Plasma Fibrinogen as a Biomarker for Mortality and Hospitalized Exacerbations in People with COPD

Online Supplement: Appendix

SAP Prepared For:
Chronic Obstructive Pulmonary Disease Biomarkers Qualification Consortium (CBQC)

Plasma Fibrinogen as a Biomarker in COPD:
Statistical Analysis Plan

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INTRODUCTION AND OVERVIEW OF THE STATISTICAL ANALYSIS PLAN (SAP)

The Chronic Obstructive Pulmonary Disease (COPD) Biomarkers Qualification Consortium (CBQC) was established to combine the expertise of representatives from industry, academia, and the government with the goal of improving the time to development of new medications used to treat COPD. CBQC is interested in identifying biomarkers such as fibrinogen that could be used to enrich clinical trial populations with patients at increased risk of clinically important COPD outcomes. To this end, the CBQC Fibrinogen working group collaborated with academic leaders and contracted with INC Research to create an integrated database comprising 6 studies that collected plasma fibrinogen, a potential biomarker of COPD exacerbations and mortality, in patients with COPD. Evidera was asked to analyze this integrated database for the purpose of supporting the qualification of plasma fibrinogen as a biomarker for COPD to enrich the study populations for future clinical trials.

The main purpose of the SAP is to describe retrospective data analyses to assess the relationship between plasma fibrinogen and hospitalized COPD exacerbations and mortality. The SAP will guide the analyses performed and the content will be developed in collaboration with the CBQC with input from academic experts and the FDA.

Background

Several studies have demonstrated that plasma fibrinogen concentrations are higher in COPD populations when compared to healthy controls after adjusting for key determinants that independently impact plasma fibrinogen concentration such as age, gender, and smoking (Duvoix et al., 2012; Dahl et al., 2001).

There have been reports from studies of the general population and COPD subjects measuring the relation between plasma fibrinogen and clinical COPD outcomes, including exacerbations and mortality. These studies have recently been reviewed by Duvoix et al. (2012) and will be briefly summarized here. In the Copenhagen City Heart Study, an adult Danish general population study (Dahl et al., 2001) reported that over a 6-year follow up period the risk for COPD hospitalization was highest for subjects with the highest plasma fibrinogen concentrations. In the COSMIC (COPD and Seretide: a Multi-Center Intervention and Characterization) study, a one-year prospective study in COPD patients, Groenewegen et al. (2008) reported that COPD subjects (with 2 or more exacerbations in the year prior to enrollment) experiencing exacerbations requiring treatment with oral steroids or exacerbations requiring hospitalization had higher plasma fibrinogen concentrations at baseline than did subjects who did not exacerbate. There was a rate ratio of 1.19 for each g/L increase in plasma fibrinogen for moderate exacerbations and a hazard ratio increase of 1.77 for severe exacerbations. In a population-based retrospective study in Sweden, Engstrom et al. (2009) reported that for each 0.8 g/L increase in plasma fibrinogen, a risk-adjusted 1.25 increase in the hazard ratio for COPD hospitalizations was observed.

In a meta-analysis of 31 individual studies, the Fibrinogen Studies Collaboration investigators (2005) assessed the relationship between plasma fibrinogen concentration and risk of mortality from cardiovascular and non-cardiovascular causes. For COPD mortality, using a case-control design, each 1

g/L increase in plasma fibrinogen was associated with a hazard ratio for mortality of 4.5 in the first 5 years of follow-up.

In an analysis combining subjects from the ARIC and CHS studies, Valvi et al. (2012) reported that mean plasma fibrinogen concentrations increased with increasing severity of COPD. Subjects whose plasma fibrinogen concentration was in the top decile for the cohort (>393.0 mg/dL) were at increased risk for COPD-related hospitalizations and death during follow-up (mean follow-up time of 9.7 years). This relation remained even after adjusting for potential confounders such as CVD (self-reported), age, gender, race, smoking status, and BMI. Mannino et al. (2012) recently reported the results of an analysis of participants in the NHANES III study. Participants with COPD, particularly GOLD stage defined stages 3 and 4 had higher plasma fibrinogen concentrations when compared to subjects with milder COPD or non-COPD subjects. Elevated plasma fibrinogen was associated with an increased risk for all-cause mortality. Information on COPD exacerbations/hospitalizations was not reported.

