulmonary disease: A case-control study. *Pulm Pharmacol Ther*. 2016; 37:89–94. [PubMed: 26996272]
39. Suzuki M, Makita H, Konno S, Shimizu K, Kimura H, Kimura H, et al. Asthma-like Features and Clinical Course of COPD: An Analysis From the Hokkaido COPD Cohort Study. *Am J Respir Crit Care Med*. 2016 Dec 1; 194(11):1358–1365. [PubMed: 27224255]
40. Bafadhel M, McKenna S, Terry S, Mistry V, Pancholi M, Venge P, et al. Blood eosinophils to direct corticosteroid treatment of exacerbations of chronic obstructive pulmonary disease: a randomized placebo-controlled trial. *Am J Respir Crit Care Med*. 2012; 186(1):48–55. [PubMed: 22447964]
41. Barnes NC, Sharma R, Lettis S, Calverley PM. Blood eosinophils as a marker of response to inhaled corticosteroids in COPD. *Eur Respir J*. 2016; 47(5):1374–82. [PubMed: 26917606]
42. Pavord ID, Lettis S, Locantore N, Pascoe S, Jones PW, Wedzicha JA, et al. Blood eosinophils and inhaled corticosteroid/long-acting beta-2 agonist efficacy in COPD. *Thorax*. 2016; 71(2):118–25. [PubMed: 26585525]
43. Holland M, Alkhalil M, Chandromouli S, Janjua A, Babores M. Eosinopenia as a marker of mortality and length of stay in patients admitted with exacerbations of chronic obstructive pulmonary disease. *Respirology*. 2010; 15(1):165–7. [PubMed: 19947999]
44. Vedel-Krogh S, Nielsen SF, Lange P, Vestbo J, Nordestgaard BG. Blood eosinophils and exacerbations in chronic obstructive pulmonary disease. The Copenhagen General Population Study. *Am J Respir Crit Care Med*. 2016; 193(9):965–74. [PubMed: 26641631]
45. Couillard S, Larivée P, Courteau J, Vanasse A. Eosinophils in COPD exacerbations are associated with increased readmissions. *Chest*. 2017; 151(2):366–73. [PubMed: 27746201]
46. Loureiro C. Blurred lines. Eosinophilic COPD: ACOS or COPD phenotype? *Revista Portuguesa de Pneumologia (English Edition)*. 2016; 22(5):279–82. [PubMed: 27013320]
47. Mesnil C, Raulier S, Paulissen G, Xiao X, Birrell MA, Pirottin D, et al. Lung-resident eosinophils represent a distinct regulatory eosinophil subset. *J Clin Invest*. 2016; 126(9):3279–95. [PubMed: 27548519]

Figure 1. Risk of mortality in COPD associated with a) CRP, b) neutrophils, c) eosinophils, d) albumin, and e) vitamin D using parametric Weibull regression. Models adjusted for age, gender, race/ethnicity, body mass index, smoking, poverty income ratio, asthma, diabetes, hypertension, and history of stroke or myocardial infarction.

Figure 2. Venn diagram for the number of participants and deaths in each category of the biomarkers associated with mortality.

Figure 3.

Receiver operating characteristic (ROC) curves showing improvement of the predictive value of the initial model by the addition of CRP, eosinophils, and NLR. The initial model included age, gender, race/ethnicity, PIR, smoking, BMI, and comorbidities (diabetes, hypertension, history of MI/stroke).

Table 1
 Characteristics of participants by blood biomarkers levels NHANES 2007–2010 (N = 431)

