Circulating Fibronectin to C-reactive Protein Ratio and Mortality: A Biomarker In COPD?

RE-REVISED PAPER

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Abstract

The balance between inflammatory and repair processes is important in maintaining lung homeostasis in chronic obstructive pulmonary disease (COPD). We determined whether or not an integrated index of a biomarker involved in inflammation, C-reactive protein (CRP), and another involved in wound repair, fibronectin, may be a good measure to predict clinical outcomes in COPD.

We measured circulating blood levels of CRP and fibronectin in 4,787 individuals with mild to moderate COPD, who were prospectively followed for more than 7 years after blood collection as part of the Lung Health Study. To assess the balance between repair and inflammation, we developed a simple ratio by dividing fibronectin by CRP levels and used a Cox proportional hazards model to determine the relationship between this ratio and all-cause and disease-specific causes of mortality.

The relationship between fibronectin to CRP ratio and all-cause mortality was L-shaped. There was an exponential decay in the adjusted hazard function (i.e. the risk of mortality) as the ratio decreased until a value of 148 was reached, beyond which point the hazard function did not significantly change. Similar results were observed for the risk of coronary and cardiovascular mortality.

Circulating fibronectin to CRP ratio is significantly associated with all-cause mortality of COPD patients. However, dissimilar to other biomarkers, the relationship appears to be L-shaped (and not linear) suggesting a threshold at ~150. While promising, future studies are needed to validate this simple index as a biomarker in COPD.

Introduction

Chronic obstructive pulmonary disease (COPD) is a highly prevalent disease in the western world, affecting 10 to 15% of the adult population over the age of 45 years [1]. Within 15 years, COPD will be the 3rd leading cause of mortality and the 5th leading cause of disability worldwide [2]. Unfortunately, there is a dearth of effective therapies that can prolong survival of COPD patients [3]. The development of novel therapeutic compounds in COPD has been impeded by a scarcity of robust intermediate end points that can track disease progression and predict morbidity and mortality [4]. The pressing need for effective intermediate end points for COPD therapeutic trials was highlighted by the American Thoracic Society/European Respiratory Society (ATS/ERS) Task Force statement on outcomes for COPD pharmacologic trials [5]. With the growing awareness of COPD as a systemic disease, there has been a shift in the emphasis of biomarker discovery towards blood specimens [6]. Serum or plasma biomarkers are attractive because blood, unlike bronchial washes or brushes, is readily available and their measurements can be easily standardized. To date most of the attention has been focused on biomarkers associated with the systemic inflammatory pathway and some of these markers (most notably C-reactive protein (CRP)) show promise [7-9]. Another important but less studied pathway is wound repair [10]. Over or under-expression of the reparative pathway can lead to disease states [11]. One possible blood biomarker of the reparative system is fibronection. Fibronectin is a high molecular weight glycoprotein, which is present in the body as two major isomers: a soluble form in the blood and as an insoluble extracellular matrix isomer [12]. While blood fibronectin has many functions, its primary role is to promote wound repair following injury or infection by mediating cellular adhesion, motility, differentiation, apoptosis, and hemostasis, and inducing the reticuloendothelial system [13]. This raises the possibility that an integrated measurable set of biomarkers that takes into account both CRP and fibronectin would perform better

than CRP or fibronectin alone in COPD. In this study, we evaluated the potential usefulness of adding measurements of blood fibronectin to serum CRP data to create an integrated index of inflammation and repair to predict clinical outcomes in a cohort of patients with mild to moderate COPD.

