Inflammatory Biomarkers Improve Clinical Prediction of Mortality in Chronic Obstructive Pulmonary Disease

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Rationale: Accurate prediction of mortality helps select patients for interventions aimed at improving outcome.

Objectives: Because chronic obstructive pulmonary disease is characterized by low-grade systemic inflammation, we hypothesized that addition of inflammatory biomarkers to established predictive factors will improve accuracy.

Methods: A total of 1,843 patients enrolled in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study were followed for 3 years. Kaplan-Meier curves, log-rank analysis, and Cox proportional hazards analyses determined the predictive value for mortality of clinical variables, while C statistics assessed the added discriminative power offered by addition of biomarkers.

Measurements and Main Results: At recruitment we measured anthropometrics, spirometry, 6-minute walk distance, dyspnea, BODE index, history of hospitalization, comorbidities, and computed tomography scan emphysema. White blood cell and neutrophil counts, serum or plasma levels of fibrinogen, chemokine ligand 18, surfactant protein D, C-reactive protein, Clara cell secretory protein-16, IL-6 and -8, and tumor necrosis factor-α were determined at recruitment and subsequent visits. A total of 168 of the 1,843 patients (9.1%) died. Nonsurvivors were older and had more severe airflow limitation, increased dyspnea, higher BODE score, more emphysema, and higher rates of comorbidities and history of hospitalizations. The best predictive model for mortality using clinical variables included age, BODE, and hospitalization history (C statistic of 0.686; $P < 0.001$). One single biomarker (IL-6) significantly improved the C statistic to 0.708, but this was further improved to 0.726 ($P = 0.003$) by the addition of all biomarkers.

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Several clinical variables are known to be associated with mortality in chronic obstructive pulmonary disease (COPD), and in some cases these have been combined into multidimensional matrices that provide better prediction than the sum of the individual components. The knowledge base on the importance of biomarkers in COPD is expanding, but the role of biomarker levels in predicting mortality is unknown.

What This Study Adds to the Field

This study investigated associations between mortality and levels of a broad range of biomarkers collected in a large cohort of subjects with COPD studied over 3 years. The addition of a panel of selected biomarkers to established clinical measures increases the capacity to predict mortality.

Conclusions: The addition of a panel of selected biomarkers improves the ability of established clinical variables to predict mortality in chronic obstructive pulmonary disease.

Clinical trial registered with www.clinicaltrials.gov (NCT00292552).

Chronic obstructive pulmonary disease (COPD) is currently the fourth highest cause of death in the world, and it is predicted to be the third by the year 2020 (1). Accurate prediction of mortality is important because it helps identify patients in whom the implementation of specific therapeutic measures can improve outcome. Several variables that predict mortality have been identified in COPD, including the severity of airflow limitation as measured by FEV_1 (2), the presence of arterial hypoxemia or hypercapnia (3), exercise performance (4, 5), degree of breathlessness (6), and a low body mass index (BMI) (7). Their integration into multidimensional indices, such as the BODE (BMI, FEV_1, dyspnea, and 6-minute walk distance [6MWD]) (8) and the ADO (age, dyspnea, and FEV_1) (9) for mortality or the DOSE (dyspnea, FEV_1, smoking status, and frequency of exacerbations) (10) for exacerbations, has been shown to predict outcome better than any of the individual variables by themselves.

This likely reflects the fact that COPD is a complex, heterogeneous disease with pulmonary and extrapulmonary manifestations (11, 12) that are not captured by a single variable. So far all predictive variables in COPD, either alone or in combination, are clinical in nature. Because COPD is also a complex and heterogeneous disease at the genetic, cellular, and molecular level, it is likely that the predictive accuracy of clinical measures can be extended with the use of biomarkers that reflect pathobiologic pathways that may be altered in this disease, as has been shown in cardiovascular diseases (13).

It is now recognized that COPD is characterized by low-grade chronic systemic inflammation (14). Several biomarkers, including C-reactive protein (CRP) in some (15) but not all (16) studies, chemokine (C-C motif) ligand 18/pulmonary and activation-regulated chemokine (CCL-18/PARC) (17), IL-6 (18), and surfactant protein-D (SP-D) (19) have been thought to be associated with increased risk of death in patients with respiratory disease. However, all of these biomarkers have been studied singly and no study has evaluated their value compared with accepted clinical predictors of death in patients with COPD. We hypothesized that the addition of a panel of biomarkers to clinical variables known to predict mortality in COPD, such as age, FEV_1, BODE, or hospitalizations because of exacerbations of the disease, will improve the accuracy for predicting the risk of death in patients with COPD. Here, we tested this hypothesis using data prospectively collected in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) study, a 3-year observational study aimed at identifying predictive surrogate endpoints in COPD.

METHODS

Study Design

The study design of ECLIPSE (Clinicaltrials.gov identifier NCT00292552; GSK study code SCO104960) has been published previously (20). Briefly, ECLIPSE is an observational, longitudinal, and controlled study where, after the baseline visit, participants were evaluated at 3 months, 6 months, and then every 6 months for 3 years. In this report we present the longitudinal analysis of mortality using the clinical and biomarker data obtained at baseline. Death was determined up to Day 1,060 of the study. All-cause mortality was used as the outcome; no attempts were made to determine cause of death. ECLIPSE complies with the Declaration of Helsinki and Good Clinical Practice Guidelines, and has been approved by the ethics committees of the participating centers. All participants provided written informed consent before the performance of all study-related assessments.

