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Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease

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Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease

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Author contributions

All authors conceived and designed the study and /or analyzed the data. All authors contributed to, and approved the final draft of the manuscript. BEC, PB, PC, HC, DAL, WM, SR, ES, JV, EW and AA collected study data. NL, JY, BEM and LDE conducted statistical analyses.

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Running Head

Mortality prediction with biomarkers in COPD

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At a glance commentary

Scientific knowledge on the subject: A number of clinical variables are known to be associated with mortality in COPD, and in some cases these have been combined into multi-dimensional matrices that provide better prediction than the sum of the individual components. The knowledge base on the importance of biomarkers in COPD is rapidly expanding, but the role of biomarker levels in predicting mortality above and beyond accepted clinical variables is currently 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 COPD subjects studied over three years. The addition of changes in a panel of biomarkers to established clinical measures increases the capacity to predict mortality.

Abstract

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 1843 patients enrolled in the ECLIPSE 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 At recruitment we measured anthropometrics, spirometry, 6 minute walk distance, dyspnea, BODE index, history of hospitalization, co-morbidities and CT 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, Interleukin-6 and -8 and tumor necrosis factor alpha were determined at recruitment and subsequent visits..

Main Results 168 of the 1843 patients (9·1 %) died. Non-survivors were older, had more severe airflow limitation, increased dyspnea, higher BODE score, more emphysema, and higher rates of co-morbidities 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 (Interleukin-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.

Conclusions The addition of a panel of selected biomarkers improves the ability of established clinical variables to predict mortality in COPD.

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Introduction

Chronic obstructive pulmonary disease (COPD) is currently the 4th highest cause of death in the world, and it is predicted to be the 3rd 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 the forced expiratory volume in one second (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 multi-dimensional 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 have 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 extra-pulmonary 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 pathobiological 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 **(**CCL-18/PARC)(17) , interleukin-6 (18), and surfactant protein-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 to 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 due to 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 three 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 1060 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 prior to the performance of all study related assessments.

Patients

ECLIPSE studied 2164 patients with COPD (GOLD stage II to IV), 337 smoking controls and 245 non-smoking controls. The current analysis includes only the patients with COPD who had full biomarker data. Inclusion criteria were as follows: male/female subjects aged 40 to 75 years, baseline post-bronchodilator FEV_1 <80% of the reference value and FEV_1/FVC (Forced Vital Capacity) of ≤0·7; and, current or ex-smokers with a smoking history of ≥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. COPD patients 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 (ATS) respiratory questionnaire, the modified Medical Research Questionnaire (mMRC) and the COPD-specific version of the St. George's Respiratory Questionnaire (SGRQ-C) were used to record clinical data. Exacerbations requiring treatment with antibiotics, oral corticosteroids, and/or hospitalization in the year prior to the study were also recorded. Co-morbidities were self-reported and registered using the ATS-DLD-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 CT Scan

All subjects underwent a low-dose computed tomography (CT) scan of the chest using multi-detector-row CT scanners (GE Healthcare or Siemens Healthcare) 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, USA).

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 1500 g for 10 to 15 minutes. Plasma (EDTA anticoagulant) was obtained by centrifugation of vacutainer tubes at 2000g for 10 to 15 minutes. Serum and plasma were stored at –80°C until analyzed. CCL-18 (PARC), surfactant protein D (SP-D), interleukin 8 (IL-8), Clara cell secretory protein 16 (CC-16), and tumor necrosis factor alpha (TNFα) 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 table 1 of the online supplement.

Statistical analysis

Demographic characteristics have been summarized as mean and standard deviation (SD) or percentage, as applicable. Blood biomarkers (excluding WBC and neutrophil counts) have been summarized using median and inter-quartile range (IQR), and have been log transformed and standardized prior to modeling to conform to the normality assumptions of the underlying models. Survivor and non-survivor 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's 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 et al.(24) Differences in C-statistics between any two models were estimated using the jackknife estimation method described in Antolini et al.(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 table 2 of the online supplement. All tests performed (SAS Version 9·1·3) 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 in total eleven academics and five 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 COPD patients included in this study is shown in figure 1. Of the 2164 patients enrolled in ECLIPSE, complete clinical and biomarker data were available in 1843 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, degree of CT emphysema but had slightly worse BODE index, SGRQ scores and oxygen saturation.