METHODS

Study Design

The present study used data from 5,887 cigarette-smoking participants of the Lung Health Study (LHS). They were between the ages of 35 to 60 years who had mild to moderate airflow obstruction on spirometry and were prospectively followed for up to 14.5 years [14]. At entry, all participants demonstrated mild to moderate airflow obstruction as defined by a forced expiratory volume in one second (FEV_1) of < 90% of but ≥ 55% of predicted, in the presence of FEV_1/forced vital capacity (FVC) ratio of < 0.70. Individuals who had a history of cancer (except carcinoma in situ or basal-cell carcinoma of the skin), myocardial infarction (in the past two years), angina, heart failure, stroke (in the past two years), renal failure, insulin-requiring diabetes mellitus, cirrhosis or other serious liver diseases, pulmonary embolism, disorders of the central nervous system, narrow-angle glaucoma, or any other major diseases which could have compromised follow-up were excluded from the cohort. After enrollment and baseline measurements, the study participants were asked to visit the study center annually for follow-up. At these visits, salivary cotinine levels were measured to objectively verify smoking status of the participants. Participants were categorized as sustained quitters if they were biochemically validated nonsmokers at each annual visit. Participants who were smokers at each annual visit were continuing smokers. Those whose behavior varied were classified as intermittent quitters. The initial LHS involved 5 years of follow-up and the results of this phase of the study have been published previously [15, 16]. At the fifth annual visit, venipuncture was carried out on participants. There were 5,413 subjects who were alive and eligible for venipuncture at this visit. Of these, 4,803 provided serum samples (89% of eligible participants), of which 4,787 had sufficient volume of serum for the fibronectin and CRP measurements. At this visit, the participants were also asked to consent for additional follow-up (LHS 3). During the

follow-up of LHS 3, the participants' vital status was captured biannually. An independent mortality and morbidity committee reviewed death certificates, autopsy reports, relevant hospital records, and summaries of interviews with attending physicians, or eyewitnesses and assigned the causes of death for all participants who died during the study. These data were supplemented by linkages with a National Death Index, which provided the date and cause of death for all U.S. study participants through the end of 2001. Vital status was successfully determined for 98.3% of the participants [14]. Mortality end points were classified into: coronary heart disease (CHD), cardiovascular disease (CVD), which also included CHD, lung cancer, other cancer, respiratory disease excluding lung cancer, other, and unknown. Because the blood samples were taken at year 5, individuals who died during the first five years of LHS follow-up and prior to blood sampling were excluded in the present analysis.

Measurements of C-reactive protein (CRP) and fibronectin

After collection, the blood samples were separated into their various components and transferred to the LHS data co-ordinating center on dry ice and were kept in -70 ^{o}C freezers until use. The serum samples were thawed and C-reactive protein (CRP) and fibronectin were determined using the SearchLight Proteome Array™ system (Pierce Biotechnology Inc, Rockford, IL). This is a highly sensitive chemiluminescent multiplexed sandwich enzyme-linked immunoassay (ELISA) analyzer that allowed quantitative measurements of fibronectin and CRP from the same aliquot of serum simultaneously.

Statistical Analysis

To assess the joint effects of CRP and fibronectin, we developed a simple ratio by dividing fibronectin by CRP levels in the systemic circulation analogous to the use of low density lipoprotein cholesterol to high-density lipoprotein cholesterol ratio for assessing CVD risk in the general population [17]. The primary relationship of interest was between serum fibronectin to CRP ratio and all-cause mortality. Other end points were considered

secondary in nature. All individuals who died or were lost to follow-up before visit 5 were excluded. Fibronectin to CRP values were divided in quintiles from the lowest to the highest levels. We compared the risk of all-cause mortality across the quintiles over the follow-up period using a Cox proportional hazards model in which the following covariates were adjusted for: age, sex, race, body mass index (BMI), pack-years of smoking, biochemically validated smoking status (i.e. continued smokers, sustained quitters, or intermittent quitters), and percentage of predicted normal value FEV_1 (in quintiles). To construct a parsimonious model, we used a stepwise selection method in which variables were considered for further evaluation when their P value was 0.25 or less and retained in the model if their P value was 0.15 or less in the multivariate analysis. Similar methods were employed for the various causes of mortality. Logarithmic transformations were used for CRP, fibronectin and the fibronectin to CRP ratio to achieve normality of distribution and to attenuate the influence of extreme outliers, which may artificially skew the data and produce misleading results. Continuous variables are presented as mean±S.D. unless otherwise specified. All analyses were performed using SAS software version 9.1 (SAS Institute, Carey, N.C.). P-values below 0.05 were considered to indicate statistical significance.