Patients

ECLIPSE studied 2,164 patients with COPD (Global Initiative for Chronic Obstructive Pulmonary Disease stages II–IV); 337 smoking control subjects; and 245 nonsmoking control subjects. The current analysis includes only the patients with COPD who had full biomarker data. Inclusion criteria were as follows: male and female subjects aged 40–75 years, baseline post-bronchodilator FEV_1 less than 80% of the reference value and FEV_1/FVC of less than or equal to 0.7, and current or ex-smokers with a smoking history of greater than or equal to 10 pack-years. Key exclusion criteria were the presence of a respiratory disorder other than COPD, other significant inflammatory diseases, or a reported COPD exacerbation within 4 weeks of enrollment. Patients with COPD were recruited from the outpatient clinics of the participating centers.

Measurements

Clinical characterization. All methods have been described in the baseline and protocol (20, 21) ECLIPSE manuscripts. In summary, the American Thoracic Society respiratory questionnaire, the modified Medical Research Questionnaire, and the COPD-specific version of the St. George's Respiratory Questionnaire were used to record clinical data. Exacerbations requiring treatment with antibiotics, oral corticosteroids, or hospitalization in the year before the study were also

recorded. Comorbidities were self-reported and registered using the American Thoracic Society–Division of Lung Diseases-78 questionnaire. Nutritional status was assessed by the BMI.

Functional measurements. Spirometry and the 6MWD test were performed according to international guidelines (22). Spirometric reference values were those of the European Community for Coal and Steel (23). The BODE index was calculated as previously reported (8).

Quantification of emphysema by computed tomography scan. All subjects underwent a low-dose computed tomography (CT) scan of the chest using multidetector-row CT scanners (GE Healthcare [Milwaukee, WI] or Siemens Healthcare [Erlangen, Germany]) as described elsewhere (20). All scans were evaluated centrally at the University of British Columbia, Vancouver, Canada. Emphysema was quantified as the percentage of lung CT voxels below a threshold of −950 Hounsfield units using the software Pulmonary Workstation 2.0 (VIDA Diagnostics, Iowa City, IA).

Inflammatory biomarkers. Whole blood was collected by venipuncture into vacutainer tubes. Serum was prepared by allowing the blood to clot for 30 minutes at room temperature followed by centrifugation at $1,500 \times g$ for 10–15 minutes. Plasma (ethylenediaminetetraacetic acid anticoagulant) was obtained by centrifugation of vacutainer tubes at $2,000 \times g$ for 10–15 minutes. Serum and plasma were stored at −80°C until analyzed. CCL-18/PARC, SP-D, IL-8, Clara cell secretory protein 16 (CC-16), and tumor necrosis factor (TNF)-α were measured in serum samples. Fibrinogen and CRP (high-sensitivity method) were measured in plasma samples. All protein biomarkers were measured by validated immunoassays. Total white blood cells (WBC) and neutrophils were counted by automated method. The biomarker performance information is presented in the online supplement (*see* Table E1).

Statistical Analysis

Demographic characteristics have been summarized as mean and SD or percentage, as applicable. Blood biomarkers (excluding WBC and neutrophil counts) have been summarized using median and interquartile range, and have been log transformed and standardized before modeling to conform to the normality assumptions of the underlying models. Survivor and nonsurvivor characteristics were compared using an analysis of variance test for continuous variables and Fisher's exact test for categorical values. Correlations between variables of interest were explored using Spearman Rho.

Kaplan-Meier curves of the individual clinical risk factors and biomarkers analyzed in this cohort are presented. To establish the relationships of biomarkers to death after adjusting for the clinical variables analyzed here (age, BODE, number of previous exacerbations) we used Cox proportional hazards regression. The added discriminative power offered by the addition of biomarkers to clinical variables was analyzed using C statistics according to the method described in Pencina and D'Agostino (24). Differences in C statistics between any two models were estimated using the jackknife estimation method described in Antolini and coworkers (25). In addition, we divided the cohort into two groups. Patients were matched on age and BODE and the C statistic analysis on the subgroups was re-run and the results are shown in the online supplement (*see* Table E2). All tests performed (SAS Version 9.1.3, SAS, Cary, NC) were two-sided tests at the 0.05 level of significance. All *P* values are nominal, as no adjustment was made for multiple comparisons.

Role of the Funding Source

The study was sponsored by GlaxoSmithKline. A Steering Committee and a Scientific Committee comprising 11 academics and 5 representatives of the sponsor developed the original study design and concept, the plan for the current analyses, approved the statistical plan, had full access to the data, and were responsible for decisions with regard to publication. The study sponsor did not place any restrictions with regard to statements made in the final paper.

RESULTS

Clinical Data

The consort diagram of the patients with COPD included in this study is shown in Figure 1. Of the 2,164 patients enrolled in ECLIPSE, complete clinical and biomarker data were available

in 1,843 of them (85.2%), 168 of whom (9.1%) died during follow-up. The clinical characteristics of the patients excluded from the analysis because of incomplete biomarker data were similar in anthropometrics, lung function, walking distance, and degree of CT emphysema but had slightly worse BODE index, St. George's Respiratory Questionnaire scores, and oxygen saturation.

Table 1 compares the baseline clinical and physiologic characteristics of survivors and nonsurvivors. The latter were older, had more severe airflow limitation, reported more dyspnea, had a lower 6MWD, had more emphysema by CT scan, had a higher BODE score, and had more comorbidities. By contrast, sex, smoking status, and BMI were not different between these two groups. Kaplan-Meier analysis showed differences in survival for different age groups, BODE index groups, and the incidence of hospitalization caused by exacerbations of COPD in the year before the study (Figure 2). These three variables were used as the baseline clinical model because the addition of any or all of the other clinical variables failed to improve the model. Age and BODE were entered into the Cox regression model as continuous covariates.