Although this prior research provides evidence of the association between high fibrinogen and COPD exacerbations, published literature is not currently available that would support the use of fibrinogen as a biomarker for increased risk of COPD-related outcomes during a time period that would be relevant to the conduct of a clinical trial. Moreover, the integration of 5 independently conducted studies will provide evidence regarding the robustness of fibrinogen as a biomarker of adverse COPD outcomes across various patient populations and time periods.

These data present considerable evidence that plasma fibrinogen is closely related to COPD and COPD exacerbations, and support a formal quantitative assessment of plasma fibrinogen as an independent predictor of clinical endpoints in patients with COPD.

Objectives

The specific objectives for this analysis are to:

1. Verify the integrity of the integrated database
2. Describe the demographic and clinical characteristics of patients in the integrated database
3. Evaluate methods of defining patients with COPD
4. Assess longitudinal variation in plasma fibrinogen between individuals and within individuals
5. Define a cut point for classifying patients as having high fibrinogen levels
6. Assess the relationship between fibrinogen and risk of mortality and hospitalized COPD exacerbations during relevant time windows

General Statistical Considerations

This is a retrospective database analysis of previously collected data from prospective clinical trials and observational studies. These data offer an opportunity to evaluate the relationship between plasma fibrinogen and adverse COPD outcomes by pooling data from multiple sources. Analyses will be conducted separately for individual studies and integrated as indicated. Data will be pooled across the

data sources as deemed appropriate for the analysis being conducted to uphold model assumptions and per variables available within each study.

Adverse COPD outcomes that will be assessed in these analyses include COPD exacerbations and all-cause mortality. The primary endpoint of clinical trials for COPD medications is a hospitalized COPD exacerbation; however, all-cause mortality does hold clinical importance in both the conduct of clinical trials and the overall management of any chronic disease.

As the data are analyzed, some deviation from the analyses outlined is anticipated (e.g., due to missing data; small sample sizes). In instances where these deviations would make the proposed analyses inappropriate, modifications to the analysis plan will be made in consultation with the CBQC and noted in the final report.

SAS statistical software version 9.2 (SAS Institute Inc., Cary, NC) will be used to conduct the analyses. All statistical tests will be two-sided and use a significance level of 0.05 unless otherwise noted. No adjustments for multiple comparisons will be performed unless otherwise noted. For all analyses, missing data will be considered randomly missing, and no data imputations or other algorithms for missing responses will be performed unless otherwise indicated. For certain procedures (such as analysis of variance) subjects with missing data will be excluded from the analysis.

Currently Available Data

The currently available data includes the following studies:

1. Atherosclerosis Risk in Communities (ARIC) study: A US general population-based study begun in 1987 (The ARIC Investigators, 1989). The study recruited 15,792 participants in four communities: Forsyth County, NC; Minneapolis, MN; Washington County, MD; and Jackson, MS. Plasma fibrinogen and spirometry (non-bronchodilator) were collected at baseline.

2. Cardiovascular Health Study (CHS): A general population-based study of US participants aged 65 years and older (Fried et al., 1991). The study was initiated in 1988. The original CHS cohort comprised 5201 subjects recruited from four communities: Forsyth County, NC; Pittsburgh, PA; Sacramento County, CA; and Washington County, MD. Plasma fibrinogen and spirometry (non-bronchodilator) were assessed at the baseline visit.

