Clinical, biochemical, and genetic predictors of coronary artery bypass graft failure

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Objectives: To identify novel predictors for coronary artery bypass grafting failure, we probed for associations with known clinical and biochemical risk factors for atherosclerosis. We also used microarray analysis to identify novel single nucleotide polymorphisms to better understand the genetics and pathogenesis of graft occlusion.

Methods: The present study was a nested case-control substudy of the Radial Artery Patency Study 5-year follow-up data. From 1996 to 2001, 87 patients underwent coronary artery bypass grafting. Of these, 26 patients (29.9%) had an occluded study graft (saphenous vein or radial artery) at 8.0 ± 1.1 years. The clinical parameters, late angiography, blood biomarker levels, and surgical outcomes data were included in a multivariate analysis to determine the independent predictors of graft failure.

Results: The risk factors of graft failure were fibrinogen (odds ratio [OR], 3.94; 95% confidence interval [CI], 1.33-11.63; $P = .01$), creatinine (OR, 1.06; 95% CI, 1.02-1.10; $P = .006$), and diabetes mellitus (OR, 5.15; 95% CI, 1.08-24.59; $P = .04$). High-density lipoprotein (OR, 0.74; 95% CI, 0.53-1.02; $P = .06$) was weakly protective; however, low-density lipoprotein and total cholesterol were not predictors. We then identified the association of several human single nucleotide polymorphisms with graft failure, including mutations in *glutathione-S-transferase α3*. Human coronary arteries and bypass grafts demonstrated increased protein expression of glutathione-S-transferase α3, a known cardioprotective factor, in the atherosclerotic regions and surrounding adventitial tissues.

Conclusions: We identified diabetes as a potential clinical predictor and plasma fibrinogen, creatinine, and high-density lipoprotein as potential novel biomarkers. These might help risk stratify patients for the development of graft failure. We also demonstrated a novel association between *glutathione-S-transferase α3* and graft failure. (J Thorac Cardiovasc Surg 2014;148:515-20)

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Coronary artery bypass grafting (CABG) is an effective treatment of complex, multivessel coronary artery disease.[1] Graft failure is a surrogate marker for future cardiac events, including repeat revascularization, myocardial infarction, and death.[2,3] The multi-institutional Collaborative Study in Coronary Artery Surgery found several clinical and angiographic patient characteristics predictive of graft occlusion, such as age, female gender, and left ventricular dysfunction.[4] However, the identification of patients at risk of graft failure continues to be challenging.

The development of a clinically validated panel of biomarkers to predict graft occlusion would have the potential to facilitate perioperative measures to prevent myocardial injury and add valuable prognostic information by predicting the risk of mortality after CABG. In the present report, we evaluated the known clinical variables and plasma levels of the biomarkers of atherosclerosis using long-term angiograms to identify novel associations that could potentially be used for the prediction of graft occlusion after CABG. Furthermore, given that 30% to

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Abbreviations and Acronyms

CABG = coronary artery bypass grafting
CI = confidence interval
CTA = computed tomography angiography
GSTα3 = glutathione-S-transferase α3
HDL = high-density lipoprotein
LDL = low-density lipoprotein
OR = odds ratio
SNP = single nucleotide polymorphism
SVG = saphenous vein graft

60% of the individual risk of coronary artery disease is hereditary, it is also likely that graft occlusion is influenced by genetics.[5] Thus, we performed a case-control genome-wide association study to identify novel single nucleotide polymorphisms (SNPs) associated with graft failure that might lead to the identification of genetic predictors of graft occlusion and novel pathogenetic molecules.

METHODS

Study Design

The present study was a nested case-control substudy of the Radial Artery Patency Study (NCT00187356).[6] This multicenter study involved 561 patients initially enrolled across 13 sites, of whom 269 patients underwent late angiography or computed tomography angiography (CTA). A total of 87 patients from a single site participated in the present substudy (of 252 total patients and 150 total patients with late angiograms or CTA). In brief, each patient had received both a radial artery graft and a study saphenous vein graft (SVG), and follow-up examinations were performed at 8.0 $\pm$ 1.1 years using angiography (n = 74) or CTA (n = 13). Peripheral blood samples were taken at follow-up angiography or CTA for biomarker analysis. All angiograms and CTAs were reviewed by an independent committee of 4 experienced cardiologists who were unaware of patient randomization (E.A.C., S.R., J.D.D., L.S.). Cases were defined as patients with $\geq$1 study grafts that were completely occluded. Controls were defined as patients with patency of both study grafts. The primary study objective was to identify the clinical variables, circulating biomarker levels, and surgical outcomes data associated with bypass graft occlusion. Our institutional review committee approved the present study, and all subjects gave written informed consent.