Biomarker Data

The levels of the biomarkers determined in the study were higher in nonsurvivors (Table 1). This was not the case for TNF-α, the levels of which were not significantly different between groups (results not shown). However, most patients had undetectable low levels of TNF-α.

Kaplan-Meier survival analysis confirmed that patients with values that were higher than the median value obtained in the control subjects in ECLIPSE of IL-6, CCL-18/PARC,

fibrinogen, CRP, and SP-D, but not CC-16, were less likely to survive at the end of 3 years ($P < 0.001$ by log-rank test) (Figure 3).

Cox regression analysis (Table 2) showed that, after adjusting for age, previous hospitalizations, and the BODE index, abnormal levels of some (WBC, neutrophils, IL-6, CCL-18/PARC, CRP, IL-8, fibrinogen, and SP-D), but not all (CC-16) biomarkers were independently and significantly associated with mortality.

To evaluate the effect of adding a panel of biomarkers to the baseline clinical model (age, BODE, and previous hospitalizations), only those individual biomarkers that were independently associated with mortality in the Cox regression model adjusted for the clinical variables (Table 2) were considered, with two exceptions. Because the correlation between WBC and neutrophils was extremely high (Rho = 0.92), neutrophils were excluded. Likewise, because of incomplete data for CCL-18/PARC (n = 1569), this marker was also excluded from the main analysis.

Table 3 shows how the addition of biomarkers improves the predictive value of the baseline clinical model using C statistics. The C statistic value of the clinical model alone (age, BODE, and hospitalizations) was 0.686. The individual addition of the biomarkers discussed previously did improve the predictive value (C statistic) of the combined index, but their contribution was relatively small (Table 3), with only IL-6 significantly improving the C statistic on its own. By contrast, when all these biomarkers were added together as a panel, the improvement in predictive value (C statistic = 0.726) was statistically significant. In the subset of 1,579 subjects with all biomarkers including CCL-18/PARC, the results were similar to the main analysis. CCL-18/PARC behaved similarly to most individual biomarkers,
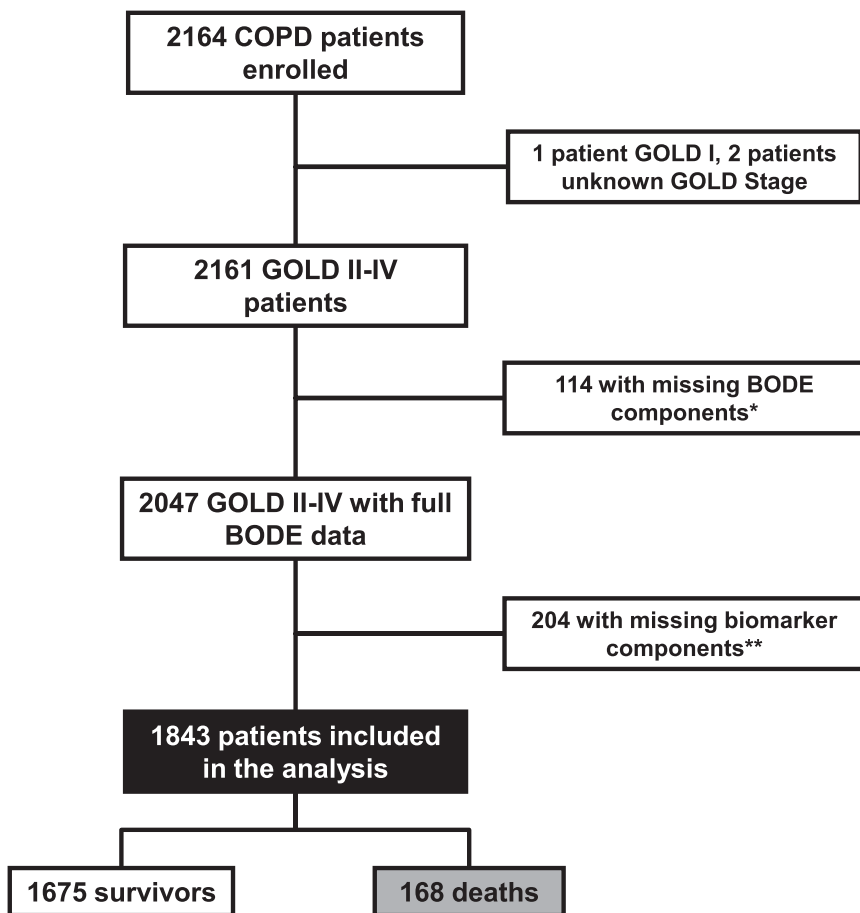
Figure 1. Consort diagram of the patients with chronic obstructive pulmonary disease (COPD) enrolled in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints observational study and participating in this study. *Four patients were missing FEV$_1$ values, 51 patients were missing 6-minute walk distance values, 62 patients were missing modified Medical Research Council values; missing components not mutually exclusive. **Patients were missing individual measures of Clara cell secretory protein-16, hs (high sensitivity) C-reactive protein, fibrinogen, IL-6, IL-8, CCL-18/PARC, surfactant protein D, tumor necrosis factor-α, neutrophils, white blood cells, or combinations thereof. GOLD = Global Initiative for Chronic Obstructive Pulmonary Disease.

