The Prognostic Significance of Biomarkers in Predicting Outcome in Patients With Coronary Artery Disease and Left Ventricular Dysfunction: Results of the Biomarker Sub-Study of the Surgical Treatment for Ischemic Heart Failure (STICH) Trials

Feldman et al: Results of the Biomarker Sub-Study of STICH Trials

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Abstract

Background—Patients with heart failure and coronary artery disease often undergo coronary artery bypass grafting (CABG) but assessment of the risk of an adverse outcome in these patients is difficult. To evaluate the ability of biomarkers to contribute independent prognostic information in these patients, we measured levels in patients enrolled in the Biomarker Sub-studies of the Surgical Treatment for Ischemic Heart Failure (STICH) trials. Patients in STICH Hypothesis 1 were randomized to medical therapy or CABG whereas those in STICH Hypothesis 2 were randomized to CABG or CABG with left ventricular reconstruction.

Methods and Results—In sub-study patients assigned to STICH Hypothesis 1 (n=606), plasma levels of sTNFR-1 and BNP were highly predictive of the primary outcome variable of mortality by univariate analysis (BNP $\chi^2=40.6; p<0.0001$: sTNFR-1 $\chi^2=38.9; p<0.0001$). When considered in the context of multivariable analysis, both BNP and sTNFR-1 contributed independent prognostic information beyond the information provided by a large array of clinical factors independent of treatment assignment. Consistent results were seen when assessing the predictive value of BNP and sTNFR-1 in patients assigned to STICH Hypothesis 2 (n=626). Both plasma levels of BNP ($\chi^2=30.3$) and sTNFR-1 ($\chi^2=45.5$) were highly predictive in univariate analysis ($p<0.0001$) as well as in multivariable analysis for the primary endpoint of death or cardiac hospitalization. In multivariable analysis, the prognostic information contributed by BNP ($\chi^2=6.0; p=0.049$) and sTNFR-1 ($\chi^2=8.8; p=0.003$) remained statistically significant even after accounting for other clinical information. Although the biomarkers added little discriminatory improvement to the clinical factors (increase in c-index $\leq 0.1$), Net Reclassification Improvement (NRI) for the primary endpoints was 0.29 for BNP and 0.21 for sTNFR-1 in the Hypothesis 1 cohort, and 0.15 for BNP and 0.30 for sTNFR-1 in the Hypothesis 2 cohort, reflecting important predictive improvement.

Conclusions—Elevated levels of sTNFR-1 and BNP are strongly associated with outcomes, independent of therapy, in two large and independent studies, thus providing important cross-validation for the prognostic importance of these two biomarkers.

Key Words: heart failure, cardiovascular disease, bypass graft
Heart failure secondary to systolic dysfunction affects over 5 million individuals in the U.S. In the majority of these patients their left ventricular dysfunction is attributable to coronary artery disease.\(^1\) Patients with heart failure and symptomatic coronary artery disease often undergo coronary artery bypass grafting (CABG) as do some patients with asymptomatic coronary disease; however, surgical intervention in patients with symptomatic disease is often associated with a high morbidity and mortality. A group of clinical indexes were developed to help assess the risk for an adverse outcome in patients undergoing CABG.\(^2\)\(^-\)\(^6\) However, when Orr et al assessed the validity of four severity-adjusted models that used clinical metrics to predict mortality following CABG, they found that the predicted mortality rate varied by a factor of 3.3 from the lowest to highest leading the investigators to suggest that the use of these models for individual patient risk estimates is “risky” because of the significant discrepancies in individual predictions created by each model.\(^7\) More recently, biomarkers have been shown to predict long-term morbidity and mortality in patients with heart failure secondary to diminished left ventricular function\(^8\)\(^-\)\(^10\); however, the association of biomarkers with outcomes in patients with heart failure who are undergoing CABG has not been defined.

The Surgical Treatment for Ischemic Heart Failure (STICH) trials provided the opportunity to test the hypothesis that biomarkers are predictive of risk in patients with heart failure undergoing CABG. The STICH trial was designed to address two areas of equipoise.\(^11\)

First, STICH Hypothesis 1 evaluated whether patients with coronary artery disease and left ventricular dysfunction benefit from the combination of optimal medical therapy and coronary artery bypass grafting (CABG) when compared with optimal medical therapy alone. In STICH Hypothesis 2, we tested whether patients with left ventricular dysfunction who were undergoing CABG benefited from the addition of left ventricular reconstruction. For patients
assigned to Hypothesis 1, the difference between medical therapy alone and medical therapy plus CABG with respect to the primary endpoint of death from any cause was not statistically significant.\textsuperscript{12} For patients assigned to STICH Hypothesis 2, the addition of surgical ventricular reconstruction to CABG did not reduce the primary outcome variable of death or hospitalization for a cardiac cause.\textsuperscript{13}

To test the hypothesis that plasma levels of biomarkers were associated with outcome in patients with ischemic heart failure being considered for surgical revascularization, we measured levels of norepinephrine, brain natriuretic peptide (BNP), and the soluble tumor necrosis factor-alpha receptor-1 (sTNFR-1) in patients enrolled in the two STICH studies who participated in the STICH biomarker sub-study. These three biomarkers were chosen because each had been shown to be associated with outcomes in patients with heart failure.\textsuperscript{8,9,10} STICH Hypothesis 1 and STICH Hypothesis 2 provided two discrete investigational groups in which to test and confirm the hypothesis that biomarkers are predictive of clinically important outcomes in ischemic heart failure patients eligible for cardiac surgery.

**Methods**

**Study Design:**

The rationale and design of the STICH trial were presented previously in detail.\textsuperscript{11-13} In brief, STICH enrolled 2,136 patients with a left ventricular ejection fraction \( \leq 35\% \) and coronary artery disease that was amenable to coronary artery bypass grafting (CABG). All patients underwent cardiac imaging for assessment of left ventricular function and wall motion with either cardiac magnetic resonance imaging, radionuclide or echocardiography, and predefined baseline variables were recorded.\textsuperscript{12} Patients were then assigned by the enrolling physician to one
of three strata. Stratum A included patients who were eligible for either medical therapy alone or medical therapy plus CABG. Stratum B included patients who were eligible for medical therapy alone, medical therapy plus CABG, or medical therapy plus CABG and surgical left-ventricular reconstruction (SVR). Stratum C patients were eligible for medical therapy plus CABG or medical therapy plus CABG and left ventricular reconstruction. Patients were then randomly assigned in equal proportions to one of the treatment options for which they were eligible. All of the patients in stratum A and some of the patients in stratum B were randomly assigned to medical therapy or medical therapy plus CABG (STICH Hypothesis 1). All of the patients in stratum C and some of the patients in stratum B were randomly assigned to medical therapy and CABG or to medical therapy plus CABG and SVR (STICH Hypothesis 2). The 76 patients randomized to CABG in Stratum B fit the criteria for assignment to either Hypothesis 1 or Hypothesis 2. Those patients and their biomarker data were analyzed with the Hypothesis 1 cohort. All patients received pharmacologic or device therapy based on consensus guideline recommendations. Approval from the institutional review board was obtained from each institution participating in the Biomarker sub-study of the STICH trial and all patients provided written informed consent.

In STICH Hypothesis 2, hospitalizations were adjudicated and classified by an independent Clinical Events Committee, since cardiovascular hospitalization was a component of the primary endpoint. In STICH Hypothesis 1, there was an identical adjudication process for reviewing and classifying hospitalizations, which continued until the completion of STICH Hypothesis 2, at which point (due to limited resources), hospitalizations were no longer adjudicated by the independent committee since that information was not part of the primary Hypothesis 1 endpoint. Thereafter, cause of hospitalization was site reported.
Sample Collection:

Blood samples for measurement of plasma biomarkers were drawn at baseline after inserting a 20 or 21 gauge butterfly needle into an arm vein and placing the subject in a quiet room in a supine position for 30 min. The first 3 ml of blood was discarded and a sample was then placed in an EDTA containing tube for measurement of norepinephrine. Blood samples for measurement of sTNFR-1 and BNP were then collected in endotoxin free EDTA tubes to minimize ex vivo production and/or catabolism of cytokines. No other biomarkers were measured. All samples were immediately stored on ice, centrifuged within 30 min, and the plasma was separated and rapidly frozen at -70°C. Samples were shipped in batches on dry ice from individual centers to the biomarker core laboratory. Samples were logged in using bar codes and stored at -80°C until they were forwarded on dry ice to laboratories for analysis of norepinephrine (M.R. Bristow, University of Colorado School of Medicine, Denver CO), BNP (A.S. Maisel, School of Medicine, University of California, San Diego, CA; A. M. Feldman, Thomas Jefferson University, Philadelphia, PA) and sTNFR-1 (D. L. Mann, Washington University School of Medicine, St. Louis, MO).