RESULTS

Study Participants

The mean age of the study participants was 53.5± 6.8 years; 3,036 (62.9%) were men and the average FEV_1 at the fifth annual visit was 2.54±0.67 L (71.0±12.4% of predicted). The average pack-year of smoking was 40.2±18.7; 1,363 (28.0%) were intermittent quitters and 2607 (54.0%) were continuous smokers at year 5. The rest were sustained quitters. The median fibronectin level was 316 mg/L (interquartile range, 227 to 456 mg/L). The median CRP was 3.12 mg/L (interquartile range, 1.26 to 7.30 mg/L) and the median fibronectin to CRP ratio was 107.2 (interquartile range, 45.29 to 253.70). The characteristics of the study participants in quintiles of fibronectin to CRP ratio are shown in Table 1. Fibronectin to CRP ratio was significantly related to the following variables: smoking status of the participants, age (β-coefficient for every 1-year increment, -0.0075; p=0.019), BMI (β-coefficient for every 1 kg/m^2 increment, -0.0666; p=0.0021), pack-years of smoking (β-coefficient for every pack-year, -0.0033 ; p<.0001), systolic blood pressure (β-coefficient for every pack-year, -0.0036 ; p=0.005), and FEV_1 (β-coefficient for every 1L increment, 0.4365; p<.0001). Compared to sustained quitters, intermittent quitters and continued smokers had decreased ratio (β-coefficient for intermittent quitters, -0.1255; p= 0.021; β-coefficient for continued smokers, -0.2729; p <.0001)

Fibronectin to CRP Ratio and Mortality

The median duration of follow-up was 7.5 year (range 21 days to 7.5 years). During follow-up, there were 329 deaths representing 6.82% of the cohort. Serum fibronectin by itself was negatively associated with all-cause mortality but it failed to reach statistical significance (hazard ratio, HR, 0.84 for one-logarithmic increase in fibronectin level; 95% confidence interval, CI, 0.70 to 1.00; p=0.055) (see web supplement table 1). Fibronectin

levels were not associated with coronary heart disease (p=0.163), cancer (p=0.232), or respiratory-specific mortality (p=0.570). Serum CRP, on the other hand, was positively associated with all-cause (p=0.004), coronary heart disease (p=0.037), and cardiovascular disease-specific mortality (p=0.017) as previously reported (see web supplement table 2) [9].

The relationship between the ratio of fibronectin to CRP and all-cause mortality was L-shaped. The lowest risk of mortality was observed in the last three quintiles, while the highest risk was observed in quintile 1 (see figure 1 and table 2). This was further supported by an analysis in which fibronectin to CRP ratio was assessed as a logarithmic continuous variable rather than in quintiles. The hazard function decreased as the ratio increased until a value of 148 was reached (see figure 1). Beyond this level, there was no significant change in the hazard function. The c-statistics (i.e. the area under the curve) was the largest at 148 providing complementary evidence of a threshold at this point (see supplementary figure 2). Similarly, the risk of coronary heart disease and cardiovascular disease mortality was highest in quintiles 1 and 2 (see table 2).

In Table 3, we summarize the relationship between mortality and fibronectin to CRP ratio according to smoking categories at the time the serum samples were obtained. The relationship between fibronectin to CRP ratio and mortality held for intermittent and continued smokers but not for sustained quitters, though a lack of statistical power may have been responsible for the latter observation (see Table 3).

Comparison of Models

We examined the goodness of fit of the 3 models (CRP alone, fibronectin alone, and fibronectin to CRP ratio models) using Baysian Information Criterion (BIC) and Akaike Information Criterion (AIC) (web supplement table 3 and web supplement for details of the AIC calculation). In general, models with the lowest AIC and BIC are preferred. For example, the AIC of the mortality model containing fibronectin alone was 5268.32, while that for the model containing fibronectin to CRP ratio was 5254.87. Since the two models have the same number of parameters, the difference in AIC, 13.45, is equivalent to a likelihood ratio of 832.98 indicating better fit of data and greater discriminatory power (p <0.001) (see web supplement for details). Indeed, for all end points, the models that contained fibronectin to CRP ratio in quintiles had the lowest AIC and BIC values, which suggests that the best model fit was achieved by using fibronectin to CRP quintiles rather than fibronectin or CRP alone in quintiles.

