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Platelet Gene Polymorphisms and Cardiac Risk Assessment in Vascular Surgical Patients

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Background: **Current perioperative cardiac risk assessment tools use historic and surgical factors to stratify patient risk. Polymorphisms in platelet glycoprotein (GP) IIIa and GPIbα are associated with myocardial ischemic risk in nonsurgical settings, but their relation to perioperative ischemia is unclear. The authors hypothesized that platelet genotype would be an independent predictor of postoperative myocardial ischemia and would improve risk assessment when added to clinical factors.**

Methods: **One hundred ninety-six patients who underwent infrainguinal, abdominal aortic, or thoracoabdominal vascular surgery were evaluated for clinical and genetic factors that might predict the development of postoperative myocardial ischemia. Genomic DNA was genotyped for the Leu33Pro polymorphism of GPIIIa and the Thr145Met polymorphism of GPIbα. Myocardial ischemic outcome was determined by review of the medical record for cardiac death or myocardial infarction and by surveillance troponin I and automated continuous 12-lead electrocardiographic analysis.**

Results: **Sixty-five patients (33%) experienced one or more ischemic endpoints (2% death, 5% myocardial infarction, 20% troponin+, 22% electrocardiogram+). The Pro33 (adjusted odds ratio [OR], 2.4 [95% confidence interval, 1.2–6.2]) and Met145 (OR 3.4 [1.4–9.3]) genotypes were independent predictors of composite ischemic outcome by multivariate regression, as were diabetes mellitus (OR 4.0 [1.7–12.5]), abdominal aortic surgery (OR 4.1 [1.7–14.4]), and thoracoabdominal aortic surgery (OR 6.4 [2.7–23.8]). The addition of platelet gene polymorphisms to clinical factors improved fit (likelihood ratio testing chi-square = 13.5, $P < 0.001$) of an ischemia prediction model. The derived risk assessment tool had a receiver operator characteristic curve of 0.73 (0.65–0.81) compared with 0.64 (0.57–0.74) for a model excluding genetic factors ($P = 0.04$). A significant relation between the GPIbα polymorphism and ischemic outcome remained after excluding electrocardiographic ischemia from the composite endpoint.**

Conclusions: **Platelet polymorphisms are independent risk factors for postoperative myocardial ischemia and improve a risk prediction model when added to historic and surgical risk factors.**

CARDIAC risk assessment tools have been developed to guide the management of surgical patients at high risk for cardiovascular morbidity. Clinical risk indices developed by Goldman *et al.*,[1] Detsky *et al.*,[2] and Lee *et al.*[3] can stratify risk; however, they have modest positive predictive value and perform poorly in patients undergoing aortic vascular surgery.[3] The addition of noninvasive cardiac testing (*e.g.*, dipyridamole or dobutamine stress testing) to clinical variables improves perioperative risk stratification in vascular surgical patients at moderate risk.[4] Current consensus guidelines of an American College of Cardiology/American Heart Association task force support the use of noninvasive cardiac testing in the majority of patients who present for vascular surgery.[5] However, the positive predictive value of noninvasive cardiac testing is less than 25%.[6,7]

The limited positive predictive power of current risk management strategies suggests that factors critical to the development of ischemic morbidity are not assessed by current algorithms. Traditionally, perioperative myocardial ischemia is thought to develop from a surgical stress–related increase in heart rate and blood pressure that causes an acute increase in myocardial oxygen consumption.[8,9] In patients with preexisting coronary artery disease, it is believed that the increased oxygenation demand can exceed the supply and cause myocardial ischemia. However, autopsy series indicate that acute coronary thrombosis contributes to perioperative ischemic morbidity as frequently as in nonoperative settings.[10]

Platelets are known to play a significant role in coronary thrombosis in nonoperative settings,[11,12] but an association between platelets and perioperative myocardial ischemia has not been defined. Polymorphisms in the genes encoding platelet glycoprotein (GP) IIIa[13,14] and GPIbα[15,16] are associated with myocardial ischemic morbidity in nonsurgical settings, but their relation to perioperative ischemia is not clear. We sought to determine the relation between genotype for GPIIIa and GPIbα and perioperative myocardial ischemia in vascular surgical patients. We hypothesized that these gene polymorphisms would be independent predictors of periop-

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erative ischemic risk and would improve risk prediction when added to clinical risk factors.