Measurement of Biomarker Levels:

Norepinephrine was assayed from -80°C stored plasma by HPLC-electrochemical detection, using an Agilent 1100 HPLC system (Palo Alto, CA), ESA Plasma Catecholamine Analysis Kits (Chelmsford, MA) and a Coulochem® III Multi-Electrode Electrochemical Detector (ESA, (Chelmsford, MA)) according to the manufacturers' specifications.

BNP assays were initially performed at the BNP core facility at the Veterans Administration Hospital, San Diego, using the Bayer ADVIACentaur BNP assay. During the ten year course of the STICH trial, the BNP core lab was moved from San Diego to the clinical chemistry laboratory at Thomas Jefferson University Hospital. There, BNP levels were measured using a similar 2 site sandwich immunoassay with a chemiluminescent indicator but we used the Biosite Triage BNP reagents and the Beckam Coulter Unicel DxI platform. The normal range BNP value for the laboratory was <125 pg/ml. There was a high degree of correlation (r = 0.964) between the two methodologies which allowed us to include all available BNP measurements by using a simple regression adjustment to standardize the measurements from one core lab to be comparable to the measurements from the other core lab. The Thomas Jefferson measurements were used as the standard for these analyses.

Plasma levels of sTNFR-1 were measured using an ELISA assay (RIO Systems, Minneapolis, MN) as previously described. To safeguard against the presence of heterophile antibodies, all cytokine assays were performed using at least one serial dilution to ensure that the samples diluted appropriately; subsequent dilutions were performed as necessary. The normal value is a mean of 1198 pg/ml. In preliminary control experiments we determined that the process of storing and shipping the plasma samples to the Core Laboratory resulted in negligible change in the detectable levels of norepinephrine, BNP or sTNFR-1.

Statistical Analysis:

Data were descriptively summarized using the median and interquartile range for continuous variables and frequencies and percentages for categorical variables.
A unique feature of STICH is that it consisted of two different studies (Hypothesis 1 and Hypothesis 2), thus enabling an examination and confirmation of the relationships of the biomarkers with clinical outcomes in two separate studies. Although we sought to acquire samples for biomarker analysis in every patient, due to the international scope of the STICH program, there were restrictions in some participating countries or centers where the collection/shipping of samples was not possible. Because samples could not be obtained in every randomized patient, we examined the baseline characteristics and outcomes of patients for whom biomarker data were available compared to the patients without samples to assess the degree to which the biomarker sub-study cohort was representative of the overall study population. The distributions of continuous variables were compared between these groups using the Wilcoxon rank-sum test, and categorical variables were compared using conventional chi-square statistics. The primary endpoint in each trial (mortality in Hypothesis 1 and death or cardiovascular hospitalization in Hypothesis 2) was also compared between patients in the biomarker sub-study compared to patients who were not included. Event rate estimates in each group and for each endpoint were calculated using the Kaplan-Meier method,\(^{17}\) and statistically compared using the Cox proportional hazards regression model.\(^{18}\) Finally the randomized treatment comparison for the primary endpoint in each trial was examined among patients in the biomarker sub-study compared to the excluded patients to assess comparability of treatment comparisons in the biomarker sub-study cohort compared with the overall trial primary results.

Treating the biomarker measurements as continuous variables, the nature (shape) and strength of the relationships of each biomarker with the clinical outcomes of (a) death and (b) death or cardiac hospitalization were examined by modeling the relationship using restricted cubic spline functions within the framework of the Cox regression model.\(^ {19}\) Where the
relationships were non-linear, a parsimonious model using piecewise linear splines was used to characterize the relationship. These relationships were assessed separately in the Hypothesis 1 and Hypothesis 2 cohorts in order to examine the consistency of the relationships across the two studies.

Once the nature of the relationship was established for each biomarker, we then examined the extent to which the biomarkers contributed independent prognostic information for death and for death or cardiac hospitalization. Using multivariable analyses with the Cox regression model and summarizing the results in terms of likelihood ratio chi-square statistics and corresponding p-values as well as hazard ratios and confidence intervals, we assessed whether any of the biomarkers (norepinephrine, BNP or sTNFR-1) contributed significant independent prognostic information to the array of other baseline clinical variables available in these patients. The other clinical variables included age, gender, race, New York Heart Association heart failure classification, history of (a) myocardial infarction, (b) stroke, (c) diabetes, (d) atrial fibrillation, and (e) hyperlipidemia, creatinine, hemoglobin, coronary anatomy characterized by the Duke Coronary Artery Disease (CAD) index, end systolic volume index (ESVI), mitral regurgitation, whether the patient could perform a six-minute walk test, randomization stratum, and randomized treatment assignment. In addition to the Cox regression assessments described above, we also used the c-index for time-to-event data (analogous to the area under the ROC curve in the case of a binary endpoint) and calculated the extent to which the biomarkers improved the discriminatory ability of the models beyond the discrimination provided by the clinical variables listed above. We also calculated the Net Reclassification Improvement (NRI) for each biomarker using the category-free extension of NRI for time-to-event data. Finally, we addressed the question of whether these biomarkers are helpful in selecting a particular
treatment strategy, i.e., whether there is a differential effect of treatment across the range of values of the biomarker. In the Hypothesis 1 cohort, we examined whether CABG + medical therapy had a greater (or lesser) effect on clinical outcomes compared to medical therapy alone depending on the value of the biomarkers. This assessment was performed by examining treatment by biomarker interactions using the Cox model, making full use of the continuous range of biomarker values and the shape of the biomarker relationships with clinical outcomes. Identical analyses were performed in the Hypothesis 2 cohort.

Because investigators often present biomarker data in the form of tertiles, we performed a secondary analysis based on tertiles of each biomarker where the tertiles were calculated separately in the Hypothesis 1 and the Hypothesis 2 sub-study cohorts. Within each tertile, Kaplan-Meier estimates of (a) death and (b) death or cardiovascular hospitalization were calculated to illustrate and compare the gradient of risk across tertiles for each biomarker and each treatment arm within each of the biomarker sub-study cohorts. Hazard ratios, 95% confidence intervals, and p-values were generated for these analyses using the Cox model. All data analysis was performed by the STICH Coordinating Center, the Duke Clinical Research Institute, Duke University, Durham, NC. All statistical analyses were performed using SAS software, version 9.2 (SAS Institute, Cary NC).