Table 1 compares the baseline clinical and physiological characteristics of survivors and non-survivors. The latter were older, had more severe airflow limitation, reported more dyspnea, had a lower 6MWD, had more emphysema by CT scan, a higher BODE score and more co-morbidities. By contrast, gender, 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 due to exacerbations of COPD in the year prior to 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 non-survivors (table 1). This was not the case for TNFα, the levels of which were not significantly different between groups (results not shown). However, most patients had undetectable low levels of TNFα.

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 three years ($p<0{\cdot}001$ 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, 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. As the correlation between WBC and neutrophils was extremely high (Rho=0.92), neutrophils were excluded. Likewise, due to incomplete data for CCL-18 (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 above 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 N=1579 subjects with all biomarkers including CCL-18, the results were similar to the main analysis. CCL-18 behaved similarly to most individual biomarkers, 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 (Table 2 of the online supplement).

Discussion

This prospective study in a large and well characterized cohort of patients with moderate to very severe COPD provides two important findings: *(1)* the level of several inflammatory biomarkers determined at recruitment was significantly higher in non-survivors over the three years of the study; and, *(2)* 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 pharmacological 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, as compared with non-smokers and smokers without airflow obstruction (15, 16, 26-28), but the relationship between CRP levels and mortality remains controversial. Whereas Dahl et al found an association between CRP levels and hospitalization and death in a population study,(15) this was not confirmed by DeTorres and co-workers(16). A recent report by Sin et al used data from the Lung Health Study and ECLIPSE, and demonstrated an association between CCL-18/PARC and increased risk of death,(17) but whether its 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 on the other hand 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 et al 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.(32) However, because the study was cross-sectional, the proteomic profile could not be related to mortality. By contrast, Man et al 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.(33) 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, CRP, IL-8, and SP-D) were not only elevated in non-survivors compared with survivors (table 1), but were also associated with mortality over three 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 TNFα or CC-16. That WBC and neutrophil counts, IL-6, IL-8, fibrinogen, CCL-18, 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 both 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. On the other hand, the addition of all the biomarkers in the panel increased the C statistic significantly suggesting that the use of integrative analyses describes 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 multi-center nature of the cohort studied, its careful clinical, radiological, functional and biological 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 TOwards a Revolution in COPD Health (TORCH) (34) and Understanding Potential Long-Term Impacts on Function with Tiotropium (UPLIFT) (35) studies showed that the cause of death can be attributed wrongly if it relies exclusively on the death certificate. However, for the health care 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. Secondly, 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 co-morbidities and their relationship to a poor outcome. This also provides room for future studies aimed at discerning the influence of co-morbidity 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 non-validated 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 utility 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 include 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 identified so far. 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 white blood cell counts and the systemic levels of IL-6, CRP, IL-8, fibrinogen, CCL-18, and SP-D improve significantly the ability of clinical variables to predict mortality in patients with COPD.

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Conflicts of interest

BRC: has received consulting fees from Altana, AstraZeneca, Boehringer-Ingelheim and GlaxoSmithKline; speaking fees from Altana, AstraZeneca, Boehringer-Ingelheim and GlaxoSmithKline; and grant support from Boehringer-Ingelheim and GlaxoSmithKline.

NL, JY, RT-S, BEM, CC and LDE*:* are full time employees of GlaxoSmithKline and hold stock or stock options in GlaxoSmithKline.

PB: has received lecture fees from AstraZeneca, GlaxoSmithKline and NycoMed; has participated in clinical research studies sponsored by GlaxoSmithKline, Pfizer and Boehringer-Ingelheim; is currently member of the Steering Committee and the Scientific Committee of the ECLIPSE study which is sponsored by GlaxoSmithKline

PC: has received fees for serving on advisory boards for GlaxoSmithKline, AstraZeneca, Nycomed, Novartis and Boehringer Ingelheim, for expert testimony for Forest/Nycomed, and has received speaker fees from GlaxoSmithKline and Nycomed; has received travel assistance from GlaxoSmithKline to attend ECLIPSE study meetings and from Boehringer Ingelheim to attend a scientific conference.