TABLE 1. BASELINE CLINICAL CHARACTERISTICS AND BIOMARKERS LEVELS OF THE PATIENTS WITH COPD WHO SURVIVED OR DIED DURING THE 3-YEAR FOLLOW-UP

	Alive at 3 Years* (n = 1,675)	Dead at 3 Years (n = 168)	P Value
Demographics			
Age, yr	63 (7)	66 (7)	<0.001
Females, %	35	30	0.232
Current smoker, %	37	35	0.558
Body mass index, kg/m^2	27 (5)	27 (6)	0.964
Clinical variables			
FEV$_1$, L[†]	1.37 (0.51)	1.14 (0.47)	<0.001
FEV$_1$,%predicted[†]	49 (15)	43 (15)	<0.001
FEV$_1$/FVC, %[†]	45 (11)	42 (12)	0.002
6-min walk distance, m	377 (117)	304 (129)	<0.001
Emphysema LAA, % (−950HU)	18 (12)	20 (12)	0.047
mMRC dyspnea score, 2+, %	51	68	<0.001
SGRQ-C total score	49 (20)	55 (20)	<0.001
BODE Index	3 (2)	4.3 (2.2)	<0.001
Percentage blood oxygen	94.7 (2.9)	93.5 (3.5)	<0.001
Comorbidities			
Hypertension, %	41	45	0.285
Cardivascular history[‡], %	32	49	<0.001
Diabetes	10	19	<0.001
Gastroesophageal reflux	26	21	0.228
Biomarkers			
White blood cells, 10^9/L	7.8 (2.2)	8.7 (3.4)	<0.001
Neutrophil count, 10^9/L	5.1 (1.9)	6 (3.2)	<0.001
IL-6, pg/ml	2 [0.6–4.3]	4.1 [1.3–8.6]	<0.001
Fibrinogen, mg/dl	444 [385–511]	504 [425–575]	<0.001
CRP, mg/L	3.1 [1.5–6.9]	4.6 [2.3–11.8]	<0.001
CCL-18/PARC, ng/ml	105 [81–135]	123 [92–164]	<0.001
SP-D, ng/ml	119 [84–168]	131 [93–188]	0.006
IL-8, pg/ml	6.9 [3.3–12.8]	9.5 [3.3–17]	0.038
CC-16, ng/ml	4.9 [3.4–6.9]	5.3 [3.6–7.7]	0.048

Definition of abbreviations: CC16 = Clara cell secretory protein-16; CCL-18/PARC = chemokine ligand 18/pulmonary and activation-regulated chemokine; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; HU = Hounsfield units; LAA = low-attenuation area; mMRC = modified Medical Research Council; SGRQ-C = St. George's Respiratory Questionnaire, COPD-specific version; SP-D = surfactant protein D.

Continuous data are shown as means (SD) and categorical variables are shown as percentages. Biomarkers data are shown as median [interquartile range].

* Three years defined as survival status on Day 1,060.
[†] Post-bronchodilator values.
[‡] Cardiovascular history as defined with the American Thoracic Society–Division of Lung Diseases 78 Questionnaire.

and contributed as part of the full biomarker panel to an improvement in predictive value (C statistic = 0.697 in the clinical model and 0.742 in the full model), which was also statistically significant. The C statistic values were similar when the cohort was split into two subgroups (*see* Table E2).