3. Evaluation of COPD Longitudinally to Identify Predictive Surrogate End-points (ECLIPSE): A 3-year prospective, observational study conducted in 46 sites in 12 countries (Vestbo et al., ERJ). The ECLIPSE cohort included 2164 COPD subjects with moderate to very severe COPD (GOLD stages II, III, and IV) along with smoking and non-smoking control groups (Agusti et al., 2010). Only the COPD subjects will be included in the proposed analysis. Plasma fibrinogen was measured at baseline and longitudinally during the 3 years of ECLIPSE.

4. Framingham Heart Study (FHS) Offspring Cohort: A non-randomized cohort study initiated in 1971 (Kannel et al., 1979). Over the ensuing decades, several follow-up examinations were conducted. The cohort initially consisted of 5124 subjects (all subjects were offspring or spouses of offspring of participants in the original Framingham Heart Study). Plasma fibrinogen and

pre-bronchodilator spirometry were collected beginning with exam visit 5 of the offspring cohort study with follow-up hospitalization and vital status information available for a 25-year period. In contrast to several of the other studies proposed for inclusion, longitudinal measurements of plasma fibrinogen and lung function are available from two subsequent exam visits.

5. National Health and Nutrition Examination Survey (NHANES) III: A general population-based cross-sectional study of a representative sample of the US population. Plasma fibrinogen concentration and spirometry (pre-bronchodilator) are available from 8342 adults aged 40 years and above.

Supplemental data:

COPDGene: A prospective cohort study conducted at 21 sites in the United States. The COPDGene cohort includes 4,000 smoker controls with normal spirometry measurements (FEV1/FVC ratio $\geq$0.7, FEV1$\geq$ 80% predicted), 2,000 patients meeting the criteria for GOLD stage 1 and GOLD stage unclassified (GOLD-U), 4,000 patients meeting the criteria for GOLD stages 2–4, and 100 non-smoker controls, for a total enrollment of 10,100 patients. Plasma fibrinogen was not collected in this study; therefore, data from this study will be used only to compare the diagnosis of patients using pre- and post-bronchodilator spirometry measurements, which were collected in the study.

Table 1. Study Population Information for Studies Proposed for Inclusion

Study	Study Population (n)	COPD Subjects (n)*	Longitudinal Fibrinogen Availability	Hospitalized COPD Exacerbations Within 12 months	Deaths within 3 Years among COPD Subjects
ARIC	15,462	1,789	No	96	86
CHS	5,730	1,292	No	236	120
ECLIPSE	2,726	2,118	Yes	335	220
Framingham Offspring Cohort	3,885	145	No	N/A	13
NHANES III	1,369	1,032	No	N/A	146

* Aged ≥40 years and GOLD Stages 2, 3, or 4 – based on pre-bronchodilator spirometry

ARIC = Atherosclerosis Risk in Communities Study; CHS = Cardiovascular Health Study; COPD = chronic obstructive pulmonary disease; ECLIPSE = Evaluation of COPD Longitudinally to Identify Predictive Surrogate Endpoints; FHS = Framingham Heart Study; NHANES = National Health and Nutrition Examination Survey

Studies have been evaluated and integrated into one database for these analyses. Analyses described here will be conducted using both the individual study datasets and the full integrated dataset.

COPD Population Definition

The Global Initiative for Chronic Lung Disease (GOLD) formed in 1998 and released a consensus report titled *Global Strategy for the Diagnosis, Management, and Prevention of COPD* (GOLD, 2011). This report was revised in 2006 and 2011, and is widely recognized among health care professionals, researchers, and other stakeholders. GOLD has published criteria used to diagnose COPD, and recommends the use of post-bronchodilator spirometry data to grade the severity of COPD to minimize variability in measurements. Only pre-bronchodilator spirometric data is available in all studies in this analysis, however, and therefore will be evaluated as to its suitability in defining COPD. Specifically, analyses will quantify the difference between pre- and post-bronchodilator spirometry in ECLIPSE to quantify any potential misclassification that may arise from using pre-bronchodilator spirometric data. Sensitivity analyses will be performed to compare the patient population defined at FEV1 levels of 60% and 70% to those meeting the criteria for GOLD stage 2 and above.