HC: has received an honorarium for serving on the steering committee for the ECLIPSE project for GlaxoSmithKline; was the co-investigator on two multi-center studies sponsored by GlaxoSmithKline and has received travel expenses to attend meetings

related to the project; has three contract service agreements with GlaxoSmithKline to quantify the CT scans in subjects with COPD and a service agreement with Spiration Inc to measure changes in lung volume in subjects with severe emphysema; was the co-investigator (D Sin PI) on a Canadian Institutes of Health – Industry (Wyeth) partnership grant; has received a fee for speaking at a conference and related travel expenses from AstraZeneca (Australia); was the recipient of a GSK Clinical Scientist Award (06/2010-07/2011).

DAL: has received grant support, honoraria and consultancy fees from GlaxoSmithKline.

AD: has no conflict of interest.

WM: has received travel assistance from GlaxoSmithKline to attend ECLIPSE study meetings.

SR: has received fees for serving on advisory boards, consulting or honoraria from Almirall, APT Pharma, Aradigm, Argenta, AstraZeneca, Boehringer Ingelheim, Chiesi, Dey, Forest, GlaxoSmitkKlein, HoffmanLaRoche, MedImmune, Mpex, Novartis, Nycomed, Oriel, Otsuka, Pearl, Pfizer, Pharmaxis, Merck and Talecris.

ES: has received an honorarium for a talk on COPD genetics, grant support for two studies of COPD genetics, and consulting fees from GlaxoSmithKline; honoraria for talks and consulting fees from AstraZeneca.

JV: has received fees for serving on advisory boards for GlaxoSmithKline, AstraZeneca, Nycomed and Boehringer Ingelheim, and has received speaker fees from GlaxoSmithKline, AstraZeneca, Pfizer, Boehringer-Ingelheim, Chiesi, Novartis and

Nycomed; has received travel assistance from GlaxoSmithKline to attend ECLIPSE study meetings; his wife has previously worked in pharmaceutical companies, including GSK and AstraZeneca.

EW: serves on an advisory board for Nycomed; has received lecture fees from GlaxoSmithKline, AstraZeneca and Novartis, and has received research grants from GlaxoSmithKline and AstraZeneca.

AA: has received travel assistance from GlaxoSmithKline to attend ECLIPSE study meetings and honorarium for speaking at conferences and participating in advisory boards from Almirall, Astra-Zeneca, Boheringer-Ingelheim, Chiesi, Esteve, GSK, Medimmune, Novartis, Nycomed, Pfizer, Roche and Procter & Gamble.

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606 ***Table 1***: Baseline clinical characteristics and biomarkers levels of the COPD
607 patients who survived or died during the 3 year follow up
608

	Alive at 3 years* (n=1675)	Dead at 3 years (n=168)	p value
Demographics			
Age, years	63 (7)	66 (7)	<0·001
Females (%)	35	30	0·232
Current smoker (%)	37	35	0·558
BMI (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-minute 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	3·0 (2·0)	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)	6·0 (3·2)	<0·001
IL-6 (pg/mL)	2·0 [(0·6 to 4·3]	4·1 [1·3- 8·6]	<0·001
Fibrinogen (mg/dL)	444 [385 to 511]	504 [425- 575]	<0·001
CRP (mg/L)	3·1 [1·5 to 6·9]	4·6 [2·3- 11·8]	<0·001
CCL-18 (ng/mL)	105 [81 to 135]	123 [92- 164]	<0·001
SP-D (ng/mL)	119 [84 to 168]	131 [93- 188]	0·006
IL-8 (pg/mL)	6.9 [3·3 to 12.8]	9.5 [3·3- 17·0]	0·038
CC-16 (ng/mL)	4.9 [3·4 to 6·9]	5.3 [3·6- 7·7]	0·048

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

*3 years defined as survival status on Day 1060; ** Cardiovascular history as defined with the ATS-DLD 78 †Post-bronchodilator values. CC16=Clara cell secretory protein-16; CCL-18= Chemokine ligand 18; CRP=C-reactive protein; FEV_1=forced expiratory volume in one second; FVC=forced vital capacity; HU=Hounsfield Units; IL-6=Interleukin-6; IL-8=Interleukin-8; LAA=low attenuation area; mMRC=modified Medical Research Council; SGRQ-C=St. George's Respiratory Questionnaire, COPD-specific version; SP-D=surfactant protein D.