DISCUSSION

This prospective study in a large and well-characterized cohort of patients with moderate to very severe COPD provides two important findings: the level of several inflammatory biomarkers determined at recruitment was significantly higher
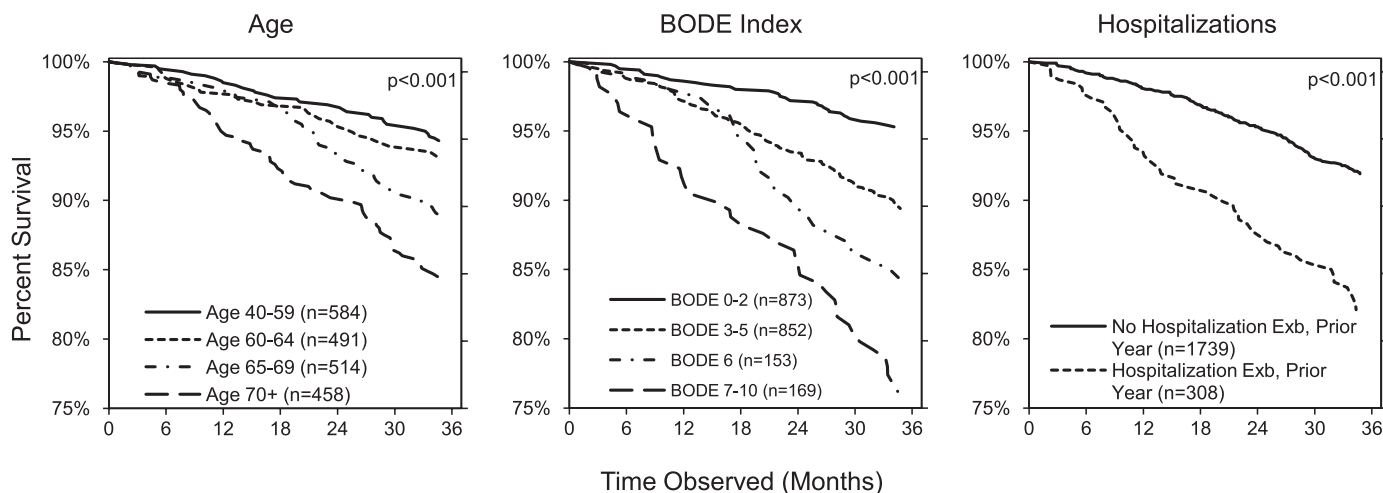
Figure 2. Kaplan-Meier survival curves for the clinical variables analyzed; age by decade; number of hospitalizations caused by an episode of chronic obstructive pulmonary disease exacerbation in the year before recruitment into the study (none vs. more than one); and the BODE index.
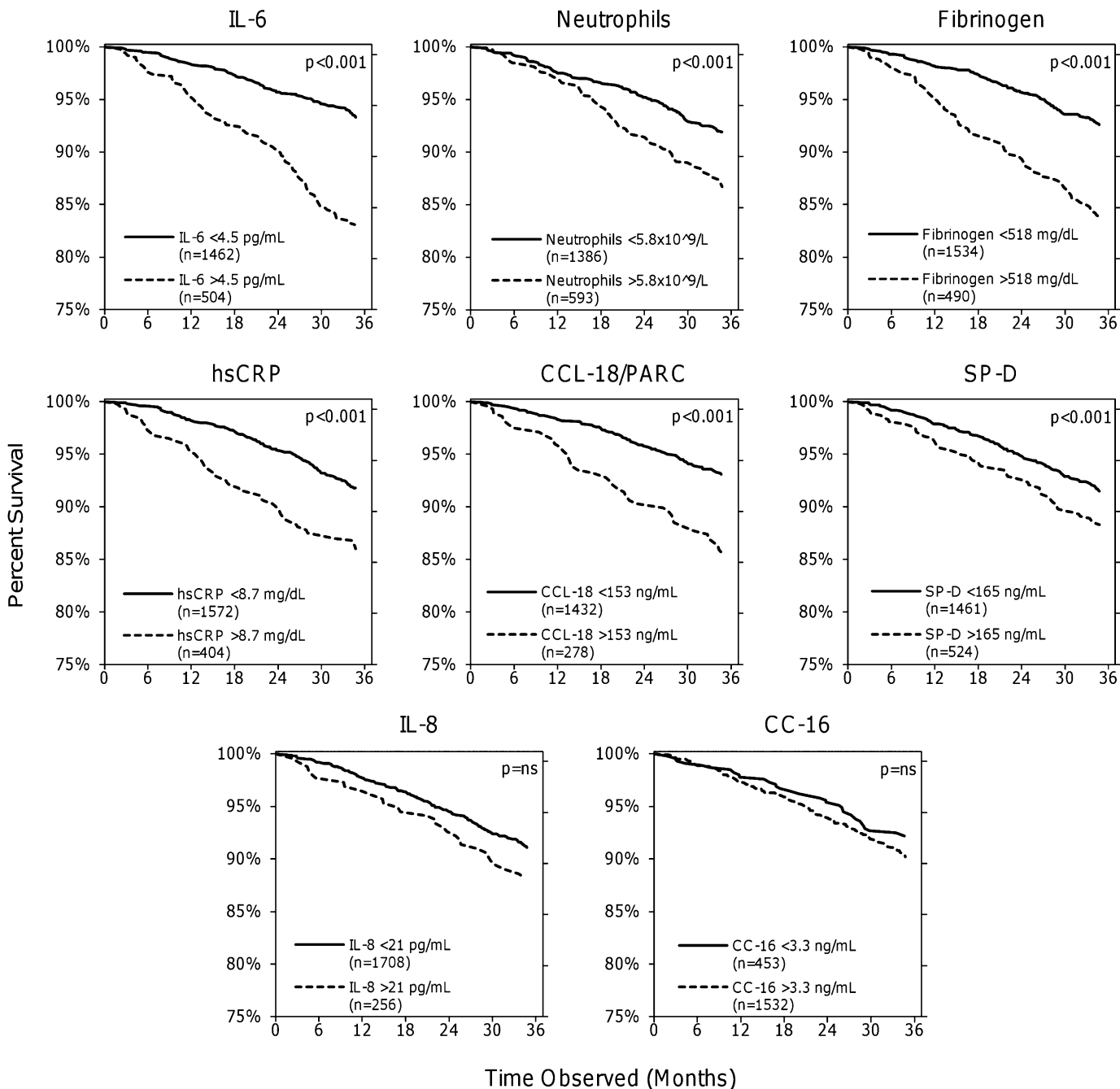
Figure 3. Kaplan-Meier survival curves for the panel of biomarkers analyzed (white blood cell curves are not included in the graph but were very similar to that of neutrophils). Cut-off values correspond to the 95% percentile determined in the nonsmoking control subjects included in the Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study. CC-16 = Clara cell secretory protein-16; CCL-18/PARC = chemokine ligand 18/pulmonary and activation-regulated chemokine; hsCRP = high-sensitivity C-reactive protein; SP-D = surfactant protein D.

in nonsurvivors over the 3 years of the study; and the addition of a selected panel of biomarkers to a model that includes well-established clinical factors improves significantly the risk stratification for all-cause mortality in these patients.

Previous Studies

Over the past few years there has been a growing interest in the field of biomarkers in COPD. Unfortunately, most of the studies have been based on existing databases of patients recruited for pharmacologic trials and/or studies that are cross-sectional in nature. CRP was the first biomarker to be investigated in COPD. Most studies have shown that CRP levels are elevated in these patients, compared with nonsmokers and smokers without airflow obstruction (15, 16, 26–28), but the relationship between CRP levels and mortality remains controversial. Whereas Dahl and coworkers found an association between CRP levels and hospitalization and death in a population study (15), this was not confirmed by DeTorres and coworkers (16). A recent report by Sin and coworkers (17) used data from the Lung Health Study and ECLIPSE and demonstrated an association between CCL-18/PARC and increased risk of death, but whether its

TABLE 2. COX PROPORTIONAL HAZARDS RATIOS FOR DEATH (ADJUSTED FOR AGE, BODE INDEX, AND HOSPITALIZATIONS) FOR THE BIOMARKERS STUDIED