Results

Biomarkers and STICH Hypothesis 1:

Description of Biomarker Cohort:

Six hundred ten of the 1,212 patients enrolled in STICH Hypothesis 1 participated in the biomarker substudy. Four hundred seventy nine patients provided a blood sample for the
measurement of norepinephrine, 607 for BNP and 607 for sTNFR-1, with 606 patients having measurements of both BNP and sTNFR-1. To provide comparative assessments of BNP and sTNFR-1 in an identical set of patients, the 606 patients with both measurements constitute the primary Hypothesis 1 biomarker substudy population. Table 1 shows selected baseline characteristics of the 606 biomarker substudy patients compared with the remainder of the STICH Hypothesis 1 patients. A full list of baseline characteristics of these two patient groups can be found in the Supplemental materials (Supplemental Table I). Although there were some baseline differences between the two groups, there was a high degree of concordance in important baseline characteristics including gender, the presence of diabetes mellitus, the presence of hypertension, severity of coronary disease, New York Heart Association Classification, and serum creatinine. Importantly, treatment assignment had the same effect on patient outcome in the patients enrolled in the STICH Hypothesis 1 biomarker sub-study as it did in the total STICH population (the hazard ratio comparing medical therapy plus CABG vs. medical therapy alone was 0.86 in the biomarker substudy patients and 0.86 in the remaining patients, identical to the treatment comparison in the overall STICH Hypothesis 1 population). (Supplemental Figure I)

In the Hypothesis 1 cohort, the median value of BNP was 313 pg/mL (180, 569; 25\textsuperscript{th} and 75\textsuperscript{th} percentiles), the median sTNFR1 was 1399 pg/ml, (1112, 1955; 25\textsuperscript{th} and 75\textsuperscript{th} percentiles) and the median norepinephrine was 507 pg/mL (337, 769; 25\textsuperscript{th} and 75\textsuperscript{th} percentiles).

Biomarker Relationships with Clinical Outcomes:

Across the continuum of BNP, there was a sharply increasing risk of death with increasing values of BNP, up to a value of approximately 400 pg/mL. Beyond that point, the level of risk exhibited no further increase. (Figure 1). For sTNFR-1, the risk was relatively level
for low values up to approximately 1200 (pg/ml), beyond which the risk increased sharply with increasing values of sTNFR-1 up to a level of approximately 2200 pg/mL. Beyond that point, no further increase was observed (Figure 2). For the composite endpoint of death or cardiac hospitalization, the patterns for the two biomarker relationships were similar to that observed for mortality alone except that there continued to be a slight further increase in risk as BNP increased beyond 400 pg/mL.

As seen in Table 2, plasma levels of sTNFR-1 and BNP were highly predictive of both the primary and secondary outcome variables in Hypothesis 1 by univariate analysis. For example, both BNP ($\chi^2 = 40.6; p < 0.0001$) and sTNFR-1 ($\chi^2 = 38.9; p < 0.0001$) contributed important prognostic information in Hypothesis 1 patients with respect to the risk of reaching the primary endpoint of death. Similar results were found for the secondary endpoint of death or cardiovascular hospitalization. Moreover, when considered in the context of multivariable analysis with the pre-defined baseline clinical variables, both BNP and sTNFR-1 contributed independent prognostic information for mortality beyond the information provided by the large array of other clinical factors (the addition of BNP to the clinical factors added a $\chi^2$ of 11.9; $p < 0.001$ and the addition of sTNFR-1 added a $\chi^2$ of 18.5; $p < 0.0001$). Translating these numbers into the increased level of risk conferred by increases in BNP or sTNFR-1, even after adjusting for the other clinical variables, an increase of 100 pg/mL in BNP (up to a level of approximately 400 pg/mL) increased the risk of death by 30% (adjusted hazard ratio 1.30, 95% confidence interval (1.11, 1.51)), and an increase in sTNFR-1 of 200 pg/mL (in the range of 1200 to 2200) elevated the risk of death by approximately 20% (adjusted hazard ratio 1.19, 95% confidence interval (1.10, 1.29)). Similarly significant results were seen when analyzing the risk of a patient reaching the secondary endpoint of death or cardiovascular hospitalization. (Table 2)
Discrimination and Net Reclassification Improvement:

The improvements in discrimination (c-index) produced by BNP or sTNFR-1 for either endpoint were small, namely 0.01 in each case. For mortality, the c-index increased from 0.67 to 0.68 for either biomarker. Such a small increase is not surprising, however, given prior experience with this measure of discrimination. Net Reclassification Improvement (NRI) for BNP beyond the clinical factors was a respectable 0.29 for mortality though only 0.17 for death or CV hospitalization. For sTNFR-1, the NRI was 0.21 for mortality and 0.37 for death or CV hospitalization.

Plasma levels of norepinephrine were not associated with the risk of meeting either the primary endpoint of mortality ($\chi^2 = <0.1, p = 0.819$) or the secondary endpoint of death or cardiovascular hospitalization ($\chi^2 = 0.1, p=0.730$).

Tertile Analysis:

We assessed the relationship between tertile levels of biomarkers and outcomes in patients enrolled in Hypothesis 1 of STICH. There was a direct relationship between the rate of death and the BNP tertile for patients randomly assigned to CABG ($\chi^2 = 21.6; p < 0.001$) with the highest tertile group having the worst prognosis and the lowest tertile group having the best prognosis. (Figure 3A) Similarly, increasing BNP tertiles were associated with a higher rate of death in Hypothesis 1 patients randomized to medical therapy. ($\chi^2 = 14.8; p = 0.001$) Tertiles of sTNFR-1 levels were also predictive of the rate of death in Hypothesis 1 patients undergoing CABG. ($\chi^2 = 17.3; p < 0.001$) as well as in those undergoing medical therapy ($\chi^2=17.0; p < 0.001$). Tertiles of sTNFR-1 levels were also highly predictive of a patient reaching the secondary endpoint of death or cardiovascular hospitalization if they were randomly...
assigned to medical therapy ($\chi^2 = 14.2; p = 0.001$) or to CABG ($\chi^2 = 13.1; p = 0.001$). In contrast, tertiles of BNP levels were only nominally useful in predicting the occurrence of death or cardiovascular hospitalization in patients allocated to CABG ($\chi^2 = 8.6; p = 0.014$), and were not significant in predicting this endpoint in patients randomized to medical therapy ($\chi^2 = 4.1; p = 0.129$).

Consistency of Prognostic Relationships Across Treatments:

There was no evidence of an interaction between treatment and any of the three biomarkers for either the primary mortality endpoint or the composite of death or hospitalization for a cardiac cause (interaction p-values were all > 0.2 and most were considerably larger, both unadjusted and adjusted for other clinical factors). Thus the relative effect of CABG was consistent across the range of all the biomarker values represented in the Hypothesis 1 cohort, and the prognostic effect of the biomarkers did not vary by treatment.

Biomarkers and STICH Hypothesis 2:

Description of Biomarker Cohort:

Although there were a total of 1000 patients enrolled in STICH Hypothesis 2, we excluded the 76 patients who were also in Hypothesis 1 and restricted the Hypothesis 2 biomarker analysis to the completely independent cohort of 924 patients. Six hundred eighty-two of the 924 patients participated in the biomarker substudy. 578 patients provided a blood sample for the measurement of norepinephrine, 676 for BNP, and 679 for sTNFR-1 measurements (with 676 patients having blood samples for both BNP and sTNFR-1 measurements). Again, to provide comparative assessments of BNP and sTNFR-1 in an identical set of patients, the 676 patients with both measurements constitute the primary Hypothesis 2 biomarker substudy population. For the Hypothesis 2 cohort the median value of
BNP was 274 pg/mL (178, 543; 25th and 75th percentiles) the median sTNFR1 was 1358 pg/ml (1063, 1761; 25th and 75th percentiles) and the median norepinephrine was 404 pg/mL (250, 583; 25th and 75th percentiles),

Table 3 presents selected baseline characteristics of these 676 patients compared to the remainder of the 924 study patients. As observed in Hypothesis 1, there was good concordance between baseline characteristics in the two groups. A full list of baseline characteristics of these two groups can be found in the Supplemental materials (Supplemental Table II). Furthermore, as seen in STICH Hypothesis 1, treatment assignment had the same effect on patient outcome in the patients enrolled in the Hypothesis 2 biomarker sub-study as it did in the total Hypothesis 2 population. There was no difference in the primary endpoint of mortality or cardiovascular hospitalization when comparing patients randomized to CABG alone versus those randomized to CABG plus SVR. (Supplemental Figure II)

Biomarker Relationships with Clinical Outcomes:

The relationships between each of the biomarkers (BNP, sTNFR-1) and the endpoints of death or cardiac hospitalization and death alone in the Hypothesis 2 cohort were remarkably similar to the relationships observed in Hypothesis 1 patients. Across the continuum of BNP, there was a sharply increasing risk of death and of death or cardiovascular hospitalization with increasing values of BNP, up to a value in this case of approximately 300 pg/mL. Beyond that point, there was only a small and gradual increase in risk with increasing values of BNP. (Figure 4) For sTNFR-1, again the risk was level up to approximately 1200 pg/mL, beyond which the risk increased sharply with increasing values of sTNFR-1 up to approximately 2200 pg/mL, beyond which no further increase was observed (Figure 5)
Table 4 demonstrates that for patients allocated to STICH Hypothesis 2, both plasma levels of BNP (χ² = 30.3) and sTNFR-1 (χ² = 45.5) were highly predictive in univariate analysis (p < 0.001) for the primary endpoint of death or cardiac hospitalization. Similarly significant results were observed when analyzing the risk of a patient reaching the secondary endpoint of death. In multivariable analysis, the prognostic information contributed by BNP (χ² = 6.0; p=0.049) and sTNFR-1 (χ² = 8.8, p=0.003) remained statistically significant even after accounting for the other clinical information, including highly prognostic factors such as ESVI. Translating these numbers into the increased level of risk conferred by increases in BNP or sTNFR-1 after adjusting for the other clinical variables, an increase of 100 pg/mL in BNP (up to a level of approximately 300 pg/mL) increased the risk of death or cardiovascular hospitalization by 26% (adjusted hazard ratio 1.26, 95% confidence interval (1.04, 1.52)), and an increase in sTNFR-1 of 200 pg/mL (in the range of 1200 to 2200) also elevated the risk of death by 26% (adjusted hazard ratio 1.26, 95% confidence interval (1.08, 1.47). Consistent results were seen when analyzing the Hypothesis 2 secondary endpoint of death. Therefore, the predictive value of BNP and sTNFR-1 observed in the independent Hypothesis 2 cohort is very similar to the results obtained in the Hypothesis 1 cohort.

Discrimination and Net Reclassification Improvement:

In the Hypothesis 2 cohort, the improvement in discrimination (c-index) produced by BNP or sTNFR-1 for either endpoint was very small, namely less than 0.01 in each case. For mortality, the c-index increased from 0.728 to 0.733 for BNP and from 0.728 to 0.731 for sTNFR-1. Net Reclassification Improvement (NRI) for BNP beyond the clinical factors was 0.13 for mortality and 0.15 for death or CV hospitalization. For sTNFR-1 the NRI was higher, namely 0.38 for mortality and 0.30 for death or CV hospitalization.
Plasma levels of norepinephrine did not have a significant relationship with the primary endpoint of death or cardiovascular hospitalization in the Hypothesis 2 patient cohort ($\chi^2 = 2.6$, $p=0.457$). There was a significant univariate association of norepinephrine with the secondary endpoint of mortality ($\chi^2 = 8.3$, $p=0.004$), which was not significant after adjusting for the clinical variables ($\chi^2 = 1.3$, $p=0.258$).

Tertile Analysis:

As observed in STICH Hypothesis 1, grouping of patients in tertiles based on their levels of BNP or sTNFR-1 demonstrated a marked gradient of risk among patients enrolled in Hypothesis 2 and randomly assigned to either CABG alone or CABG plus left ventricular reconstruction when assessing the primary endpoint of death or cardiovascular hospitalization (Figure 6A and 6B). There was a direct relationship between the rate of death or cardiovascular hospitalization and the BNP tertile for Hypothesis 2 patients randomly assigned to CABG ($\chi^2 = 8.3 ; p = 0.016$) with the lowest tertile group having the best prognosis. (Figure 6A) Similarly, increasing BNP tertiles were associated with a higher rate of death or cardiovascular hospitalization in Hypothesis 2 patients randomized to CABG plus SVR. ($\chi^2 = 20.7; p < 0.001$)

Tertiles of sTNFR-1 levels were also predictive of the rate of death or cardiovascular hospitalization in Hypothesis 2 patients undergoing CABG. (Figure 6B: $\chi^2 = 20.5; p < 0.001$) as well as in those undergoing the combination of CABG plus SVR ($\chi^2 = 23.7; p < 0.001$). Tertiles of sTNFR-1 levels were also highly predictive of a patient reaching the secondary endpoint of death if they were randomly assigned to CABG ($\chi^2 = 17.5; p < 0.001$) or to CABG plus SVR ($\chi^2 = 31.4 ; p < 0.001$). In contrast, tertiles of BNP levels were only marginally associated with the secondary endpoint of death in patients allocated to CABG ($\chi^2=4.1 ; p = 0.128$) and strongly associated with death in patients allocated to CABG plus SVR ($\chi^2 = 31.8; p < 0.001$).
Consistency of Prognostic Relationships Across Treatments:

Similar to the Hypothesis 1 cohort, there was no evidence of an interaction between treatment and any of the three biomarkers for either endpoint (large interaction p-values). Thus the absence of a treatment effect of CABG + surgical ventricular reconstruction was consistent across the range of biomarker values represented in the Hypothesis 2 cohort, and the predictive ability of the biomarkers did not vary by treatment arm.

Discussion

Investigators have developed a group of risk indexes that are predictive of postoperative mortality, morbidity and hospital stay in patients undergoing CABG. These risk indexes have used a variety of clinical and laboratory evaluations but have questionable predictive value, limited accuracy, and are of uncertain value as a clinical tool. We report results from two independent but closely related clinical trials demonstrating that sTNFR-1 and BNP, but not norepinephrine, are strongly associated with outcome in patients with ischemic heart failure undergoing CABG or the combination of CABG and left ventricular reconstruction when assessed both by univariate and multivariable analyses. BNP and sTNFR-1 levels were also predictive of outcomes in patients receiving optimal medical therapy. However, the highly prognostic value of these two biomarkers was independent of treatment. Importantly, the addition of biomarkers to a group of historical, morphologic, hemodynamic and functional parameters significantly improved the predictive value of this group of clinical variables. Interestingly, sTNFR-1 appeared in these analyses to generally be more strongly associated with outcome, either alone or in combination with clinical co-variables, than was BNP.
Our finding that norepinephrine was not a useful predictor of outcome in patients with heart failure is in contrast to previous studies.8,23-25 This disparity could be explained by several factors. Earlier studies were all in relatively advanced heart failure patients, a substantial number of whom would not have been eligible for the STICH protocol. Accurate assessment of plasma norepinephrine levels requires that patients be free of external stimuli that would enhance adrenergic drive at the time of sample collection. Many of the samples for STICH were drawn contemporaneously with a hospitalization for CABG, a time when patients might be expected to have a high level of adrenergic drive. Finally, we assessed norepinephrine levels in a group of patients with ischemic heart failure whereas earlier studies evaluated norepinephrine levels in patients with both ischemic and non-ischemic disease, another possible confounding factor.

Our finding that elevated plasma levels of BNP were associated with a worse outcome in a heart failure population undergoing consideration for CABG is consistent with earlier studies in patients with both ischemic and non-ischemic heart failure. Measurement of the levels of BNP and NTproBNP are useful in the diagnosis of heart failure and risk stratification in the emergency department, at the time of hospital admission, and at the time of hospital discharge when used as a complement to the clinical examination and other available diagnostic tests including renal function and body mass index.9,26 More recently, Huftless and colleagues reported that high pre-operative BNP levels predicted postoperative complications and one-year mortality after CABG; although they reported that BNP levels were best used in conjunction with multivariable risk indexes.27 BNP has also been shown in a group of observational cohort studies to be a marker of cardiac dysfunction and both short and long-term survival in patients undergoing CABG.28
Plasma levels of sTNFR-1 were strongly associated with clinical outcome in patients with heart failure while exceeding the predictive value of levels of BNP in Hypothesis 2 and proving slightly stronger than the predictive value of BNP in Hypothesis 1. These findings were both unanticipated and novel. The circulating levels of the proinflammatory cytokines TNFα, IL-1b, and IL-6 and the soluble fragments of their cognate receptors, sTNF-R1, STNF-R2, IL-2sR and IL-6sR are elevated in patients with heart failure and are thought to play a role in the development of progressive left ventricular dysfunction and remodeling.29-33 The soluble portion of the type 1 (sTNFR-1, p55, TNFSF1A) and type 2 (TNFR2, p75, TNFSF1B) TNFα receptors are shed into the circulation in response to a variety of stimuli, including TNFα. These soluble receptors are thought to bind to TNF and block the activity of this cytokine when it is released into the circulation.34 Thus far, the role of sTNFR-1 in predicting outcome in patients with heart failure has received far less attention than the role of BNP. Although both cytokines and cytokine receptors have been found to be independently predictive of mortality in patients with advanced heart failure,10, 16, 35 their relationship to outcomes in patients with ischemic disease undergoing surgical revascularization or medical therapy has not been defined. It is unclear why sTNFR-1 was more strongly predictive of risk than BNP in this group of patients with heart failure and coronary artery disease; however, it might relate, at least in part, to the greater stability of sTNFR-1 in the plasma36 and the fact that unlike BNP, it is not influenced by gender and age.