Table 2: Cox proportional hazard ratios for death (adjusted for age, BODE index and hospitalizations) for the biomarkers studied

Parameter	Increment	Hazard ratio	95% CI	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

*Calculated from N=1569, all other values calculated from entire biomarker population (N=1843). CC-16=Clara cell secretory protein-16; CCL-18 (PARC)=Chemokine ligand 18; CRP=C-reactive protein; IL-6=Interleukin-6; IL-8=Interleukin-8; SP-D=surfactant protein D;

634 ***Table 3***: C statistic value for the prediction of death. The base model value represents
635 that obtained by the use of age and BODE. For further explanations, see text

Model	C statistic	Difference from base	95%CI for difference from base model	p value v reference
Age + BODE + COPD Hosp	0·686			
+ IL-6	0·708	0·023	(0·003, 0·043)	0·027
+ Neutrophils	0·699	0·013	(–0·001, 0·028)	0·078
+ White blood cells	0·698	0·012	(–0·003, 0·028)	0·119
+ CRP	0·697	0·012	(–0·005, 0·028)	0·168
+ Fibrinogen	0·698	0·012	(–0·007, 0·031)	0·207
+ SP-D	0·692	0·006	(–0·006, 0·018)	0·309
+ IL-8	0·690	0·005	(–0·005, 0·013)	0·371
+ All biomarkers	0·726	0·041	(0·014, 0·067)	0·003
Sensitivity model (N=1579)				
Age + BODE + COPD Hosp	0·697			
+ CCL-18 (PARC)	0·706	0.009	(-0·008, 0·026)	0.294
+ All biomarkers	0·742	0·045	(0·010, 0·079)	0.011

636 CRP=C-reactive protein; IL-6=Interleukin-6; IL-8=Interleukin-8; SP-D=surfactant protein
637 D. Biomarkers included here correspond to those identified by the Cox proportional
638 hazard ratios analysis (adjusted for clinical variables) as significantly associated with the
639 risk of death (WBC, neutrophils, IL-6, IL-8, fibrinogen, CRP, SP-D) (table 2).
640
641
642

FIGURE LEGENDS

***Figure 1*: Consort diagram of the patients with COPD enrolled in the ECLIPSE observational study and participating in this study**

***Figure 2*: Kaplan-Meier survival curves for the clinical variables analyzed, age by decade, number of hospitalizations due to an episode of COPD exacerbation in the year prior to recruitment into the study (none *vs* more than 1) and the BODE index**

***Figure 3*: Kaplan-Meier survival curves for the panel of biomarkers analyzed (WBC 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 non-smoking controls included in ECLIPSE**

Inflammatory biomarkers improve clinical prediction of mortality in chronic obstructive pulmonary disease

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Online supplement.

The biomarker assay performance is summarized in a tabluar form on table 1S. The abbreviations are as follows:

[1]LLOQ, lower limit of quantification at the minimum required dilution for sample testing