Parameter	Increment	Hazard Ratio	95% Confidence Interval	P Value
IL-6	1 log SD increase	1.47	(1.25–1.72)	<0.001
Neutrophils	1 SD increase	1.26	(1.14–1.40)	<0.001
White blood cells	1 SD increase	1.26	(1.13–1.42)	<0.001
Fibrinogen	1 log SD increase	1.33	(1.13–1.56)	<0.001
CRP	1 log SD increase	1.27	(1.09–1.48)	0.002
CCL-18/PARC*	1 log SD increase	1.3	(1.08–1.56)	0.005
SP-D	1 log SD increase	1.22	(1.04–1.43)	0.016
IL-8	1 log SD increase	1.17	(1.00–1.36)	0.045
CC-16	1 log SD increase	1.04	(0.88–1.23)	0.674

Definition of abbreviations: CC-16 = Clara cell secretory protein-16; CCL-18/PARC = chemokine ligand 18/pulmonary and activation-regulated chemokine; CRP = C-reactive protein; SP-D = surfactant protein D.
*Calculated from n = 1,569; all other values calculated from entire biomarker population (n = 1,843).

addition yielded any prognostic value to clinical variables already known to predict outcome was not investigated. Other candidate biomarkers studied in COPD include circulating levels of CC-16 (29) and SP-D (30). The former, a marker of Clara cell toxicity, appears reduced in patients with COPD and its levels were associated with rate of decline of FEV_1 in the same ECLIPSE cohort (31). The lung-derived protein SP-D, however, is associated with presence of pulmonary inflammation and is elevated in smokers (with or without COPD). None of these studies evaluated the relationship between the levels of these biomarkers and survival in stable patients with COPD and whether they add value to accepted predictors of survival. Using a protein microarray platform, Pinto-Plata and coworkers (32) identified a panel of 24 markers of inflammation, tissue destruction, and repair that were significantly related to lung function, exercise capacity, the BODE index, and exacerbation frequency. However, because the study was cross-sectional, the proteomic profile could not be related to mortality. By contrast, Man and coworkers (33) analyzed data from the Lung Health Study and reported an association between a high ratio of CRP (inflammatory marker) to fibronectin (repair marker) with mortality. Yet, this study used serum collected midway through an interventional study, included patients primarily with mild COPD, and had a very low mortality rate. To our knowledge, our study is the first to investigate if the addition of biomarker levels to well-established clinical predictors of outcome adds relevant prognostic information.

Interpretation of Findings

Our results show that a panel of selected biomarkers (WBC counts, IL-6, fibrinogen, CCL-18/PARC, CRP, IL-8, and SP-D) were not only elevated in nonsurvivors compared with survivors (Table 1), but were also associated with mortality over 3 years (Figure 3) after adjusting for clinical variables known to predict death in COPD (Table 2), whereas this was not the case for other biomarkers previously thought to be potential predictors of outcome in COPD, such as TNF-α or CC-16. That WBC and neutrophil counts, IL-6, IL-8, fibrinogen, CCL-18/PARC, CRP, and SP-D do add independent predictive information is further supported by the results of the Cox proportional analyses (Table 2) and the use of C statistic (Table 3). Using C statistics, only IL-6 independently added predictive power to the basic clinical model, whereas the other biomarkers individually improved the model only marginally. We determined WBC and neutrophils and the correlation between them is extremely high (Spearman rho = 0.92), these two values are essentially interchangeable, and no value is added by combining the two measures together. However, the addition of all the biomarkers in the panel increased the C statistic significantly suggesting that the use of integrative analyses describes

TABLE 3. C STATISTIC VALUE FOR THE PREDICTION OF DEATH

Model	C Statistic	Difference from Base	95% Confidence Interval for Difference from Base Model	P Value Versus Reference
Age + BODE + COPD Hosp	0.686			
+ IL-6	0.708	0.023	(0.003 to 0.043)	0.027
+ Neutrophils	0.699	0.013	(−0.001 to 0.028)	0.078
+ White blood cells	0.698	0.012	(−0.003 to 0.028)	0.119
+ CRP	0.697	0.012	(−0.005 to 0.028)	0.168
+ Fibrinogen	0.698	0.012	(−0.007 to 0.031)	0.207
+ SP-D	0.692	0.006	(−0.006 to 0.018)	0.309
+ IL-8	0.690	0.005	(−0.005 to 0.013)	0.371
+ All biomarkers	0.726	0.041	(0.014 to 0.067)	0.003
Sensitivity Model (n = 1,579)				
Age + BODE + COPD Hosp	0.697			
+ CCL-18/PARC	0.706	0.009	(−0.008 to 0.026)	0.294
+ All biomarkers	0.742	0.045	(0.010 to 0.079)	0.011

Definition of abbreviations: CCL-18/PARC = chemokine ligand 18/pulmonary and activation-regulated chemokine; COPD = chronic obstructive pulmonary disease; CRP = C-reactive protein; SP-D = surfactant protein D.
Biomarkers included here correspond to those identified by the Cox proportional hazards ratios analysis (adjusted for clinical variables) as significantly associated with the risk of death (white blood cells, neutrophils, IL-6, IL-8, fibrinogen, CRP, and SP-D) (Table 2).
The base model value represents that obtained by the use of age and BODE index. For further explanations, *see* text.

better the complexity of COPD. It is impossible to determine the proportion or number of patients whose abnormal biomarker expression would identify increased risk of death above those detected using clinical variables because the magnitude of increase provided by the C statistic has not been related to precise clinical metrics. However, the magnitude of the additional predictive power for mortality provided by the biomarkers in this study is similar to that described recently in patients with cardiovascular disease using different biomarkers (13).