We analyzed the biomarkers as continuous variables because it did not require grouping patients arbitrarily or choosing arbitrary cut points and as illustrated by the data, the risk varies over a continuous range. The lowest values for each biomarker were at the lowest risk, and the highest values were at the highest risk, but the risk did not increase linearly over the entire range.
of the biomarkers. Different clinicians have different threshold levels of risk, and therefore providing the data in this format allows physicians to decide what cut-off levels for risk stratification might be most appropriate. The measurement of these biomarkers must also be viewed in the context of each patient. For example, a patient with a BNP of greater than 1,000 pg/mL certainly has a surgical risk that is quite significant; however, a patient who is severely limited by their angina might be willing to undergo CABG in order to improve their quality of life – recognizing the significant operative risk.

In summary, heart failure secondary to coronary artery disease continues to be associated with significant morbidity and mortality. Analysis of the biomarker sub-study of the STICH trial, which incorporated patients in Hypothesis 1 and Hypothesis 2, shows that elevated levels of 2 biomarkers, sTNFR-1 and BNP, were strongly associated with outcomes in two large and independent studies of patients who received either medical therapy or surgical revascularization, thus providing important cross-validation for the prognostic importance of these two biomarkers. It bears emphasis that elevated levels of sTNFR-1 and BNP also enhanced the predictive abilities of a well-established set of time honored clinical and hemodynamic variables. The finding that the predictive value of levels of both BNP and sTNFR-1 did not increase linearly across the full range of biomarker values raises questions about the importance of modest changes in biomarker levels in those with very high levels at baseline and the rationale for performing serial biomarker levels in these patients. Since there were no differential treatment effects across the range of these biomarkers in either the Hypothesis 1 or Hypothesis 2 cohort, the prognostic value of these biomarkers is applicable and consistent for each of the different treatment options. Taken together, the results of this study provide a strong rationale for using validated biomarkers to assess prognosis in this challenging group of patients.
Sources of Funding

This work was supported by grants from the National Heart, Lung and Blood Institutes, nos. HL69015, HL-069012, HL070011, HL072430, HL-069009, HL-069010, HL-069012, HL-069011, HL-069009, HL-069010, HL-069012, HL-069011, HL-069013, and HL-072683

Disclosures

None.

References


Table 1. Baseline Characteristics of STICH Hypothesis 1 Patients in Biomarker Substudy

<table>
<thead>
<tr>
<th></th>
<th>Biomarker Substudy (n = 606)</th>
<th>Not in Substudy (n = 606)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.) median (25th, 75th percentiles)</td>
<td>62 (56, 69)</td>
<td>58 (52, 66)</td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>Male gender</td>
<td>86%</td>
<td>89%</td>
<td>0.160</td>
</tr>
<tr>
<td>Previous MI</td>
<td>78%</td>
<td>77%</td>
<td>0.682</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>10%</td>
<td>5%</td>
<td>0.002</td>
</tr>
<tr>
<td>Diabetes</td>
<td>41%</td>
<td>38%</td>
<td>0.347</td>
</tr>
<tr>
<td>Hypertension</td>
<td>61%</td>
<td>59%</td>
<td>0.558</td>
</tr>
<tr>
<td>Current smoker</td>
<td>20%</td>
<td>22%</td>
<td>0.561</td>
</tr>
<tr>
<td>Current NYHA HF class</td>
<td></td>
<td></td>
<td>0.068</td>
</tr>
<tr>
<td>I</td>
<td>13%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>53%</td>
<td>51%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>31%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>3%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL), median 25th 75th percentiles</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.1 (1.0, 1.3)</td>
<td>0.706</td>
</tr>
<tr>
<td>Number of diseased vessels (≥ 75%)</td>
<td></td>
<td></td>
<td>0.217</td>
</tr>
<tr>
<td>≤ 1</td>
<td>28%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>37%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>LVEF</td>
<td>26 (21, 32)</td>
<td>28 (23, 34)</td>
<td>0.0004</td>
</tr>
<tr>
<td>ESVI</td>
<td>82 (62, 112)</td>
<td>76 (57, 97)</td>
<td>0.0001</td>
</tr>
</tbody>
</table>
Table 2. Prognostic Information Contributed by BNP and/or sTNFR-1 in Univariable and Multivariable Cox Regression Model Analyses (Hypothesis 1, N=606)

<table>
<thead>
<tr>
<th>Predictor(s) in Cox Model</th>
<th>Primary Endpoint: Death (Events=249)</th>
<th>Secondary Endpoint: Death or Cardiovascular Hospitalization (Events=439)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-Square</td>
<td>DF</td>
</tr>
<tr>
<td>BNP (univariate)</td>
<td>40.6</td>
<td>1</td>
</tr>
<tr>
<td>TNFR (univariate)</td>
<td>38.9</td>
<td>1</td>
</tr>
<tr>
<td>BNP + sTNFR-1</td>
<td>62.1</td>
<td>2</td>
</tr>
<tr>
<td>Covariates</td>
<td>82.4</td>
<td>21</td>
</tr>
<tr>
<td>Covariates + BNP</td>
<td>94.2</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>(17.9)</td>
<td>(1)</td>
</tr>
<tr>
<td>Covariates + sTNFR-1</td>
<td>100.9</td>
<td>22</td>
</tr>
<tr>
<td></td>
<td>(18.5)</td>
<td>(1)</td>
</tr>
<tr>
<td>Covariates + BNP + sTNFR-1</td>
<td>109.0</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>(26.6)</td>
<td>(2)</td>
</tr>
<tr>
<td></td>
<td>(8.1)</td>
<td>(1)</td>
</tr>
<tr>
<td></td>
<td>(14.7)</td>
<td>(1)</td>
</tr>
</tbody>
</table>

1. The covariates include treatment assigned, stratum, age, gender, race, HF class at baseline, history of MI, ESVI, Duke CAD Index, creatinine, hemoglobin, mitral regurgitation, hyperlipidemia, diabetes, history of stroke, history of atrial flutter/fibrillation, and whether or not a patient can do the baseline 6-minute walk. These are variables either prospectively specified in STICH protocol or with significant prognostic effect.
Table 3. Baseline Characteristics of STICH Hypothesis 2 Patients in Biomarker Substudy