[2]RE, relative error

[3]CV, coefficient of variation

n.d.: not done

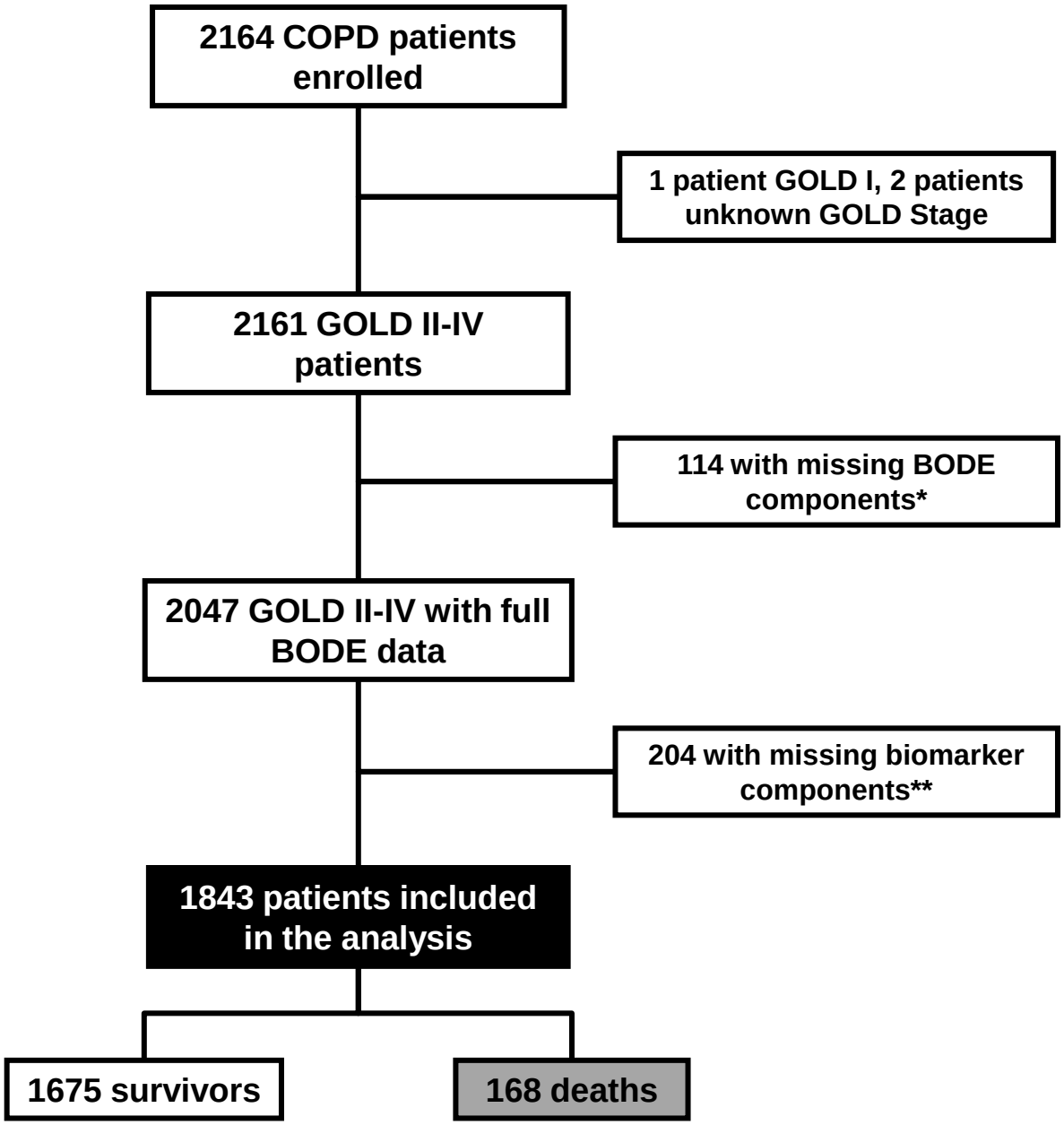
Table 1 S. Biomarker Assay Performance Information

		Mean Intra-Assay Variability		Mean Inter- Assay Variability	
Analyte	**LLOQ[1]**	**%RE[2]**	**%CV[3]**	**%RE**	**%CV**
CRP	0.1 ug/mL	-0.3	3.9	n.d.	3.8
CC-16	80 pg/mL	-6.5	3.0	-6.33	4.2
CCL18	1.0 ng/mL	6.0	10.9	5.0	6.8
Fibrinogen	5.4 mg/dL	2.4	1.2	n.d.	1.9
IL-6	0.4 pg/mL	-9.8	15.5	n.d.	6.3
IL-8	0.8 pg/mL	-24.4	15.4	n.d.	12.5
SP-D	1.6 ng/mL	-5.3	3.6	-6.5	5.2
TNFα	4.7 pg/mL	-28.6	9.2	n.d.	8.0

Table 2. To test the validity of the model, we divided the cohort into two groups. Patients were matched on age (+/-2 years) and BODE (+/-1 unit). This captured 863 per subgroup (1726 total). The remaining unmatched 117 patients were assigned to one or another group randomly and the analysis on the subgroups was re-run. The table below shows the results. The results are consistent across the two subgroups with C statistic values that are similar in the group as a whole and in the two subgroups.