Strengths and Limitations

The large sample size and multicenter nature of the cohort studied, its careful clinical, radiologic, functional, and biologic characterization, and its prospective design and long follow-up time are clear strengths of this study. However, several potential limitations deserve comment. First, there was no adjudication committee to specify the correct cause of death. The Toward a Revolution in COPD Health (34) and Understanding Potential Long-Term Impacts on Function with Tiotropium (35) studies showed that the cause of death can be attributed wrongly if it relies exclusively on the death certificate. However, for the healthcare provider, it is still important to be able to predict all-cause mortality risk and evaluate the potentially modifiable factors to help guide individual patient management and therapeutic approaches. Second, the panel of biomarkers selected did not include some that have been thought to be important in the pathobiology of COPD, such as the metalloproteinases and growth factors (36). This does not negate the value of our findings, and actually provides room to improve accuracy if in due time these other biomarkers also are shown to relate to poor outcome. Third, the same could be said about the number of comorbidities and their relationship to a poor outcome. This also provides room for future studies aimed at discerning the influence of comorbidity on biomarker levels and their relationship to the risk of death. Fourth, it could be argued that there was no derivative and validating cohort. However, this is customary for nonvalidated biomarkers, whereas in this study we compared validated clinical and serum biomarkers modeled on studies in the cardiovascular arena. Fifth, although the C statistic is the method most commonly used to assess model discrimination, its use for the evaluation of biomarkers as risk predictors has been questioned. This is because significant increases in the C statistic require very large independent associations of the marker with the outcome of interest. Thus, the significant increments in the C statistic observed in the present study (Table 3) indicate that a multimarker approach represents a substantial improvement in the performance of the model. The fact that the Cox analysis and the C statistics agreed in the added predictive value of the biomarkers provides strong support to this approach. The panel of clinical predictors studied here includes age; the BODE index (that in turn considers BMI, FEV_1, dyspnea, and exercise tolerance); and hospitalizations. These variables cover most but not all clinical risk factors thus far identified. For instance, we did not include in the analysis arterial blood gases, pulmonary hemodynamics, or heart function, because they were not determined in ECLIPSE. Yet, the clinical variables included in the model are readily available to most practicing physicians.

Conclusions

The addition of WBC counts and the systemic levels of IL-6, CRP, IL-8, fibrinogen, CCL-18/PARC, and SP-D improve significantly the ability of clinical variables to predict mortality in patients with COPD.

References

1. Mannino DM, Buist AS. Global burden of COPD: risk factors, prevalence, and future trends. *Lancet* 2007;370:765–773.
2. Anthonisen NR, Wright EC, Hodgkin JE. Prognosis in chronic obstructive pulmonary disease. *Am Rev Respir Dis* 1986;133:14–20.
3. Nocturnal Oxygen Therapy Trial Group. Continuous or nocturnal oxygen therapy in hypoxemic chronic obstructive lung disease: a clinical trial. *Ann Intern Med* 1980;93:391–398.
4. Cote CG, Casanova C, Marin JM, Lopez MV, Pinto-Plata V, de Oca MM, Dordelly LJ, Nekach H, Celli BR. Validation and comparison of reference equations for the 6-min walk distance test. *Eur Respir J* 2008;31:571–578.
5. Oga T, Nishimura K, Tsukino M, Sato S, Hajiro T. Analysis of the factors related to mortality in chronic obstructive pulmonary disease: role of exercise capacity and health status. *Am J Respir Crit Care Med* 2003;167:544–549.
6. Nishimura K, Izumi T, Tsukino M, Oga T. Dyspnea is a better predictor of 5-year survival than airway obstruction in patients with COPD. *Chest* 2002;121:1434–1440.
7. Schols AM, Broekhuizen R, Weling-Scheepers CA, Wouters EF. Body composition and mortality in chronic obstructive pulmonary disease. *Am J Clin Nutr* 2005;82:53–59.
8. Celli BR, Cote CG, Marin JM, Casanova C, Montes de Oca M, Mendez RA, Pinto Plata V, Cabral HJ. The body-mass index, airflow obstruction, dyspnea, and exercise capacity index in chronic obstructive pulmonary disease. *N Engl J Med* 2004;350:1005–1012.
9. Puhan MA, Garcia-Aymerich J, Frey M, ter Riet G, Antó JM, Agustí AG, Gómez FP, Rodríguez-Roisín R, Moons KG, Kessels AG, *et al.* Expansion of the prognostic assessment of patients with chronic obstructive pulmonary disease: the updated BODE index and the ADO index. *Lancet* 2009;374:704–711.
10. Jones RC, Donaldson GC, Chavannes NH, Kida K, Dickson-Spillmann M, Harding S, Wedzicha JA, Price D, Hyland ME. Derivation and validation of a composite index of severity in chronic obstructive pulmonary disease: the DOSE Index. *Am J Respir Crit Care Med* 2009;180:1189–1195.
11. Rabe KF, Hurd S, Anzueto A, Barnes PJ, Buist SA, Calverley P, Fukuchi Y, Jenkins C, Rodriguez-Roisin R, van Weel C, *et al.*; Global Initiative for Chronic Obstructive Lung Disease. Global strategy for the diagnosis, management, and prevention of chronic obstructive pulmonary disease: GOLD executive summary. *Am J Respir Crit Care Med* 2007;176:532–555.
12. Celli BR, MacNee W. Standards for the diagnosis and treatment of patients with COPD: a summary of the ATS/ERS position paper. *Eur Respir J* 2004;23:932–946.
13. Zethelius B, Berglund L, Sundstrom J, Ingelsson E, Basu S, Larsson A, Venge P, Arnlöv J. Use of multiple biomarkers to improve the prediction of death from cardiovascular causes. *N Engl J Med* 2008;358:2107–2116.
14. Barnes PJ. Small airways in COPD. *N Engl J Med* 2004;350:2635–2637.
15. Dahl M, Vestbo J, Lange P, Bojesen SE, Tybjaerg-Hansen A, Nordestgaard BG. C-reactive protein as a predictor of prognosis in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2007;175:250–255.
16. de Torres JP, Pinto-Plata V, Casanova C, Mullerova H, Córdoba-Lanús E, Muros de Fuentes M, Aguirre-Jaime A, Celli BR. C-reactive protein levels and survival in patients with moderate to very severe COPD. *Chest* 2008;133:1336–1343.
17. Sin DD, Miller B, Duvoix A, Man SF, Zhang X, Silverman EK, Connett JE, Anthonisen NA, Wise RA, Tashkin D, *et al.* ECLIPSE Investigators. Serum PARC/CCL-18 concentrations and health outcomes in chronic obstructive pulmonary disease. *Am J Respir Crit Care Med* 2011;183:1187–1192.
18. Mehrotra N, Freire AX, Bauer DC, Harris TB, Newman AB, Kritchevsky SB, Meibohm B, Health ABC. Study. Predictors of mortality in elderly subjects with obstructive airway disease: the PILE score. *Ann Epidemiol* 2010;20:223–232.
19. Calfee CS, Ware LB, Glidden DV, Eisner MD, Parsons PE, Thompson BT, Matthay MA; National Heart, Blood, and Lung Institute Acute