DISCUSSION

We found that reduced levels of fibronectin relative to CRP were associated with increased all-cause and CVD mortality. Overall the relationship was L-shaped. There was an exponential decay of the hazard function as the ratio increased until a value of ~150 was reached. Beyond this point, the hazard function did not change significantly, suggesting a threshold at this value. Future studies will be needed to validate this threshold in a variety of different clinical settings (e.g. severe COPD populations). Similar L- or reverse L-shaped relationships have been described in other conditions. For instance, the risk of mortality is relatively constant until a BMI of 28 kg/m^2 is reached beyond which point the risk increases sharply [18]. Similarly the risk of cardiovascular mortality increases exponentially only beyond a serum CRP level of 3 mg/L [19]. Although we observed a relationship between blood levels of fibronectin/CRP and mortality, it is uncertain, however, how pulmonary repair and inflammation are reflected in the circulating blood, and how these molecules contribute to the increased mortality and morbidity of the subjects.

Both CRP and fibronectin play relevant roles in tissue injury and repair. CRP is an acute phase protein, which is synthesized predominantly by hepatocytes. CRP activates complements as part of the innate immune response, enhances opsonization of particles and microbes by macrophages and promotes endothelial activation [20]. Over-expression of CRP locally amplifies tissue damage and increases infarction size in myocardium and brain [21]. Fibronectin, on the other hand is an adhesive glycoprotein that limits tissue injury and mediates cellular repair following tissue insult by promoting hemostasis and wound healing [22, 23]. Additionally, circulating fibronectin upregulates anti-apoptotic proteins such as BCL-2 thus preventing cellular death and limiting infarction size following ischemic injury [24]. Circulating fibronectin also enhances

macrophages' ability to phagocytose particles and microbes. Depletion of fibronectin increases lung deposits of blood-borne particles and microbes, which may cause disruptions of the lung microcirculation [25, 26]. Interestingly, CRP interacts with fibronectin by binding with it to initiate the repair process, which in turn creates a negative feedback loop [27].

To our knowledge, there are no published studies of the fibronectin/CRP ratio. However, there are examples of where biomarkers of repair (e.g. fibronectin) and inflammation (e.g. CRP or IL-6) have been associated with poor clinical outcomes. For example, reduced blood fibronectin is associated with coronary artery disease, which is generally associated with elevated CRP levels [28]. In meningococcal disease, children with the worst prognosis are those who have depressed blood fibronectin levels but elevated serum interleukin-6 levels [29]. Similarly, in sepsis syndrome, patients with the lowest blood fibronectin levels have generally the worst prognosis while nearly all of them have elevated CRP levels [30, 31].

Although the local lung expression of fibronectin is usually increased [32], systemic (circulating) levels are paradoxically decreased in COPD [33]. The reduced levels are associated with increased symptoms and severity of disease [33]. The reduced systemic levels may reflect increased extravasation of circulating fibronectin to sites of local injury and accelerated turn-over owing to chronic inflammation [34]. Cigarette smoking, which is the main cause of COPD, may further reduce fibronectin levels by inhibiting local production [35]. However, it is unclear based on our study whether the reduced fibronectin to CRP ratio was a cause or an effect of COPD.

There are several implications from the present study. Firstly, it highlights the importance of considering both the inflammatory and repair pathways in assessing health risks in patients with COPD. CRP and fibronectin are linked with COPD progression but do not appear to be the main drivers of the progression. Secondly, unlike other markers of inflammation and repair, CRP and fibronectin are biomarkers uniquely useful to risk stratify COPD patients because they are stable over long periods of time, have little diurnal variations and can be measured relatively inexpensively with commercially available assays [36, 37]. Traditionally, FEV_1 has been used as the "gold standard" for determining prognosis in COPD patients. The present analysis shows that the fibronectin to CRP ratio provides incremental and independent prognostic information beyond that achieved by FEV_1 alone justifying their utility in risk-stratification and prognostication.