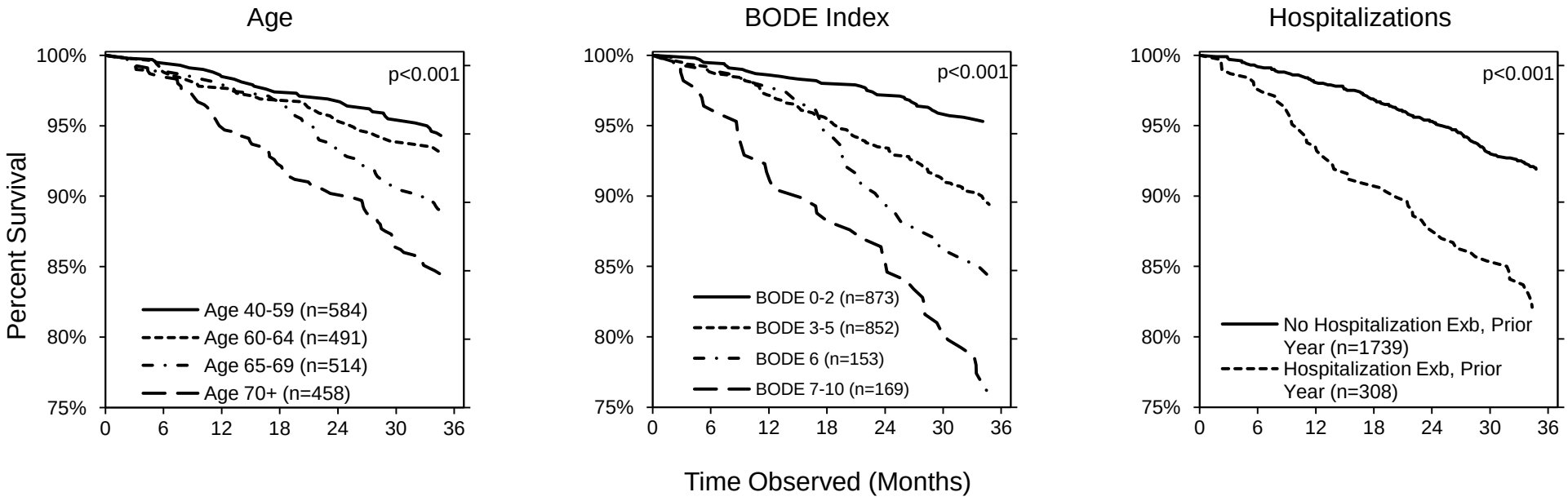
Table 2 S.

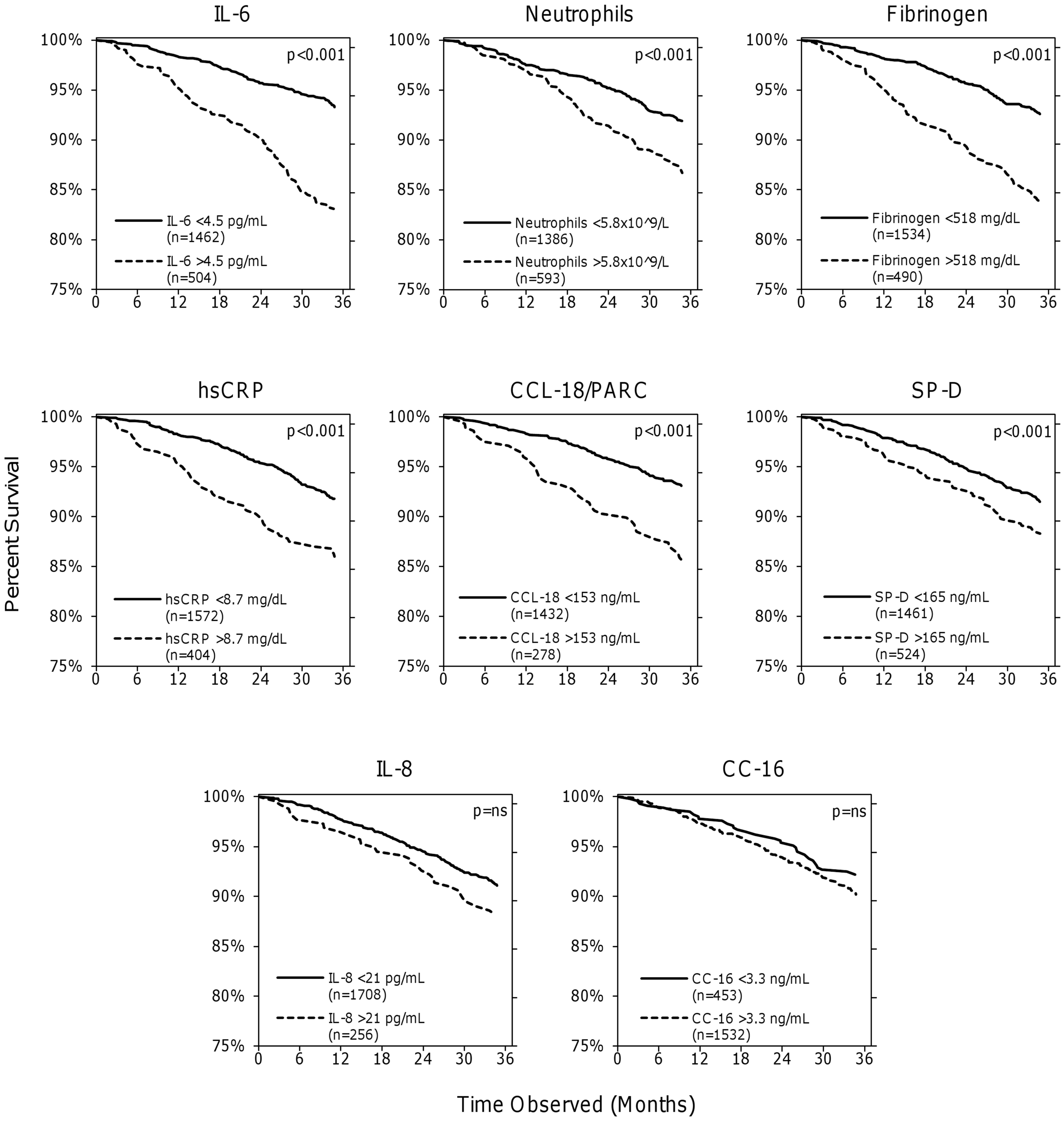
	# deaths (%)	Base model C-Statistic	Base + biomarker C-statistic	Difference (95%CI)	p-value vs reference
Full result (N=1843)	168 (9.1%)	0.686	0.728	0.042 (0.016, 0.069)	0.002
Subset 1 (N=923)	90 (9.8%)	0.697	0.724	0.026 (-0.012, 0.066)	0.166
Subset 2 (N=920)	78 (8.5%)	0.675	0.730	0.055 (0.008, 0.102)	0.021