The following GOLD COPD severity definitions will be used in analyses:

1. Gold Stage 2: FEV1/FVC <0.70 and FEV1 50–79% of normal
2. Gold Stage 3: FEV1/FVC <0.70 and FEV1 30–49% of normal
3. Gold Stage 4: FEV1/FVC <0.70 and FEV1 <30% of normal
    a. Note: Stage 4 also includes patients with a FEV1 <50% of normal with chronic respiratory failure present but the data does not allow for determining if chronic respiratory failure is present. Therefore, it will not be included in the definition used in SAP.

The following definitions for COPD will be used for all analyses among patients with at least one fibrinogen result unless otherwise stated:

1. COPD patients will be defined as those ≥40 years of age with moderate, severe, or very severe COPD, as defined by meeting the criteria for GOLD Stage 2+, as described above.
2. A second COPD definition will include the above criteria (i.e., age ≥40 years, GOLD stage 2+), but include only patients with a history of COPD exacerbations to identify a patient sample that is similar to those used in clinical trials of medications developed to treat COPD. This definition can only be used in ECLIPSE, however, as other studies did not collect history of prior COPD exacerbations.

FEV1 predicted and FEV1 % predicted values were not provided within the NHANES and FHS datasets. FEV1 predicted values were calculated using the equations published by Hankinson et al (1999). Race and ethnicity information was not captured in the FHS dataset but all three Framingham Cohorts (Original Cohort, Offspring Cohort, and Third-Generation Cohort) have been characterized as predominantly white (Splansky et al., 2007); therefore, the prediction equation for Caucasians was used for all patients in FHS. Race/ethnicity was captured in the NHANES data as ‘white’ vs. ‘non-white’; therefore, the prediction

equation for Caucasians was used for NHANES patients characterized as 'white', and the prediction equation for African Americans was used for NHANES patients characterized as 'non-white'.

Fibrinogen Classification

Based on preliminary analyses of the distribution of fibrinogen among patients with COPD, four thresholds of baseline fibrinogen will be assessed in these analyses: 250 mg/dL, 300 mg/dL, 350 mg/dL, and 400 mg/dL. The terms "high" and "low" fibrinogen will refer to the level of fibrinogen in relation to the threshold(s) being assessed in the analysis.

Outcome Ascertainment

Exacerbations and other clinical events were recorded differently in each study depending on the study objectives and population. In ARIC and CHS, exacerbations were identified as hospitalizations with an International Classification of Diseases, 9th Revision, Clinical Modification (ICD-9-CM) code of (490.x, 491.x, 492.x and 496.x) in any position (principal or supporting diagnosis). In ECLIPSE, hospitalized COPD exacerbations were recorded in the supplemental clinical event file as 'EXACHOSP'. COPD exacerbations were not recorded outcomes in NHANES or FHS. All-cause mortality was recorded in each study in the integrated database.

Assay Methods

Several laboratory methods are available for the determination of plasma fibrinogen and these can be classified into two general categories:

1. *Functional methods* that measure "clottable" fibrinogen (Clauss method)
2. *Direct methods* that quantify the fibrinogen protein (Immunologic method)