Respiratory Distress Syndrome Network. Use of risk reclassification with multiple biomarkers improves mortality prediction in acute lung injury. *Crit Care Med* 2011;39:711–717.

20. Vestbo J, Anderson W, Coxson HO, Crim C, Dawber F, Edwards L, Hagan G, Knobil K, Lomas DA, MacNee W, *et al.*; ECLIPSE investigators. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE). *Eur Respir J* 2008;31:869–873.

21. Agusti A, Calverley PM, Celli B, Coxson HO, Edwards LD, Lomas DA, MacNee W, Miller BE, Rennard S, Silverman EK, *et al.*; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) investigators. Characterisation of COPD heterogeneity in the ECLIPSE cohort. *Respir Res* 2010;11:122.

22. Brusasco V, Crapo R, Viegi G. Coming together: the ATS/ERS consensus on clinical pulmonary function testing. *Eur Respir J* 2005;26:1–2.

23. Roca J, Burgos F, Sunyer J, Saez M, Chinn S, Antó JM, Rodríguez-Roisin R, Quanjer PH, Nowak D, Burney P. References values for forced spirometry. Group of the European Community Respiratory Health Survey. *Eur Respir J* 1998;11:1354–1362.

24. Pencina MJ, D'Agostino RB. Overall C as a measure of discrimination in survival analysis: model specific population value and confidence interval estimation. *Stat Med* 2004;23:2109–2123.

25. Antolini L, Boracchi P, Biganzoli E. A time-dependent discrimination index for survival data. *Stat Med* 2005;24:3927–3944.

26. Broekhuizen R, Wouters EF, Creutzberg EC, Schols AM. Raised CRP levels mark metabolic and functional impairment in advanced COPD. *Thorax* 2006;61:17–22.

27. 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;61:849–853.

28. Pinto-Plata VM, Mullerova H, Toso JF, Feudjo-Tepie M, Soriano JB, Vessey RS, Celli BR. C-reactive protein in patients with COPD, control smokers and non-smokers. *Thorax* 2006;61:23–28.

29. Lomas DA, Silverman EK, Edwards LD, Miller BE, Coxson HO, Tal-Singer R; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints (ECLIPSE) investigators. Evaluation of serum CC-16 as a biomarker for COPD in the ECLIPSE cohort. *Thorax* 2008;63:1058–1063.

30. Lomas DA, Silverman EK, Edwards LD, Locantore NW, Miller BE, Horstman DH, Tal-Singer R; Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints study investigators. Serum surfactant protein D is steroid sensitive and associated with exacerbations of COPD. *Eur Respir J* 2009;34:95–102.

31. Vestbo J, Edwards LD, Scanlon PD, Yates JC, Agusti A, Bakke P, Calverley PMA, Celli B, Coxson HO, Crim C, *et al.*, for the ECLIPSE Investigators. Changes in forced expiratory volume in 1 second over time in COPD. *N Engl J Med* 2011;365:1184–1192.

32. Pinto-Plata V, Toso J, Lee K, Park D, Bilello J, Mullerova H, De Souza MM, Vessey R, Celli B. Profiling serum biomarkers in patients with COPD: associations with clinical parameters. *Thorax* 2007;62:595–601.

33. Man SF, Xing L, Connett JE, Anthonisen NR, Wise RA, Tashkin DP, Zhang X, Vessey R, Walker TG, Celli BR, *et al.* Circulating fibronectin to C-reactive protein ratio and mortality: a biomarker in COPD? *Eur Respir J* 2008;32:1451–1457.

34. Calverley PM, Anderson JA, Celli B, Ferguson GT, Jenkins C, Jones PW, Yates JC, Vestbo J; TORCH investigators. Salmeterol and fluticasone propionate and survival in chronic obstructive pulmonary disease. *N Engl J Med* 2007;356:775–789.

35. Tashkin DP, Celli B, Senn S, Burkhart D, Kesten S, Menjoge S, Decramer M; UPLIFT Study Investigators. A 4-year trial of tiotropium in chronic obstructive pulmonary disease. *N Engl J Med* 2008;359:1543–1554.

36. Barnes PJ. Emerging pharmacotherapies for COPD. *Chest* 2008;134:1278–1286.