Materials and Methods

This study was approved by the Johns Hopkins Institutional Review Board (Baltimore, Maryland) and all subjects gave written informed consent. Between June 1997 and May 2002, 196 nonconsecutive vascular surgical patients were studied. Patients were eligible if they underwent elective infrainguinal (IIS), abdominal aortic (AAS), or descending thoracic aortic (TAS) vascular surgery at Johns Hopkins Hospital and consented to study participation including genetic testing. The patients were part of a prospective study to determine the benefit of continuous 12-lead electrocardiography for detection of perioperative myocardial ischemia.

Clinical management was conducted according to the standards of the attending staff and included the routine use of perioperative β blockade. Management of aspirin was at the discretion of the attending staff, and most patients were requested to stop aspirin during the week before surgery. In addition to routine surveillance for myocardial ischemia, patients were assessed for ischemia as follows: (1) serum troponin I was performed on postoperative days 1–3 and 7,[17] and (2) computerized 12-lead electrocardiographic ST-segment analysis (Solar 6000/ST-Guard; GE/Marquette Electronics, Milwaukee, WI) was assessed during the first 48 h after surgery. The sensitivity and specificity of this methodology are described in detail elsewhere.[18,19]

The independent variables were clinical and genetic risk factors (table 1) for myocardial ischemia. Clinical risk factors were obtained from patient interview and review of the medical record. Genomic DNA was isolated from EDTA-anticoagulated whole blood using commercially available kits (Gentra Systems, Minneapolis, MN). DNA was stored at −80°C until genotyping was performed. DNA quality was verified by spectrophotometric analysis at 260/280, and a ratio greater than 1.7 was deemed acceptable. Genotyping for the Leu33Pro polymorphism of GPIIIa and Thr145Met polymorphism of GPIbα was performed on genomic DNA using restriction fragment length polymorphism analysis and fluorescent single nucleotide polymorphism detection. Genomic DNA was amplified using primers 5'-AGCTCTGATT GCTGGACTTC-3' and 5'-biotin-GAGGGGCCTGTCCTCTAG-3' to detect the T-to-C base substitution in exon 2 of the GPIIIa gene,[20] corresponding to leucine/proline at position 33 of the protein. The C-to-T transition at nucleotide 1018 of the GPIbα gene, which corresponds to threonine/methionine at position 145 of the GPIbα protein, was amplified using primers 5'-CCTGCTCTCACCGTCCTG -3' and 5'-biotin-AAGTTGTTGTTAGCCAGACTGA-3'.[21]

For restriction fragment analysis, Leu/Pro33 and Thr/Met145 amplicons were restriction enzyme digested with *Msp*I or Bsa HI (New England Biolabs, Beverly, MA), respectively. Digestion products were visualized after electrophoresis on 2% NuSieve (FMC BioProducts, Vallensbaek, Denmark), 1% agarose (Life Technologies, Rockville, MD) gels. For polymorphism analysis, allele-specific reporters and stabilizers were designed using gene sequences from GenBank. Wild-type and variant reporters were 5'-labeled with Cy3 and Cy5 fluorophores, respectively. Polymorphism detection was performed on a NanoChip® Molecular Biology Workstation (Nanogen, Inc., San Diego, CA). Desalted amplicons were electronically addressed onto a 10 × 10 microarray chip covered by a hydrogel permeation layer containing streptavidin. The chip was hybridized using 500 nM of each Cy3 and Cy5–labeled reporter probe and 1 μM stabilizer. The chip was placed in the reader, and thermal stringency was applied through a series of decreasing temperatures to a final discrimination temperature