Characteristics	CRP			Neutrophil count			Eosinophil count			Serum albumin			Serum vitamin D		
	Normal	High	P	Normal	High	P	<2%	2%	P	Normal	Low	P	Low	Normal	P
Prevalence, %	91.4	8.6		87.6	12.4		30.7	69.3		98.5	1.5		17.9	82.1	
Age, %			0.010			0.67			0.89			0.071			0.93
20 – 39 years	8.8	1.6		8.2	7.7		9.6	7.6		8.3	0.0		7.0	8.3	
40 – 59 years	49.3	33.9		47.0	54.2		47.7	48.0		48.1	20.2		50.0	47.7	
60+ years	41.9	64.5		44.8	38.1		42.7	44.4		43.5	79.8		43.1	44.1	
Gender, %			0.15			0.33			0.0005			0.014			0.015
Men	70.0	58.1		67.9	74.9		52.1	75.1		70.0	31.3		54.7	71.6	
Women	30.0	41.9		32.1	25.1		47.9	24.9		30.0	68.7		45.3	28.4	
Race/ethnicity, %			0.066			0.40			0.0084			0.17			< 0.0001
Hispanics	2.6	5.3		2.7	3.6		2.9	2.8		3.0	2.1		3.3	2.6	
Non-Hispanic Whites	85.9	82.2		84.9	89.4		86.4	85.1		85.7	79.7		72.0	88.9	
Non-Hispanic Blacks	6.7	12.5		7.8	4.4		10.3	6.3		6.9	18.2		21.4	3.9	
Other	4.7	0.0		4.6	2.6		0.5	5.8		4.4	0.0		3.2	4.6	
BMI, %			0.41			0.71			0.0007			0.77			0.05
Normal	35.8	33.7		34.7	42.3		51.5	29.7		35.4	26.1		33.6	35.4	
Overweight	39.2	29.0		38.4	37.3		28.5	42.0		38.6	36.7		26.7	40.9	
Obese	23.9	35.3		25.6	19.8		19.7	26.8		24.8	37.3		39.3	22.2	
Missing	1.1	1.9		1.3	0.6		0.3	1.6		1.2	0.0		0.4	1.4	
PIR, %			0.14			0.80			0.15			0.073			0.20
1	7.9	11.4		8.2	10.2		11.9	7.1		7.9	31.1		11.7	6.9	
>1	86.1	75.1		85.2	84.1		79.7	87.1		85.5	55.8		84.2	86.5	
Missing	6.0	13.5		6.7	5.7		8.4	5.8		6.6	13.2		4.1	6.5	
Cigarette smoking, %			0.22			0.042			0.014			0.30			0.96
Asthma, %	73.3	82.3		72.1	87.9		83.7	70.4		74.3	88.3		73.4	73.8	
Diabetes, %	11.9	19.8		12.9	11.4		9.5	13.9		12.5	20.2		25.5	10.0	0.005
Hypertension, %	9.7	19.9		10.5	10.7		8.9	11.4		10.1	41.7		18.3	8.9	0.048
History of MI or stroke, %	38.8	69.8		40.8	46.5		41.3	41.6		40.3	89.3		53.8	40.2	0.11
	6.6	23.2		7.6	12.0		9.1	7.7		7.3	61.2		20.6	5.7	0.008

Characteristics	CRP			Neutrophil count			Eosinophil count			Serum albumin			Serum vitamin D		
	Normal	High	P	Normal	High	P	<2%	2%	P	Normal	Low	P	Low	Normal	P
FEV1 (L), GM (SE)	<u>2.60</u>	<u>2.00</u>	<u>0.014</u>	2.83	2.53	0.067	<u>2.33</u>	<u>2.64</u>	<u>0.0062</u>	<u>1.25</u>	<u>2.58</u>	<u>0.035</u>	<u>2.28</u>	<u>2.60</u>	<u>0.047</u>
Mortality, %	4.2	27.3	<0.0001	5.2	13.1	0.029	11.3	4.2	0.0078	5.8	36.7	<0.0001	7.8	5.7	0.51

CRP normal if 1 mg/dL and high if >1 mg/dL. neutrophil count normal if 70% or high if >70%. Serum albumin as low if <30 g/dL or normal if 30 g/dL. Serum vitamin D low if <50 nmol/L and normal if 50 nmol/L. CRP, C-reactive protein; BMI, body mass index; PIR, poverty income ratio; FEV, forced expiratory volume; GM, geometric mean; SE, standard error; MI, myocardial infarction.