<table>
<thead>
<tr>
<th></th>
<th>Biomarker Substudy (n = 676)</th>
<th>Not in Substudy (n = 248)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.) median (25th, 75th percentiles)</td>
<td>62 (56, 70)</td>
<td>60 (53, 68)</td>
<td>0.013</td>
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<tr>
<td>Male gender</td>
<td>87%</td>
<td>80%</td>
<td>0.007</td>
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<tr>
<td>Previous MI</td>
<td>87%</td>
<td>89%</td>
<td>0.483</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>5%</td>
<td>5%</td>
<td>0.960</td>
</tr>
<tr>
<td>Diabetes</td>
<td>34%</td>
<td>38%</td>
<td>0.286</td>
</tr>
<tr>
<td>Hypertension</td>
<td>58%</td>
<td>64%</td>
<td>0.072</td>
</tr>
<tr>
<td>Current smoker</td>
<td>19%</td>
<td>26%</td>
<td>0.026</td>
</tr>
<tr>
<td>Current NYHA HF class</td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>I</td>
<td>9%</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>46%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>40%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>5%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL), median 25th 75th percentiles</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.1 (0.9, 1.3)</td>
<td>0.427</td>
</tr>
<tr>
<td>Number of diseased vessels (≥ 75%)</td>
<td></td>
<td></td>
<td>0.324</td>
</tr>
<tr>
<td>≤ 1</td>
<td>18%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>42%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>LVEF</td>
<td>27 (21, 33)</td>
<td>28 (25, 33)</td>
<td>0.026</td>
</tr>
<tr>
<td>ESVI</td>
<td>80 (58, 106)</td>
<td>79 (60, 100)</td>
<td>0.386</td>
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</table>
Table 4. Prognostic Information Contributed by BNP and/or sTNFR-1 in Univariable and Multivariable Cox Regression Model Analyses (Hypothesis 2, N=676)

<table>
<thead>
<tr>
<th>Predictor(s) in Cox Model</th>
<th>Primary Endpoint: Death or Cardiovascular Hospitalization (Events=410)</th>
<th>Secondary Endpoint: Death (Events=196)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Chi-Square</td>
<td>DF</td>
</tr>
<tr>
<td>BNP (univariate)</td>
<td>30.3</td>
<td>2</td>
</tr>
<tr>
<td>sTNFR-1 (univariate)</td>
<td>45.5</td>
<td>1</td>
</tr>
<tr>
<td>BNP + sTNFR-1</td>
<td>62.4</td>
<td>3</td>
</tr>
<tr>
<td>Covariates¹</td>
<td>144.7</td>
<td>21</td>
</tr>
<tr>
<td>Covariates + BNP</td>
<td>150.8</td>
<td>23</td>
</tr>
<tr>
<td>Contributed by BNP</td>
<td>152.0</td>
<td>22</td>
</tr>
<tr>
<td>Covariates + sTNFR-1</td>
<td>156.3</td>
<td>24</td>
</tr>
<tr>
<td>Contributed by BNP + sTNFR-1</td>
<td>150.8</td>
<td>23</td>
</tr>
<tr>
<td>Independent contribution of BNP</td>
<td>152.0</td>
<td>22</td>
</tr>
<tr>
<td>Independent contribution of sTNFR-1</td>
<td>156.3</td>
<td>24</td>
</tr>
</tbody>
</table>

¹: The covariates include treatment assigned, stratum, age, gender, race, HF class at baseline, history of MI, ESVI, Duke CAD Index, creatinine, hemoglobin, mitral regurgitation, hyperlipidemia, diabetes, history of stroke, history of atrial flutter/fibrillation, and whether or not a patient can do the baseline 6-minute walk. These are variables either prospectively specified in STICH protocol or with significant prognostic effect.
Figure Legends

Figure 1. The relationship between BNP levels and all-cause mortality in patients enrolled in STICH Hypothesis 1. Biomarker levels were treated as a continuous variable, and the relationship was modeled using restricted cubic spline functions within the framework of the Cox regression model. The solid curve represents the modeled relationship, and the hatched lines represent 95% confidence bounds for the relationship.

Figure 2. The relationship between sTNFR-1 levels and all-cause mortality in patients enrolled in STICH Hypothesis 1. Biomarker levels were treated as a continuous variable, and the relationship was modeled using restricted cubic spline functions within the framework of the Cox regression model. The solid curve represents the modeled relationship, and the hatched lines represent 95% confidence bounds for the relationship.

Figure 3A. Kaplan-Meier estimates of mortality rate by BNP tertiles for patients enrolled in STICH Hypothesis 1. Mortality curves are presented in the upper panel for patients randomized to CABG and in the lower panel for patients randomized to medical therapy.

Figure 3B. Kaplan-Meier estimates of mortality rate by sTNFR-1 tertiles for patients enrolled in STICH Hypothesis 1. Mortality curves are presented in the upper panel for patients randomized to CABG and in the lower panel for patients randomized to medical therapy.

Figure 4. The relationship between BNP levels and the primary endpoint of death or cardiovascular hospitalization in patients enrolled in STICH Hypothesis 2. BNP levels were treated as a continuous
variable, and the relationship was modeled using restricted cubic spline functions within the framework of the Cox regression model. The solid curve represents the modeled relationship, and the hatched lines represent 95% confidence bounds for the relationship.

Figure 5. The relationship between sTNFR-1 levels and the primary endpoint of death or cardiovascular hospitalization in patients enrolled in STICH Hypothesis 2. sTNFR-1 levels were treated as a continuous variable, and the relationship was modeled using restricted cubic spline functions within the framework of the Cox regression model. The solid curve represents the modeled relationship, and the hatched lines represent 95% confidence bounds for the relationship.

Figure 6A. Kaplan Meier estimates of mortality or cardiovascular hospitalization rates by BNP tertiles for STICH Hypothesis 2 patients. Event-rate curves are presented in the upper panel for patients randomized to CABG and in the lower panel for patients randomized to the combination of CABG and left ventricular reconstruction.

Figure 6B. Kaplan Meier estimates of mortality or cardiovascular hospitalization rates by sTNFR-1 tertiles for STICH Hypothesis 2 patients. Event-rate curves are presented in the upper panel for patients randomized to CABG and in the lower panel for patients randomized to the combination of CABG and left ventricular reconstruction.
H1 Patients Randomized to CABG
Log-rank Chi-square = 21.60  , P-value= <0.001

<table>
<thead>
<tr>
<th>BNP Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>2.02</td>
<td>1.23, 3.34</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>3.05</td>
<td>1.87, 4.95</td>
</tr>
</tbody>
</table>

Death Rate

Years Following Randomization

Patients at Risk:
- Tertile 1: 105, 99, 97, 94, 79, 52, 31
- Tertile 2: 102, 89, 79, 76, 64, 45, 27
- Tertile 3: 103, 82, 70, 64, 49, 32, 17
H1 Patients Randomized to MED
Log-rank Chi-square = 14.75, P-value= 0.001

<table>
<thead>
<tr>
<th>BNP Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>1.94</td>
<td>1.21, 3.09</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>2.43</td>
<td>1.52, 3.87</td>
</tr>
</tbody>
</table>

Death Rate vs. Years Following Randomization

Patients at Risk:
- Tertile 1: 96, 93, 88, 80, 64, 42, 25
- Tertile 2: 104, 92, 81, 75, 62, 40, 23
- Tertile 3: 96, 79, 71, 61, 45, 32, 16

Diagram showing survival rates over time for different tertiles with hazard ratios and 95% confidence intervals.
H1 Patients Randomized to CABG

Log-rank Chi-square = 17.25, P-value = <0.001

<table>
<thead>
<tr>
<th>TNFR1 Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>1.46</td>
<td>0.90, 2.34</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>2.51</td>
<td>1.59, 3.96</td>
</tr>
</tbody>
</table>

Death Rate

Patients at Risk:
- Tertile 1: 110, 104, 96, 94, 70, 53, 33
- Tertile 2: 105, 92, 83, 79, 73, 47, 26
- Tertile 3: 95, 74, 67, 61, 49, 29, 16

Years Following Randomization
H1 Patients Randomized to MED
Log-rank Chi-square = 17.02 , P-value= <0.001

<table>
<thead>
<tr>
<th>TNFR1 Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>1.36</td>
<td>0.83, 2.20</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>2.32</td>
<td>1.48, 3.63</td>
</tr>
</tbody>
</table>

Death Rate

Years Following Randomization

Patients at Risk:

<table>
<thead>
<tr>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>90</td>
<td>102</td>
<td>104</td>
</tr>
<tr>
<td>81</td>
<td>97</td>
<td>86</td>
</tr>
<tr>
<td>75</td>
<td>88</td>
<td>77</td>
</tr>
<tr>
<td>69</td>
<td>81</td>
<td>66</td>
</tr>
<tr>
<td>59</td>
<td>62</td>
<td>50</td>
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<tr>
<td>51</td>
<td>36</td>
<td>27</td>
</tr>
<tr>
<td>23</td>
<td>19</td>
<td>22</td>
</tr>
</tbody>
</table>
4-Year Mortality or Cardiovascular Hospitalization Rate vs. TNFR1
(Hypothesis 2 Patients)
H2 Patients Randomized to CABG
Log-rank Chi-square = 8.27 ,  P-value = 0.016