Sample Collection and Biochemical Assay

The peripheral blood samples were centrifuged (15 minutes, 3000 rpm) and analyzed in triplicate for high-sensitivity C-reactive protein (Roche Diagnostics, Basel, Switzerland), fibrinogen (Roche Diagnostics), homocysteine (Roche Diagnostics), interleukin-6 (R&D Systems, Minneapolis, Minn), interleukin-18 (MBL, Co, Ltd, Nagoya, Japan), lipoprotein a (Abcam, Cambridge, Mass), total cholesterol (Roche Diagnostics), high-density lipoprotein (HDL; Roche Diagnostics), low-density lipoprotein (LDL; Roche Diagnostics), apolipoprotein B (Roche Diagnostics), and triglycerides (Roche Diagnostics).

DNA Extraction and SNP Characterization

Peripheral blood leukocytes were collected from flash frozen samples. The DNA was extracted (DNeasy kit, Qiagen, Hilden, Germany) and multiplex polymerase chain reaction performed. Purified primer extension reactions were submitted for hybridization to the Illumina 660W-Quad Infinium DNA Analysis BeadChips (San Diego, Calif). The criteria for SNPs were a call rate >95%, SNPs in Hardy-Weinberg equilibrium in control ($P < .0001$), a minor allele frequency >1%, and individual samples with a genotype presence >95%.

Immunohistochemistry

Formalin-fixed and paraffin-embedded human coronary artery and bypass graft tissues from the autopsy cases were submitted for immunohistochemistry using anti-glutathione-S-transferase α3 (GSTα3; 1:5000; Abcam, Cambridge, Mass), biotinylated secondary antibody (1:10,000), and Vector Black Alkaline Phosphatase Substrate Kit (Vector Laboratories, Burlingame, Calif). All sections were reviewed by a cardiovascular pathologist (B.M.M.). Quantitative imaging analysis was performed using ImageProPlus (Media Cybernetics, Rockville, Md). The positive and negative controls are shown in Figure E1.

Statistical Analysis

Statistical analyses were performed with SPSS (IBM, Somers, NY). Univariate analyses were performed using the χ^2 or Fisher exact test for categorical variables, an independent 2-sample t test for normally distributed continuous variables, and Wilcoxon rank sum test for continuous variables, with a nonparametric distribution. The variables with univariate $P < .25$ or those of known clinical importance were included in a multivariable logistic regression model to calculate the risk-adjusted predictors of graft occlusion. Model discrimination was evaluated by the area under the receiver operating characteristic curve, and calibration was assessed using the Hosmer-Lemeshow goodness-of-fit statistic ($P = .7$).[7] The model was evaluated for collinearity using the variance inflation factor, in which multicollinearity is considered positive if the variance inflation factor is >2.5. All variance inflation factors in our model were <2.5. The data are presented as the median and range or mean $\pm$ standard deviation.

RESULTS

Patient Characteristics, Preoperative Details, and Operative Outcomes

A total of 87 patients who had undergone CABG from June 1996 to January 2001 were enrolled in the present study. The baseline characteristics of the total study population at enrollment are listed in Table 1. The cases had greater baseline creatinine levels than the controls (97.0 $\pm$ 21.2 vs 88.1 $\pm$ 17.1; $P = .05$).

The intraoperative variables and postoperative outcomes are listed in Table 2. A trend was seen for a greater number of grafts in the case group than in the control group (3.9 $\pm$ 0.7 vs 3.6 $\pm$ 0.6; $P = .05$) associated with a longer cardiopulmonary bypass time (116.9 $\pm$ 25.4 vs 105.3 $\pm$ 26.7 minutes; $P = .06$). No in-hospital mortalities occurred.

Late Angiography and CTA

Angiography or CTA was performed at 8.0 $\pm$ 1.1 years of follow-up for all 87 patients, 7 of which were clinically directed. Of the 26 patients with an occluded study graft, 5 (5.7%) had an occluded radial artery, 19 (21.8%) an occluded SVG, and 2 (2.3%) an occluded radial artery and SVG. Regarding the target vessels, of the occluded grafts, 16 (57.1%) had been grafted to the circumflex artery and 12 (42.9%) to the right coronary artery.