There were several limitations to the present study. Firstly, serum CRP and fibronectin levels were measured only at one time point. Thus, the impact of changes in CRP and fibronectin levels over time on mortality of COPD patients is unknown. Any bias resulting from misclassification of the exposure variable would have diluted the relationship between the biomarkers and mortality. Thus, our findings are likely conservative. As well, it is uncertain how the blood levels of these molecules are linked to the pathology of the lung disease in COPD. Notably, neither CRP nor fibronectin is lung-specific; thus their blood levels may not correlate with what is happening in the lungs. Secondly, based on the current work, it is uncertain whether CRP or fibronectin is part of the causal pathway leading to mortality in COPD patients or just an epiphenomenon of other more salient molecules in the inflammatory and/or repair pathways. Thirdly, it is not clear whether the present data can be applied to other patient populations at risk for cardiac events (e.g. diabetes, obesity). Future studies will be needed in these specific populations to determine the potential utility of the fibronectin to CRP ratio in predicting

cardiac events in these patients. Moreover, because we studied only patients with COPD, comparisons of the current findings with those in the general population are not possible. As recommended by the ATS/ERS Task Force, large population-based studies are thus needed in the future to establish "normative" values of blood biomarkers [5]. Finally, because we studies patients with mild to moderate CRP, the current findings may not be generalizable to patients with severe disease.

In sum, we found in a large cohort of well-characterized patients with mild to moderate COPD that fibronectin-CRP ratio was independently associated with mortality (especially cardiac mortality) more than 7 years of follow-up and that the relationship was L-shaped raising the possibility that this ratio may help clinicians and researchers to better risk-stratify patients with mild to moderate COPD for prognosis and intervention.

ACKNOWLEDGEMENT

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Figure Legend

Figure 1. Fitted Adjusted Mortality Curve Evaluating the Relationship Between Fibronectin to CRP Ratio and the Hazard Function for All-Cause Mortality

The hazard function was generated using a Cox proportional hazards model in which the relationship between fibronectin to CRP ratio as a continuous variable and all-cause mortality was evaluated adjusted for various covariates (see methods). The fitted curve is presented. The x-axis plots the logarithmic function of fibronectin to CRP ratio. The natural units of the ratio are shown in brackets.

The arrow indicates a potential threshold at ~150 beyond which point the hazard function does not change significantly.

Hazard ratios can be calculated from figure 1 by determining the hazard function at a particular "x" value (e.g. 2.7) and dividing it by the hazard function of the reference value (e.g. 148). In this example, the hazard ratio would be 3.
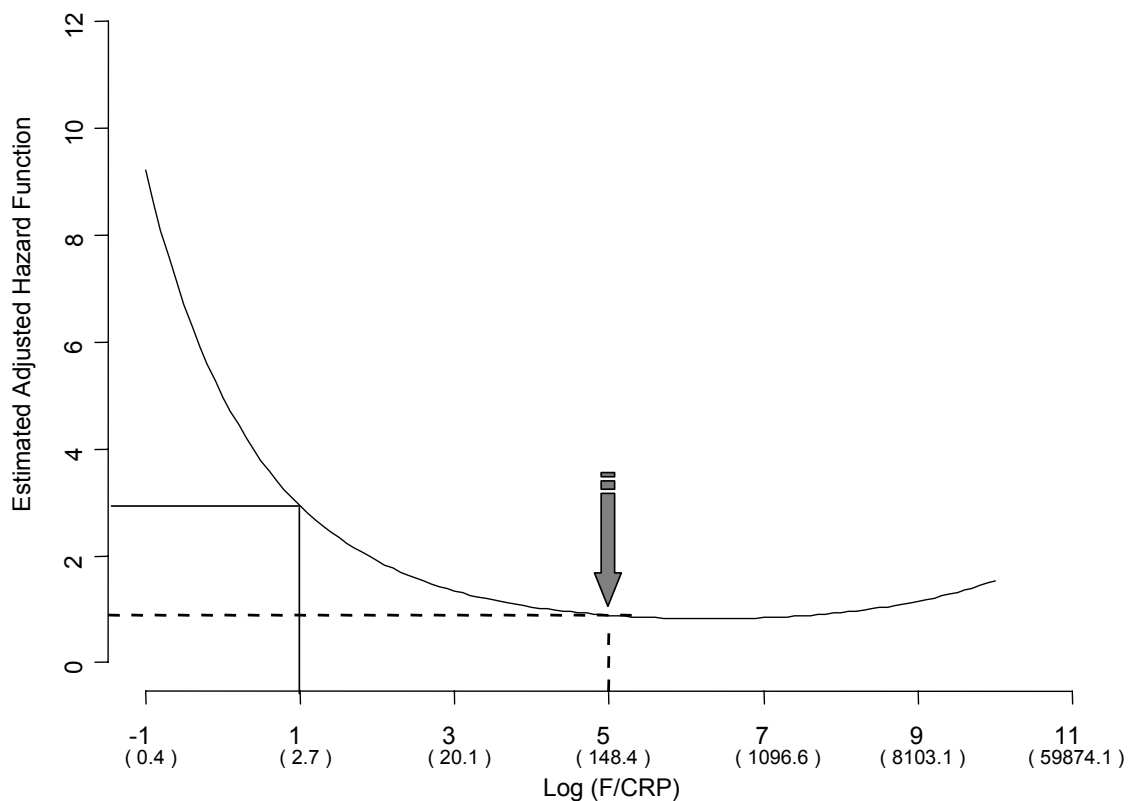
Figure 2. Cumulative Cardiovascular Mortality Of Participants With Mild to Moderate COPD Stratified According to Quintiles Of Fibronectin To CRP Ratio.