In routine clinical laboratory practice, plasma fibrinogen is most commonly measured by a functional method that determines thrombin-clottable protein in plasma samples derived from whole blood collected with sodium citrate as the anticoagulant. These methods are based on the original method of Clauss and are frequently referred to as the Clauss or modified Clauss methods (Kamath and Lip, 2003). Direct methods, primarily methods that measure immunoreactive fibrinogen protein, are also available, and several have been developed for use with clinical samples. The immunologic methods have the advantage that they can be used with plasma prepared with a variety of anticoagulants (e.g., sodium citrate, EDTA), whereas the functional assays are most appropriately limited to plasma samples prepared with anticoagulants that have no impact on clottable fibrinogen, e.g., sodium citrate. Both the functional-based and immunologic-based methods can be calibrated against accepted international standards allowing for results from different laboratories, different methods, or different testing occasions to be properly compared (Whitton et al., 2000; Raut and Hubbard, 2010). For clinical studies, plasma fibrinogen concentration is typically measured in laboratories that adhere to accepted practices for the handling and testing of clinical samples and have well established and documented quality control processes for determining and monitoring assay performance. These assay methods were the subject of a literature review that was performed concurrently with these analyses.

Unpublished analyses have been conducted by industry to directly compare the fibrinogen results of samples tested using both the functional and direct assay methods, in order to identify an appropriate adjustment factor to account for the differences in fibrinogen results between these methods. Fibrinogen values for ECLIPSE have been corrected by -13.6% to account for the use of EDTA plasma instead of citrate plasma, based on data provided by the manufacturer of the assay used in ECLIPSE (Pacific Biometrics Inc., 2007).

DESCRIPTIVE ANALYSES

Objectives:

We will conduct several data validation steps to ensure the accuracy of the integrated dataset. UBC will attempt to reproduce patient counts from individual studies that have been either previously published in papers that give a clear description of the methods used to obtain those counts or data validation documents that have been released by the study coordinators. UBC will also note lab values that are out of range and verify these values with INC Research or the appropriate study coordinators.

These analyses will:

- Identify illogical, missing and out of range data values.
- Compare obtained counts with counts previously published or verified as accurate by the individual study coordinators to demonstrate reproducibility with source data.

Next, we will conduct univariate descriptive statistics on demographic and clinical information across all datasets and the integrated dataset. Although similar analyses may have been conducted previously in individual study datasets, the analytic population for the proposed analyses in this SAP may differ from the original studies. UBC will examine these sample descriptive statistics in each dataset and combined datasets, as they will be useful in future dissemination work to describe and characterize the patient population evaluated in subsequent analyses. Analyses will be performed among all patients included in the data, as well as those who meet the definition of COPD as previously described.

These descriptive analyses will:

- Identify important covariates
- Inform model development
- Characterize the study sample for use in future dissemination of research

Proposed Analyses:

1. Baseline characteristics

    1.1. Demographic characteristics

        Analyses:

Compute descriptive statistics for baseline demographic characteristics in each dataset and combined datasets. Results will report missing values, as well as mean (SD), median, and range (minimum to maximum) for continuous variables and frequencies (count and percentage) for categorical variables. Certain variables of interest are only available in a limited number of studies in the integrated database; when applicable, the studies in which these variables are available are noted below.

Variables:

- Age
- Gender
- Race
- Ethnicity
- Weight
- Height
- Body Mass Index (BMI)
- Systolic blood pressure (SBP)/ Diastolic blood pressure (DBP)
- Smoking history
- Heart rate : ARIC, CHS, ECLIPSE
- Pulse: NHANES
- Blood oxygen saturation (SPo2): ECLIPSE
- Medical History
    - Including history of COPD
    - Including history of COPD exacerbations

1.2. Baseline clinical characteristics

Analyses:

Descriptive statistics for baseline clinical characteristics in each dataset and combined datasets will be computed. Results will report mean (SD), median, and range (minimum to maximum) for continuous variables and frequencies (count and percentage) for categorical variables. The distribution of fibrinogen will additionally be summarized using interquartile ranges and the geometric mean, to better describe the skewed nature of the data.