Table 1. Clinical Characteristics of the GPIIIa and GPIbα Genotype Cohorts

	GPIIIa Polymorphism		GPIbα Polymorphism	
	Pro33+ n = 51 (26%)	Leu33 n = 145 (74%)	Met145+ n = 40 (20%)	Thr145 n = 156 (80%)
Age	69 ± 10	68 ± 11	66 ± 11	69 ± 11
Sex				
Male	28 (55)	63 (63)	28 (70)	91 (58)
Female	23 (45)	54 (37)	12 (30)	65 (42)
Race				
White	35 (69)	110 (76)	24 (40)	121 (78)
African-American	13 (25)	33 (23)	14 (35)	32 (20)
Other	3 (6)	2 (1)	2 (5)	3 (2)
Hypertension	39 (76)	111 (76)	29 (72)	121 (78)
Diabetes mellitus	16 (31)	26* (18)	11 (28)	31 (20)
Cigarette smoking	39 (76)	119 (82)	30 (75)	128 (82)
Angina	9 (18)	18 (12)	4 (10)	23 (15)
MI	12 (24)	44 (30)	12 (30)	44 (28)
MI < 6 months	4 (8)	6 (4)	3 (8)	7 (4)
CABG	12 (24)	26 (18)	8 (20)	30 (19)
Percutaneous coronary intervention	6 (12)	24 (16)	10 (25)	20 (13)
CHF	8 (16)	12 (8)	3 (8)	17 (11)
Aortic stenosis	1 (2)	6 (4)	1 (2)	6 (4)
Creatinine, mg/dl	1.2 ± 1.0	1.4 ± 1.7	1.7 ± 2.6	1.2 ± 1.2
Creatinine > 2.0 mg/dl	4 (8)	14 (10)	6 (15)	12 (8)
Preoperative β blockers	23 (45)	58 (40)	19 (48)	62 (40)
Preoperative aspirin	14 (27)	69* (48)	18 (45)	65 (42)
Preoperative statin	18 (35)	50 (34)	15 (38)	53 (34)
Type of surgery				
Infrainguinal	24 (47)	64 (44)	19 (48)	69 (44)
Abdominal aortic	15 (29)	42 (29)	13 (32)	44 (28)
Thoracic aortic	12 (24)	39 (27)	8 (20)	43 (28)
Postoperative β blockers	39 (76)	111 (76)	30 (75)	120 (77)
Postoperative aspirin	23 (45)	68 (47)	20 (50)	71 (46)

* Chi-square *P* value < 0.05 *vs.* Pro33+.

CABG = coronary artery bypass grafting; CHF = congestive heart failure; Leu33 = Leu33/Leu33 genotype; Met145+ = Met145/Met145 and Thr145/Met145 genotypes; MI = myocardial infarction; Pro33+ = Pro33/Pro33 and Leu33/Pro33 genotypes; Thr145 = Thr145/Thr145 genotype.

used to differentiate between wild-type and variant alleles. The restriction fragment and fluorescent polymorphism methods yielded concordant genotype results in more than 95% of analyses. Genotype in discordant cases was determined by fluorescent dideoxy terminator sequencing of polymerase chain reaction products using a 3730xl DNA Analyzer (Applied Biosystems Division, Foster City, CA).

The main dependent variable was a composite measure of myocardial ischemic outcome defined as any one of the following within 30 days of surgery: (1) cardiac death, (2) myocardial infarction (MI), (3) increased surveillance troponin I, or (4) prolonged myocardial ischemia on automated ST-segment analysis. Cardiac death was defined as death attributable to MI, heart failure, or arrhythmia. MI was defined according to standard World Health Organization criteria.[22] Cardiac death and MI were determined by the clinical team and verified by the investigators through review of medical records. Increased surveillance troponin I was defined as any troponin I that exceeded the diagnostic cutoff for MI, *i.e.*, exceeded the 99th percentile of the core laboratory reference range for the test. Different troponin assays were used during the study period such that the criteria for MI was greater than 1.5 ng/ml for patients enrolled before October 1, 1999 and greater than 0.15 ng/ml for patients enrolled afterward. Prolonged myocardial ischemia was defined as 20 min or more of continuous ST elevation or depression ($>$ 1 mm in two or more contiguous leads) on automated 12-lead electrocardiographic monitoring.[23] A secondary composite outcome was defined as cardiac death or myocardial injury, where myocardial injury was defined as clinical MI or increased surveillance troponin I, excluding patients whose only evidence of ischemia was by automated [1]electrocardiography.

Statistical Analysis

Univariate analyses of clinical and genotypic factors were determined by chi-square test (or Fisher exact test) for categorical variables and *t* test for continuous variables (mean $\pm$ SD). *P* values were two tailed, and values less than 0.05 were considered significant. Forward stepwise multivariate logistic regression was used to fit composite myocardial ischemic outcome to clinical and genotypic risk factors. Odds ratios (ORs), 95% confidence intervals (shown in brackets), and *P* values are reported for regression analyses. Confidence intervals were determined by nonparametric bootstrapping, and *P* values were determined from parametric Wald statistics. With a sample size of 196 and the SE for a risk factor regression coefficient of 0.40, our study was adequately powered to detect an OR of 2.2 at the $P < 0.05$ level (SEs for the GPIIIa and GPIbα regression coefficients were 0.39 and 0.41, respectively). To build an ischemia prediction model, each clinical variable was added in forward stepwise fashion, followed by each of the genetic variables, and the contribution of each variable to the model was assessed by likelihood ratio testing (LRT). Each variable with an LRT *P* value less than 0.10 was retained. Colinearity of variables was assessed. Each of the variables identified by LRT was assigned an integer risk score in proportion to its multivariate OR to create an additive ischemia risk model. Accuracy of the final predictive model was assessed by receiver operator characteristic (ROC) analysis. The ROC model was validated by leave-one-out cross-validation.[24] Statistical analysis was performed using the R software package.‡‡