The survival curves are significantly different from each other (p<0.001 by log-rank test).
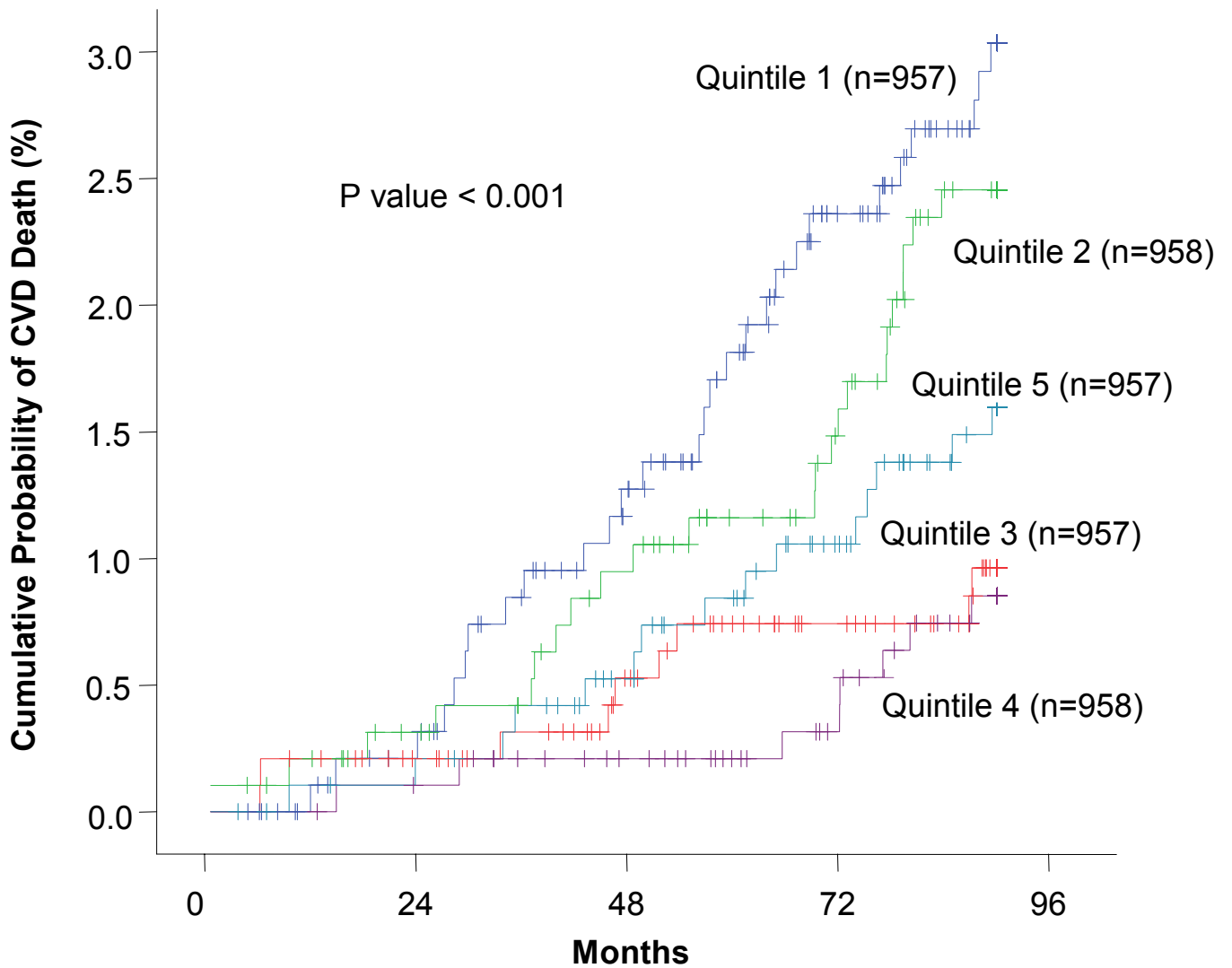
Table 1: Clinical Characteristics of Study Participants Stratified In Quintiles of Fibronectin (F) To C-Reactive Protein (CRP) Ratio