Variables:

- Plasma fibrinogen
- Spirometry

  - Pre-bronchodilator Forced Expiratory Volume in 1 second (preFEV1)
  - preFEV1 (% predicted)
  - Pre-bronchodilator Forced Vital Capacity (preFVC)
  - preFVC (% predicted)
  - preFEV1/FVC ratio
  - Post-bronchodilator Forced Expiratory Volume in 1 second (postFEV1)
  - Post-bronchodilator Forced Vital Capacity (postFVC)
  - postFEV1 (% predicted)
  - postFVC (% predicted)
  - postFEV1/FVC ratio
- COPD exacerbations
  - Hospitalization
  - Death
  - Reported exacerbation

ASSESSMENT OF KEY ASSUMPTIONS

The use of this integrated dataset to accomplish the study objectives is predicated upon at least 2 assumptions:

1. Pre- and post-bronchodilator spirometric measurements each can be used to identify patients with COPD. Both measures are available only in the ECLIPSE and COPDGene studies. Utility of other studies requires the assumption that differences between pre- and post-bronchodilator measurements are not clinically important and that similar results are obtained using either method of defining COPD.
2. Most patients categorized at baseline as having ‘high fibrinogen’ maintain this classification during a period relevant to the conduct of a clinical trial.

Objectives:

These analyses will:

- Compare COPD definitions using pre- and post-bronchodilator spirometric data within the ECLIPSE and COPDGene data.
- Assess longitudinal variation in plasma fibrinogen within individuals and between individuals using data from ECLIPSE.

Analyses

1. Compare COPD definitions.

   Analyses:

   Two analyses are proposed to compare and validate the substitution of pre-bronchodilator spirometry measurements for post-bronchodilator measurements, which are recommended by GOLD but not available in all databases. First, we will compare patients categorized as having COPD using post-bronchodilator measurements (GOLD standard) with those classified as having COPD using pre-bronchodilator measurements. This analysis will permit calculation of error rates to determine the accuracy of using pre-bronchodilator measurements to define COPD in ECLIPSE. A similar analysis will be performed using data from the COPDGene study, which also collected pre- and post-bronchodilator spirometry measurements.

   This comparative analysis of pre- and post-spirometry measurements will first include patients meeting the criteria for GOLD stage 2+ (FEV1/FVC <0.70 and FEV1 <80% of normal). We will compare the n and % of patients classified as having COPD by using the pre-bronchodilator spirometry data with those classified as having COPD using the post-bronchodilator spirometry data in studies with both measurement available. If pre-bronchodilator spirometry measurements are a reasonable proxy for post-bronchodilator measurements in patients with moderate to severe COPD, a similar n and % of patients will be categorized as having COPD when using either pre- or post-bronchodilator measurements. Misclassification (error) rates, sensitivity, and specificity will also be calculated for this analysis. Sensitivity analyses will be performed to assess whether error rates can be reduced by further restricting the sample of patients included in analyses to those with either FEV1 <70% of normal or FEV1 <60% of normal.

   Variables for consideration:

   - Number of patients
   - Pre- and post-bronchodilator spirometry measurements

2. Assess longitudinal variation in plasma fibrinogen between individuals and within individuals over 3 years.

   Analyses:

   The purpose in assessing the longitudinal variation in plasma fibrinogen is to determine if patients can be meaningfully categorized into groups of those with ‘high’ and normal levels of fibrinogen for a duration corresponding to the likely maximum duration of most Phase 2 or Phase 3 COPD trials. A single fibrinogen measurement may be a suitable indicator of fibrinogen levels over a time period relevant to a clinical trial if it can be determined that the variability among fibrinogen measurements is not clinically important over time. Longitudinal data are required to assess intra-patient variability in fibrinogen levels over time and to help determine the stability of plasma fibrinogen concentration. Analyses assessing the stability of fibrinogen over time will be performed in ECLIPSE, the only study with longitudinal fibrinogen measurements.