<table>
<thead>
<tr>
<th>BNP Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>1.69</td>
<td>1.19, 2.41</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>1.37</td>
<td>0.94, 1.99</td>
</tr>
</tbody>
</table>

Death or CV Hospitalization Rate

Patients at Risk:

<table>
<thead>
<tr>
<th>Tertile 1</th>
<th>Tertile 2</th>
<th>Tertile 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients at Risk:</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tertile 1</td>
<td>98</td>
<td>73</td>
</tr>
<tr>
<td>Tertile 2</td>
<td>108</td>
<td>61</td>
</tr>
<tr>
<td>Tertile 3</td>
<td>99</td>
<td>57</td>
</tr>
</tbody>
</table>
H2 Patients Randomized to CABG+SVR
Log-rank Chi-square = 20.71,  P-value < 0.001

<table>
<thead>
<tr>
<th>BNP Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>1.38</td>
<td>0.98, 1.95</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>2.09</td>
<td>1.50, 2.90</td>
</tr>
</tbody>
</table>

Death or CV Hospitalization Rate

Patients at Risk:
- Tertile 1: 123
- Tertile 2: 123
- Tertile 3: 125

Years Following Randomization
H2 Patients Randomized to CABG
Log-rank Chi-square = 20.46, P-value < 0.001

<table>
<thead>
<tr>
<th>TNFR1 Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>0.90</td>
<td>0.62, 1.30</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>1.79</td>
<td>1.27, 2.53</td>
</tr>
</tbody>
</table>

Death or CV Hospitalization Rate

Years Following Randomization

Patients at Risk:
- Tertile 1: 97, 73, 57, 46, 26, 7
- Tertile 2: 105, 73, 61, 53, 21, 6
- Tertile 3: 103, 45, 36, 31, 19, 2
H2 Patients Randomized to CABG+SVR
Log-rank Chi-square = 23.66, P-value < 0.001

<table>
<thead>
<tr>
<th>TNFR1 Tertiles</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tertile 2 : Tertile 1</td>
<td>1.49</td>
<td>1.06, 2.09</td>
</tr>
<tr>
<td>Tertile 3 : Tertile 1</td>
<td>2.20</td>
<td>1.58, 3.06</td>
</tr>
</tbody>
</table>

Death or CV Hospitalization Rate

Patients at Risk:
- Tertile 1: 126
- Tertile 2: 125
- Tertile 3: 120

Years Following Randomization
The Prognostic Significance of Biomarkers in Predicting Outcome in Patients With Coronary Artery Disease and Left Ventricular Dysfunction: Results of the Biomarker Sub-Study of the Surgical Treatment for Ischemic Heart Failure (STICH) Trials
Arthur M. Feldman, Douglas L. Mann, Lilin She, Michael R. Bristow, Alan S. Maisel, Dennis M. McNamara, Ryan Walsh, Dorellyn L. Lee, Stanislaw Wos, Irene Lang, Gretchen Wells, Mark H. Drazner, John F. Schmedtje, Jr., Daniel F. Pauly, Carla A. Sueta, Michael Di Maio, Irving L. Kron, Eric J. Velazquez and Kerry L. Lee

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http://circheartfailure.ahajournals.org/content/suppl/2013/04/12/CIRCHEARTFAILURE.112.000185.DC1

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http://circheartfailure.ahajournals.org/subscriptions/
### Supplemental Table I.
**Extended Table of Baseline Characteristics of STICH Hypothesis 1 Patients in Biomarker Substudy**

<table>
<thead>
<tr>
<th>Biomarker Substudy (n = 606)</th>
<th>Not in Substudy (n = 606)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.) median (25th, 75th percentiles)</td>
<td>62 (56, 69)</td>
<td>58 (52, 66)</td>
</tr>
<tr>
<td>Male gender</td>
<td>86%</td>
<td>89%</td>
</tr>
<tr>
<td>Previous MI</td>
<td>78%</td>
<td>77%</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>10%</td>
<td>5%</td>
</tr>
<tr>
<td>Diabetes</td>
<td>41%</td>
<td>38%</td>
</tr>
<tr>
<td>Hypertension</td>
<td>61%</td>
<td>59%</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>68%</td>
<td>53%</td>
</tr>
<tr>
<td>Current smoker</td>
<td>20%</td>
<td>22%</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>17%</td>
<td>13%</td>
</tr>
<tr>
<td>Chronic renal insufficiency</td>
<td>9%</td>
<td>6%</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>16%</td>
<td>9%</td>
</tr>
<tr>
<td>Depression</td>
<td>8%</td>
<td>4%</td>
</tr>
<tr>
<td>Previous PCI</td>
<td>16%</td>
<td>9%</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>3%</td>
<td>3%</td>
</tr>
<tr>
<td>Inpatient at initial evaluation</td>
<td>50%</td>
<td>72%</td>
</tr>
<tr>
<td>Current CCS angina class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No Angina</td>
<td>44%</td>
<td>29%</td>
</tr>
<tr>
<td>I</td>
<td>18%</td>
<td>13%</td>
</tr>
<tr>
<td>II</td>
<td>33%</td>
<td>54%</td>
</tr>
<tr>
<td>III</td>
<td>4%</td>
<td>4%</td>
</tr>
<tr>
<td>IV</td>
<td>&lt; 1%</td>
<td>1%</td>
</tr>
</tbody>
</table>
### Supplemental Table I
Extended Table of Baseline Characteristics of STICH Hypothesis 1 Patients in Biomarker Substudy (continued)

<table>
<thead>
<tr>
<th></th>
<th>With Data (n = 606)</th>
<th>Without Data (n = 606)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Current NYHA HF class</td>
<td></td>
<td></td>
<td>0.068</td>
</tr>
<tr>
<td>I</td>
<td>13%</td>
<td>10%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>53%</td>
<td>51%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>31%</td>
<td>37%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>3%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL), median 25\textsuperscript{th} 75\textsuperscript{th} percentiles)</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.1 (1.0, 1.3)</td>
<td>0.706</td>
</tr>
<tr>
<td>Number of diseased vessels (&gt; 75%)</td>
<td></td>
<td></td>
<td>0.217</td>
</tr>
<tr>
<td>≤ 1</td>
<td>28%</td>
<td>23%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>37%</td>
<td>39%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>35%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>Left main stenosis (&gt; 50%)</td>
<td>4%</td>
<td>1%</td>
<td>0.004</td>
</tr>
<tr>
<td>Proximal LAD stenosis (&gt; 75%)</td>
<td>64%</td>
<td>72%</td>
<td>0.002</td>
</tr>
<tr>
<td>Duke CAD index (0-100)</td>
<td>65 (39, 77)</td>
<td>65 (39, 77)</td>
<td>0.065</td>
</tr>
<tr>
<td>LVEF</td>
<td>26 (21, 32)</td>
<td>28 (23, 34)</td>
<td>0.0004</td>
</tr>
<tr>
<td>ESVI</td>
<td>82 (62, 112)</td>
<td>76 (57, 97)</td>
<td>0.0001</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td></td>
<td></td>
<td>0.021</td>
</tr>
<tr>
<td>None or trace</td>
<td>39%</td>
<td>34%</td>
<td></td>
</tr>
<tr>
<td>Mild (≤ 2+)</td>
<td>42%</td>
<td>50%</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>16%</td>
<td>14%</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>4%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Able to do 6-min walk test</td>
<td>85%</td>
<td>88%</td>
<td>0.057</td>
</tr>
<tr>
<td>Region</td>
<td></td>
<td></td>
<td>&lt; 0.0001</td>
</tr>
<tr>
<td>USA</td>
<td>14%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>18%</td>
<td>2%</td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>60%</td>
<td>51%</td>
<td></td>
</tr>
<tr>
<td>Asia Pacific</td>
<td>8%</td>
<td>31%</td>
<td></td>
</tr>
<tr>
<td>South America</td>
<td>0%</td>
<td>9%</td>
<td></td>
</tr>
</tbody>
</table>
## Supplemental Table II
Extended Table of Baseline Characteristics of STICH Hypothesis 2 Patients in Biomarker Substudy