Table 2

Blood biomarkers levels NHANES 1999–2010 (N = 431)

Biomarkers	Hazard Ratio (95% CI)	P
<u>Increasing CRP^a</u>		
Increasing CRP	4.45 (1.91, 10.37)	0.001
High versus Normal	3.95 (1.27, 12.31)	0.019
<u>Increase in neutrophils</u>		
Increasing neutrophil count	1.07 (1.03, 1.11)	0.002
NLR	1.48 (1.28, 1.72)	<0.001
High versus Normal	2.53 (1.07, 6.00)	0.035
<u>Decrease in eosinophils^a</u>		
Decreasing eosinophil count	7.03 (2.05, 24.01)	0.003
Low versus High	2.54 (1.06, 6.08)	0.037
<u>Decrease in Albumin</u>		
Decreasing albumin concentration	2.49 (0.63, 9.79)	0.183
Low versus Normal	1.67 (0.22, 12.57)	0.604
<u>Decrease in Vitamin D</u>		
Decreasing vitamin D concentration	1.01 (0.99, 1.03)	0.280
Low versus Normal	0.50 (0.18, 1.34)	0.162

Hazard ratios calculated using parametric Weibull regression. Models adjusted for age, gender, race/ethnicity, BMI, smoking, PIR, asthma, diabetes, hypertension, history of stroke or MI. CRP, C-reactive protein.

^aConcentration of biomarker log-transformed to improve normal distribution.

Table 3

C-Statistics for the prediction of mortality in COPD

<i>Model</i>	<i>C-statistic (95% CI)</i>	<i>Effect on initial C-statistic</i>	<i>P-value</i>
<i>Initial model</i>	0.78 (0.71, 0.86)		
<i>+ Log-CRP</i>	0.81 (0.74, 0.88)	+0.017	0.243
<i>+ CRP (Categorical)</i>	0.80 (0.73, 0.88)	+0.016	0.387
<i>+ Log-Eosinophils</i>	0.79 (0.71, 0.87)	+0.007	0.769
<i>+ Eosinophils (Categorical)</i>	0.79 (0.71, 0.86)	+0.008	0.655
<i>+ Neutrophils</i>	0.80 (0.73, 0.88)	+0.021	0.295
<i>+ Neutrophils (Categorical)</i>	0.79 (0.71, 0.86)	+0.006	0.666
<i>+ NLR</i>	0.80 (0.72, 0.88)	+0.019	0.325
<i>+ Albumin</i>	0.78 (0.71, 0.86)	-0.004	0.727
<i>+ Albumin (Categorical)</i>	0.78 (0.71, 0.86)	-0.004	0.455
<i>+ Vitamin D</i>	0.80 (0.73, 0.87)	+0.008	0.478
<i>+ Vitamin D (Categorical)</i>	0.80 (0.73, 0.87)	+0.006	0.668
<i>+ Log-CRP + Log-Eosinophils</i>	<i>0.84 (0.78, 0.90)</i>	<i>+0.059</i>	<i>0.027</i>
<i>+ Log-CRP + Neutrophils</i>	<i>0.83 (0.77, 0.90)</i>	<i>+0.047</i>	<i>0.035</i>
<i>+ Log-CRP + NLR</i>	<i>0.84 (0.78, 0.91)</i>	<i>+0.057</i>	<i>0.030</i>
<i>+ Log-Eosinophils + Neutrophils</i>	0.80 (0.72, 0.88)	+0.015	0.481
<i>+ Log-Eosinophils + NLR</i>	0.80 (0.72, 0.88)	+0.017	0.420
<i>+ Log-CRP + Log-Eosinophils + Neutrophils</i>	<i>0.84 (0.78, 0.91)</i>	<i>+0.060</i>	<i>0.023</i>
<i>+ Log-CRP + Log-Eosinophils + NLR</i>	<i>0.85 (0.78, 0.91)</i>	<i>+0.063</i>	<i>0.019</i>

Initial model included age, gender, race/ethnicity, BMI, smoking, PIR, asthma, diabetes, hypertension, history of stroke or MI. CRP, C-reactive protein; NLR, neutrophils lymphocytes ratio.