Continuous variables are expressed as mean (SD) and dichotomous variables are expressed as number (% column totals).

F/CRP ratio quintiles	Quintile 1 (n = 957)	Quintile 2 (n = 958)	Quintile 3 (n = 957)	Quintile 4 (n = 958)	Quintile 5 (n = 957)	Total	P values
F/CRP ratio	**20.57 (9.16)**	**55.25 (11.46)**	**108.91 (21.28)**	**218.12 (45.97)**	**802.39 (1069.96)**	**241.01 (558.99)**	**<0.001**
Fibronectin (ug/ml)	315.40 (210.77)	366.01 (212.21)	404.48 (445.54)	416.81 (291.30)	441.18 (522.02)	388.78 (361.75)	<0.001
CRP (ug/ml)	22.24 (36.55)	6.96 (4.52)	3.82 (3.89)	2.01 (1.52)	0.77 (0.87)	7.16 (18.32)	<0.001
Age, year	54.71 (6.34)	53.9 (6.59)	53.67 (6.79)	53.07 (6.82)	52.06 (7.02)	53.50 (6.78)	<0.001
Men	511 (53%)	612 (64%)	635 (66%)	632 (66%)	623 (65%)	3036 (63%)	<0.001
White	899 (94%)	917 (96%)	929 (97%)	930 (97%)	931 (97%)	4644 (96%)	0.001
Pack-years of smoking	41.81 (17.77)	42.12 (20.07)	40.02 (18.27)	39.2 (18.31)	37.44 (18.36)	40.15 (18.71)	<0.001
BMI, kg/m^2	26.19 (4.22)	26.36 (3.86)	25.67 (3.87)	25.12 (3.61)	24.46 (3.62)	25.56 (3.91)	<0.001
FEV$_1$, L	2.33 (0.6)	2.5 (0.64)	2.56 (0.66)	2.62 (0.67)	2.68 (0.7)	2.54 (0.67)	<0.001
FEV$_1$, % predicted	69.05 (11.94)	70.25 (11.82)	70.82 (12.97)	72.21 (12.34)	72.66 (12.64)	70.99 (12.41)	<0.001
Intermittent smokers	259 (27%)	255 (27%)	276 (29%)	273 (28%)	291 (30%)	1363 (28%)	0.739
Continued smokers	582 (61%)	536 (56%)	518 (54%)	478 (50%)	470 (49%)	2607 (54%)	<0.001
Daily sputum	311 (32%)	281 (29%)	306 (32%)	297 (31%)	274 (29%)	1477 (31%)	0.580
Daily Cough	365 (38%)	319 (33%)	342 (36%)	334 (35%)	326 (34%)	1697 (35%)	0.416
Diastolic BP, mm Hg	78.1 (9.37)	78.48 (8.77)	78.59 (9.74)	77.94 (8.89)	77.25 (9.54)	78.08 (9.27)	0.029
Systolic BP, mm Hg	123.5 (15.42)	123.35 (14.26)	122.4 (15.84)	121.42 (13.96)	119.74 (14.7)	122.10 (14.91)	<0.001
Theophylline	27 (2.82%)	25 (2.61%)	17 (1.78%)	14 (1.46%)	15 (1.57%)	98 (2.03%)	0.218
Inhaled Corticosteroids	15 (1.57%)	9 (0.94%)	19 (1.99%)	7 (0.73%)	12 (1.25%)	62 (12.8%)	0.226
Beta-blockers	41 (4.28%)	40 (4.18%)	36 (3.76%)	22 (2.30%)	27 (2.82%)	167 (3.46%)	0.136
Diabetics	29 (3.03%)	19 (1.98%)	16 (1.67%)	14 (1.46%)	10 (1.05%)	88 (1.82%)	0.037