Histograms will be used to present the initial fibrinogen levels of patients in the integrated database and individual studies. Additional histograms will also be presented in these samples, and fibrinogen distributions will be presented for patients with and without the presence of an outcome during follow-up (i.e., a hospitalized COPD exacerbation within 12 months, or mortality within 36 months). For histograms stratified by the presence of an outcome of interest during follow-up, the non-parametric Kolmogorov-Smirnov (KS) two-sample test statistic and corresponding p-value is also presented. This test compares the location and shape of the distribution of fibrinogen at baseline between subjects with and without outcomes. A statistically significant p-value (<0.05) indicates the two samples are derived from populations with a different distribution.

These histograms will help to inform the choice of threshold by identifying the distribution of fibrinogen measurements; the mean, geometric mean, median, and interquartile range of fibrinogen measurements in this cohort of patients will also be calculated. A logistic regression model using any exacerbation as an outcome and baseline fibrinogen levels as the independent variable will be used to determine if the fibrinogen threshold is associated with future COPD exacerbations. The purpose of these analyses is to assess the intra-patient variability of longitudinal fibrinogen measurements and not to identify the final fibrinogen threshold. For the purposes of this analysis, fibrinogen will be considered to be a stable biomarker if there is not an appreciable difference in the number of patients who are categorized as having high fibrinogen at the initial fibrinogen measurement when compared to subsequent measurements within 3 years.

We will compare the n and % of patients classified as having high fibrinogen at baseline with those classified as high fibrinogen at subsequent visits within 3 years. If fibrinogen is a stable biomarker, a similar n and % of patients will be categorized as having high fibrinogen at subsequent visits when compared to the initial measurement. Misclassification (error) rates, sensitivity, and specificity at each threshold of fibrinogen will also be calculated for this analysis.

The frequency of patients categorized as having high or low fibrinogen at each time point during the ECLIPSE study will also be tabulated. This analysis requires the exclusion of patients who are missing fibrinogen measurements at any time point for reasons which include death or other loss-to-follow-up. As such, patients with missing fibrinogen measurements are not randomly missing. The summary of loss to follow up due to mortality or other causes will also be summarized with these analyses for reference. This analysis will be repeated for each fibrinogen threshold assessed.

Scatter plots will also be generated using data from ECLIPSE to graphically present the level of fibrinogen at baseline and subsequent time points (6 months, 1 year, 2 years, and 3 years). Pearson correlation coefficients will be calculated to assess the correlation between baseline and subsequent fibrinogen levels at each time point.

A categorical analysis of variance (ANOVA) will be used to assess the variability of fibrinogen over time. Analyses will be performed using ECLIPSE data, using the temporary threshold as defined previously, within time periods of 3 years. PROC CATMOD will be used for the categorical analysis at multiple visit lengths. To perform an analysis of variance, only subjects with continuous visits are kept.

Variables for consideration:

- Gender
- Fibrinogen values

RELATIONSHIP BETWEEN FIBRINOGEN AND RISK OF ADVERSE COPD OUTCOMES

Objectives

The primary purpose of this analysis is to assess the relationship between plasma fibrinogen concentration and adverse COPD clinical outcomes within varying time points that are relevant to a conduct of a clinical trial, and to support the Cox models described later. The association between fibrinogen levels and COPD exacerbations will be evaluated at time points of 12 months and 18 months. The association between fibrinogen levels and mortality will be evaluated at time points of 2 years and 3 years. The nature of the relationship will be critical to the determination of an appropriate cut-point or threshold defining “high” fibrinogen.

These analyses will:

- Define a cut-point for patients with high fibrinogen
- Describe and compare patients with high fibrinogen and patients with low fibrinogen according to demographic characteristics and covariates
- Assess the relation between high fibrinogen and the risk of COPD exacerbations within 12 and 18 months and mortality within 2 and 3 years
    - Identify predictors using recommended cut points
- Assess the time-to-event (COPD exacerbation and/or all-cause mortality) among patients with high fibrinogen
- Assess the impact of fibrinogen on the sample size of a hypothetical clinical trial

Proposed Analyses

1. Assess the time-varying relationship between high fibrinogen and the risk of adverse COPD outcomes.

   Analysis:

   Cox proportional hazards models will be used to identify hazard risk ratios with corresponding 95% confidence intervals associated with fibrinogen levels as well as demographic and clinical characteristics. These models will account for the time-varying nature of the relationship between fibrinogen levels and exacerbations/all-cause mortality; as such, they can only be performed in studies that recorded the dates of outcomes of interest. There will be two main groups considered in the modeling process:

1. Age 40+, GOLD stage 2+, history of exacerbations unspecified

2. Age 40+, GOLD stage 2+, and any prior COPD exacerbation (ECLIPSE)

Analyses using hospitalized exacerbations as an endpoint will be completed for outcomes which are recorded within 12 months of the initial fibrinogen measurement. Analyses with mortality will be completed for outcomes which are recorded within 3 years of the initial fibrinogen measurement. Analyses will be conducted using the fibrinogen thresholds determined previously (250 mg/dL, 300 mg/dL, 350 mg/dL, and 400 mg/dL).

Each outcome model will be constructed as follows:

1. The association between baseline characteristics and each outcome will be examined using univariate models. These models will be fitted on data of patients with high and low fibrinogen, and will result in a set of baseline characteristics that are predictive of the outcome and will be used as candidates for the final model.

2. To identify confounders of the cohort-outcome effect (e.g., the association between high vs. low fibrinogen and a given outcome), all baseline characteristics will be tested individually in a model with cohort as the only other independent variable. Those variables modifying the cohort-outcome effect size by at least 10% will create an additional set of candidates for the final model.

3. To obtain the fully adjusted model, the first step will be to add the candidate variables from step 2 to the unadjusted model one at a time, in descending order of strength of the confounder (i.e., the percentage change in the hazard ratio that results from adding that variable to the unadjusted model). Variables that still modify the cohort-outcome effect size by at least 10% will be retained, as will any that are significant predictors of the outcome at $P<0.05$.

4. The second step in constructing the fully adjusted model will be to take the model resulting from step 3, which will include all of the confounders and significant predictors forced into the model. Into that model, all of the significant predictor variables identified in step 1 that were not tested in step 3 will be entered and backward selection with $P<0.05$ used to select from among these additional variables. This will allow inclusion of significant predictors in the model that do not necessarily act as confounders.

5. Finally, a correlation matrix will be created for all variables included in the model in step 4. If two or more variables are found to be highly correlated with one another (i.e., a correlation of 0.90 or greater), the variable that acts as the stronger confounder or the stronger predictor of the outcome will be selected and the other variable dropped from the model.

Hazard ratios and 95% confidence intervals will be presented for each of the final outcome models.

Variables for consideration:

- Demographic and clinical characteristics

1. Assess the relation between high fibrinogen and the risk of adverse COPD outcomes over time.

   Analysis:

   Time-to-event analyses will be performed using survival analysis methods for patients with high fibrinogen in studies which recorded dates of hospitalized exacerbations (ARIC, CHS, and ECLIPSE) and all-cause mortality (ARIC, CHS, ECLIPSE, FHS, and NHANES). PROC PHREG will be used to create Kaplan Meier plots for each outcome, and plots will be repeated for each threshold assessed. Means, standard deviations, medians, and ranges of time-to-events will also be calculated.

   Variables for consideration:

   - Demographic and clinical characteristics

2. Assess the impact of using a fibrinogen threshold to select patients at higher risk of outcomes on the sample size of a hypothetical clinical trial

   Analysis:

   Survival estimates from the Cox models produced for each outcome will be used to generate power analyses to present the sample sizes of a hypothetical clinical trial designed to assess hospitalized exacerbations within 12 months or mortality within 36 months. Sample sizes will be presented for each fibrinogen threshold and will also be presented for hazard ratios of 0.6, 0.7, and 0.8. This analysis will assume a power of 0.8 and a 10% loss to follow-up. Equal sample sizes will be assumed for both “control” and “treatment” groups.

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