<table>
<thead>
<tr>
<th></th>
<th>Biomarker Substudy (n = 676)</th>
<th>Not in Substudy (n = 248)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (yrs.) median (25\textsuperscript{th}, 75\textsuperscript{th} percentiles)</td>
<td>62 (56, 70)</td>
<td>60 (53, 68)</td>
<td>0.013</td>
</tr>
<tr>
<td>Male gender</td>
<td>87%</td>
<td>80%</td>
<td>0.007</td>
</tr>
<tr>
<td>Previous MI</td>
<td>87%</td>
<td>89%</td>
<td>0.483</td>
</tr>
<tr>
<td>Previous stroke</td>
<td>5%</td>
<td>5%</td>
<td>0.960</td>
</tr>
<tr>
<td>Diabetes</td>
<td>34%</td>
<td>38%</td>
<td>0.286</td>
</tr>
<tr>
<td>Hypertension</td>
<td>58%</td>
<td>64%</td>
<td>0.072</td>
</tr>
<tr>
<td>Hyperlipidemia</td>
<td>72%</td>
<td>70%</td>
<td>0.580</td>
</tr>
<tr>
<td>Current smoker</td>
<td>19%</td>
<td>26%</td>
<td>0.026</td>
</tr>
<tr>
<td>Peripheral vascular disease</td>
<td>14%</td>
<td>18%</td>
<td>0.131</td>
</tr>
<tr>
<td>Chronic renal insufficiency</td>
<td>8%</td>
<td>9%</td>
<td>0.570</td>
</tr>
<tr>
<td>Atrial fibrillation</td>
<td>10%</td>
<td>15%</td>
<td>0.055</td>
</tr>
<tr>
<td>Depression</td>
<td>7%</td>
<td>4%</td>
<td>0.185</td>
</tr>
<tr>
<td>Previous PCI</td>
<td>21%</td>
<td>13%</td>
<td>0.004</td>
</tr>
<tr>
<td>Previous CABG</td>
<td>3%</td>
<td>3%</td>
<td>0.794</td>
</tr>
<tr>
<td>Inpatient at initial evaluation</td>
<td>72%</td>
<td>81%</td>
<td>0.006</td>
</tr>
<tr>
<td>Current CCS angina class</td>
<td></td>
<td></td>
<td>0.020</td>
</tr>
<tr>
<td>No Angina</td>
<td>23%</td>
<td>27%</td>
<td></td>
</tr>
<tr>
<td>I</td>
<td>6%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>19%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>44%</td>
<td>42%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>8%</td>
<td>13%</td>
<td></td>
</tr>
</tbody>
</table>
## Supplemental Table II
### Extended Table of Baseline Characteristics of STICH Hypothesis 2 Patients in Biomarker Substudy (continued)

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>With Data (n = 676)</th>
<th>Without Data (n = 248)</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Current NYHA HF class</strong></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>I</td>
<td>9%</td>
<td>8%</td>
<td></td>
</tr>
<tr>
<td>II</td>
<td>46%</td>
<td>32%</td>
<td></td>
</tr>
<tr>
<td>III</td>
<td>40%</td>
<td>56%</td>
<td></td>
</tr>
<tr>
<td>IV</td>
<td>6%</td>
<td>5%</td>
<td></td>
</tr>
<tr>
<td>Creatinine (mg/dL), median 25\textsuperscript{th} 75\textsuperscript{th} percentiles)</td>
<td>1.1 (0.9, 1.3)</td>
<td>1.1 (0.9, 1.3)</td>
<td>0.427</td>
</tr>
<tr>
<td><strong>Number of diseased vessels (\geq 75%)</strong></td>
<td></td>
<td></td>
<td>0.324</td>
</tr>
<tr>
<td>\leq 1</td>
<td>18%</td>
<td>22%</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>42%</td>
<td>40%</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>40%</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>Left main stenosis (\geq 50)</td>
<td>21%</td>
<td>21%</td>
<td>0.808</td>
</tr>
<tr>
<td>Proximal LAD stenosis (\geq 75%)</td>
<td>73%</td>
<td>83%</td>
<td>0.002</td>
</tr>
<tr>
<td>Duke CAD index (0-100)</td>
<td>65 (43, 91)</td>
<td>65 (39, 91)</td>
<td>0.378</td>
</tr>
<tr>
<td>LVEF</td>
<td>27 (21, 33)</td>
<td>28 (25, 33)</td>
<td>0.026</td>
</tr>
<tr>
<td>ESVI</td>
<td>80 (58, 106)</td>
<td>79 (60, 100)</td>
<td>0.386</td>
</tr>
<tr>
<td>Mitral regurgitation</td>
<td></td>
<td></td>
<td>0.050</td>
</tr>
<tr>
<td>None or trace</td>
<td>38%</td>
<td>31%</td>
<td></td>
</tr>
<tr>
<td>Mild (\leq 2+)</td>
<td>43%</td>
<td>53%</td>
<td></td>
</tr>
<tr>
<td>Moderate</td>
<td>15%</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td>Severe</td>
<td>4%</td>
<td>3%</td>
<td></td>
</tr>
<tr>
<td>Able to do 6-min walk test</td>
<td>69%</td>
<td>69%</td>
<td>0.923</td>
</tr>
<tr>
<td><strong>Region</strong></td>
<td></td>
<td></td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>USA</td>
<td>20%</td>
<td>19%</td>
<td></td>
</tr>
<tr>
<td>Canada</td>
<td>17%</td>
<td>12%</td>
<td></td>
</tr>
<tr>
<td>Europe</td>
<td>58%</td>
<td>57%</td>
<td></td>
</tr>
<tr>
<td>Asia Pacific</td>
<td>5%</td>
<td>6%</td>
<td></td>
</tr>
<tr>
<td>South America</td>
<td>0%</td>
<td>7%</td>
<td></td>
</tr>
</tbody>
</table>
Supplemental Figure I
Kaplan-Meier Estimates of All-Cause Mortality Rates for CABG vs. MED Patients
(Comparing Hypothesis 1 Patients with BNP and TNFR1 Data vs. Those without Data)

<table>
<thead>
<tr>
<th>Sub-group</th>
<th>N</th>
<th>Events</th>
<th>Hazard Ratio</th>
<th>95% CI</th>
<th>M E D Group</th>
<th>C A B G Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>With Data</td>
<td>606</td>
<td>249</td>
<td>0.86</td>
<td>0.67, 1.10</td>
<td>39.7 %</td>
<td>35.3 %</td>
</tr>
<tr>
<td>Without Data</td>
<td>606</td>
<td>213</td>
<td>0.86</td>
<td>0.66, 1.13</td>
<td>41.0 %</td>
<td>34.6 %</td>
</tr>
</tbody>
</table>

Interaction with Treatment
P-value = 0.971
Supplemental Figure II
Kaplan-Meier Estimates of Mortality or Cardiovascular Hospitalization Rates for CABG+SVR vs. CABG Patients
(Comparing Hypothesis 2 Patients with BNP and TNFR1 Data vs. Those without Data)

With Data
- CABG (187 events)
- CABG+SVR (223 events)

Without Data
- CABG (61 events)
- CABG+SVR (66 events)

Sub-group | N | Events | Hazard Ratio | 95% CI | 3 Year Rates
--- | --- | --- | --- | --- | ---
With Data | 676 | 410 | 0.97 | 0.80, 1.18 | 54.4% vs. 53.7%
Without Data | 248 | 127 | 1.03 | 0.72, 1.45 | 47.7% vs. 48.3%

Interaction with Treatment
P-value = 0.817