Novel Markers of Thrombotic Risk

Clinical Trial Registration: NCT02505217

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Objectives:

1. To determine whether four novel biomarkers identified in blood early after myocardial infarction will be evident in the same subjects 6 months later. The biomarkers are increased platelet expression of FcγRIIa, the ratio TF:TFPI, and the reduction in coagulation factors II and X, and thrombin generation.
2. To determine whether the four biomarkers defined above are associated with a greater risk of cardiovascular events (stroke, myocardial infarction and death) and a greater risk of bleeding.

Protocol

After informed consent is obtained, the patients will be interviewed and their medical records will be reviewed. We will ask about previous medical problems and medications. Clinical characteristics will be recorded in a manner that protects confidentiality. Blood (15 ml, 1 tablespoon) will be taken by peripheral venipuncture. The following biochemical assays will be performed

1. Platelet expression of FcγRIIa with the use of fluorochrome labelled antibodies and flow cytometry
2. The concentration of coagulation factors in blood will be quantified.
3. A thrombin generation assay will be performed. In this assay, thrombin production will be initiated by lipidated tissue factor in both platelet rich and platelet poor plasma. Thrombin formed will hydrolyze a specific substrate to give a fluorescent signal that is continuously recorded. This analysis will assess the entire process of thrombin generation with respect to the initiation, propagation, and termination phases of the reaction.

Patients will have an ambulatory visit 6 months later. This visit will be a research visit but we will attempt to perform the research visit in the context of a clinical visit. During this visit, the patient will be interviewed (focusing on current medications and clinical events that occurred during the previous 6 months), medical records will be reviewed, and clinical characteristics will be recorded in a manner that protects confidentiality. Blood (15 ml, 1 tablespoon) will be taken by peripheral venipuncture. The biochemical assays above will be performed a second time.

Patients will be contacted by telephone 1 year after study initiation and a final time after the last patient enrolled completes 1 year of follow-up. The patient will be interviewed and, if appropriate, medical records will be reviewed. We will ask about medical conditions focusing on events that occurred during the previous year. Clinical characteristics will be recorded in a manner that protects confidentiality.

Clinical care of patients will not be altered by participation in this study. Participation will end after telephone interview performed 1 year after the last patient is enrolled.

Statistical Plan

Sample Size Calculations: We propose to enroll 200 subjects. We have not yet established a specific thresholds to identify high platelet reactivity, high TF:TFPI, depletion of coagulation factors, or altered thrombin generation. For the purposes of this study we will define the

upper/lower 33% of the group as a whole as expressing 'high' values or depletion of coagulation factors. Power calculations are based on the association of the novel biomarkers with subsequent clinical events. Based on recent trial results, we anticipate that at least 10-12% of the entire group will have a cardiovascular event during the subsequent year. Based on our previous retrospective results we hypothesize that high expression of FcγRIIa will be ≥11,000 molecules/platelet. For the hypotheses above to have clinical utility, they must identify substantial differences. Accordingly we powered the study to identify groups that exhibit a 2-fold increase in risk. An event rate of 14% in the high risk group and 7% in the control group can be identified with a power of 95% ($\alpha<0.05$) when 200 subjects are enrolled.

Statistical Rationale: Our primary analysis will test each biomarker and associated hypothesis independently. Because an acute event such as a myocardial infarction may amplify the extent of alteration (i.e. enhance the elevation or depletion) the initial analysis will determine whether the initially identified groups are significantly different at the time of the second analysis. In addition we will determine the extent of change for each of the biomarkers over time, assessing all subjects (the total group) and the originally defined groups. Secondary analysis will assess the extent of overlap between the originally defined groups and the relative predictive value of each parameter as well as correlating each of the parameters with subsequent risk of cardiovascular events. Sensitivity analyses will be performed to assess additional thresholds to identify high and low expression of biomarkers.

Primary Endpoint Analysis: We will compare results between the 2 groups with analysis of variance (ANOVA). Multiple analysis of variance with time as a covarate will be used to assess changes over time in all subjects and each of the originally defined groups.

Secondary Endpoint Analysis: The incidence of clinical events in the originally defined groups will be compared with the use of chi squared analysis. A logistic regression model will be implemented to examine the joint relationship of clinical endpoint incidence relative to baseline group classifications. Potential confounding measures will be included to adjust for confounding group differences. A full time-to-event analysis will start with the estimation of Kaplan-Meier plots to characterize the subject data for the full follow-up time period. This analysis will be followed by primary survival analysis modeling performed with the use of Cox regression methods.