*Four patients were missing FEV_1 values, 51 patients were missing 6MWD values, 62 patients were missing mMRC values; missing components not mutually exclusive

** Patients were missing individual measures of CC-16, hsCRP, Fibrinogen, IL-6, IL-8, PARC, SP-D, TNF-a, Neutrophils, WBC or combinations thereof


Age
BODE Index
Hospitalizations
Percent Survival
100%
95%
90%
85%
80%
75%
0
6
12
18
24
30
36
p<0.001
Age 40-59 (n=584)
Age 60-64 (n=491)
Age 65-69 (n=514)
Age 70+ (n=458)
BODE 0-2 (n=873)
BODE 3-5 (n=852)
BODE 6 (n=153)
BODE 7-10 (n=169)
No Hospitalization Exb, Prior Year (n=1739)
Hospitalization Exb, Prior Year (n=308)
Time Observed (Months)


IL-6
p<0.001
IL-6 <4.5 pg/mL
(n=1462)
IL-6 >4.5 pg/mL
(n=504)
Neutrophils
p<0.001
Neutrophils <5.8x10^9/L
(n=1386)
Neutrophils >5.8x10^9/L
(n=593)
Fibrinogen
p<0.001
Fibrinogen <518 mg/dL
(n=1534)
Fibrinogen >518 mg/dL
(n=490)
hsCRP
p<0.001
hsCRP <8.7 mg/dL
(n=1572)
hsCRP >8.7 mg/dL
(n=404)
CCL-18/PARC
p<0.001
CCL-18 <153 ng/mL
(n=1432)
CCL-18 >153 ng/mL
(n=278)
SP-D
p<0.001
SP-D <165 ng/mL
(n=1461)
SP-D >165 ng/mL
(n=524)
IL-8
p=ns
IL-8 <21 pg/mL
(n=1708)
IL-8 >21 pg/mL
(n=256)
CC-16
p=ns
CC-16 <3.3 ng/mL
(n=453)
CC-16 >3.3 ng/mL
(n=1532)
Percent Survival
100%
95%
90%
85%
80%
75%
0
6
12
18
24
30
36
Time Observed (Months)