Abbreviations: F/CRP, fibronectin to C-reactive protein; BMI, body mass index; FEV$_1$, forced expiratory volume in one second; BP, blood pressure

Table 2. The Relationship Between The Fibronectin to CRP Ratio In Quintiles and All-Cause and Specific Causes Of Mortality

End Points	Fibronectin to CRP Ratio (Quintiles)				
	Q1 (lowest) (N=957)	Q2 (N=958)	Q3 (N=957)	Q4 (N=958)	Q5 (highest) (N=957)
All-cause	2.08 (1.46, 2.98) [103 | 10.76%]	1.42 (0.97, 2.09) [64 | 6.68%]	1.39 (0.95, 2.05) [64 | 6.69%]	1 [39 | 4.07%]	1.33 (0.89, 1.99) [54 | 5.64%]
Coronary Heart Disease	5.07 (1.46, 17.61) [17 | 1.78%]	5.17 (1.50, 17.76) [16 | 1.67%]	1.62 (0.39, 6.77) [5 | 0.52%]	1 [2 | 0.21%]	3.80 (1.05, 13.83) [10 | 1.04%]
Cardiovascular	2.60 (1.34, 5.05) [35 | 3.66%]	2.42 (1.24, 4.72) [31 | 3.24%]	1.01 (0.46, 2.22) [13 | 1.36%]	1 [10 | 1.04%]	1.78 (0.86, 3.69) [18 | 1.88%]
Cancer	1.46 (0.98, 2.17) [66 | 6.90%]	0.98 (0.63, 1.52) [41 | 4.28%]	1.21 (0.80, 1.84) [51 | 5.33%]	1 [37 | 3.86%]	0.97 (0.61, 1.53) [36 | 3.76%]
Respiratory	2.20 (0.77, 7.20) [9 | 0.94%]	3.02 (0.96, 9.53) [11 | 1.15%]	2.03 (0.62, 6.61) [9 | 0.94%]	1 [3 | 0.31%]	2.70 (0.85, 8.60) [10 | 1.04%]

Each cell expresses an adjusted hazards ratio (95% confidence interval) using quintile 4 as the referent, adjusted for covariates selected from age, sex, FEV_1 (% of predicted normal values) current smoking status, pack-years of smoking, body mass index, medications and other covariates (see methods).

The first number in the [ ] is the number of death in that cell and the second number is the crude death rate as a percentage.

Abbreviation: CRP, C-Reactive Protein

Table 3. The Relationship Between Fibronectin to CRP Ratio and All-Cause and Specific Causes Of Mortality In Various Smoking Categories

Each cell expresses a hazard ratio comparing quintiles 1 and 2 against quintiles 3, 4 and 5.

End Points	All Participants (n=4809)	Sustained Quitters (n=853)	Intermittent Quitters (n=1360)	Continued Smokers (n=2596)
All-cause	1.46 (1.18, 1.82)	0.91 (0.47, 1.77)	1.57 (1.00, 2.46)	1.56 (1.18, 2.04)
Coronary Heart Disease	2.81 (1.56, 5.05)	4.29 (0.76, 24.33)	3.74 (1.13, 12.35)	2.33 (1.12, 4.84)
Cardiovascular	2.26 (1.53, 3.33)	2.17 (0.53, 8.81)	2.36 (1.06, 5.23)	2.25 (1.40, 3.60)
Cancer	1.17 (0.91, 1.52)	0.92 (0.42, 2.01)	0.97 (0.59, 1.62)	1.33 (0.96, 1.86)
Respiratory	1.30 (0.71, 2.37)	2.16 (0.35, 13.33)	2.61 (0.78, 8.72)	0.85 (0.38, 1.87)

All hazards ratio (95% confidence interval) using quintile 3, 4 and 5 as the referent, adjusted for age, sex and FEV_1 (% of predicted normal values) (see methods).