Results

A total of 196 patients were studied, 61% of whom were men and 39% of whom were women. The mean age of the population was 68 $\pm$ 11 yr. Forty-five percent of patients underwent infrainguinal revascularization (IIS), 29% had surgery involving the abdominal aorta (AAS), and 26% underwent thoracoabdominal aneurysm surgery (TAS). Clinical characteristics of the GPIIIa and GPIbα genotype cohorts are shown in table 1. There was a higher frequency of diabetes and a lower frequency of aspirin use among patients with a Pro33 genotype of GPIIIa.

Sixty-five patients (33%) experienced the main composite ischemic outcome measure. Forty-one patients (21%) experienced the secondary endpoint of cardiac death or myocardial injury. Cardiac death and clinically detected MI occurred in 2% and 5% of patients, respectively. Thirty-nine patients (20%) had evidence of myocardial injury by surveillance troponin I, and 44 patients (22%) had prolonged ischemia on automated electrocardiogram. Both surveillance troponin I and automated electrocardiographic ischemia were associated with clinically detected MI (OR 46.8 [5.7–383] and 9.4 [2.3–38.1], respectively) and cardiac death (13.0 [1.3–129] and 3.6 [0.5–26.1], respectively).

The relation between clinical and genetic factors and the main composite ischemic outcome is shown in table 2. A history of angina was significantly related to the main outcome on univariate analysis. A strong trend between postoperative ischemia and a history of MI or diabetes mellitus (DM) was also observed. The type of surgical procedure was significantly related to postoperative ischemia by univariate analysis, with risk of TAS $>$ AAS $>$ IIS. Angina (OR 3.2 [1.3–7.6]; $P = 0.01$), DM (OR 2.4 [1.1–5.0]; $P = 0.03$), and TAS (OR 3.8 [1.6–4.0]; $P < 0.01$) were significantly related to the secondary outcome on univariate analysis, and there was a trend toward a relation between AAS (OR 2.0 [0.9–4.8]; $P = 0.10$) and the secondary outcome.

‡‡ Free software available from the R Foundation for Statistical Computing, at www.r-project.org. Accessed April 2004.

Table 2. Relation between Clinical and Genetic Factors and Composite Ischemic Outcome

	+Ischemia n = 65 (33%)	−Ischemia n = 131 (67%)	*P* Value*	Univariate OR (95% CI)	Multivariate OR (95% CI)
Age	68 ± 11	68 ± 11	0.86	1.0 (0.97–1.02)	
Sex					
Male	43 (66)	76 (58)	0.27	F: 1.0	
Female	22 (34)	55 (42)		M: 1.4 (0.8–2.7)	
Race					
White	48 (74)	97 (74)	0.80	W: 1.0	
African-American	16 (25)	30 (23)		NW: 1.0 (0.5–2.0)	
Other	1/65 (1)	4 (3)			
Hypertension	48 (74)	102 (78)	0.85	0.8 (0.4–1.6)	
Diabetes mellitus	19 (29)	23 (18)	0.06	1.9 (1.0–3.9)	4.0 (1.7–12.5)
Cigarette smoking	49 (75)	109 (83)	0.19	0.6 (0.3–1.3)	
Angina	15 (23)	12 (9)	< 0.01	3.0 (1.3–6.8)	2.2 (0.7–7.2)
MI	24 (36)	32 (24)	0.07	1.8 (1.0–3.4)	2.4 (1.0–5.9)
MI < 6 months	4 (6)	6 (4)	0.64	1.4 (0.4–5.0)	
CABG	16 (25)	22 (17)	0.19	1.6 (0.8–3.4)	
Percutaneous coronary intervention	12 (18)	18 (14)	0.39	1.4 (0.6–3.2)	
CHF	8 (12)	12 (9)	0.49	1.4 (0.5–3.6)	
Aortic stenosis	4 (6)	3 (2)	0.17	2.8 (0.6–12.8)	
Creatinine, mg/dl	1.3 ± 1.3	1.3 ± 1.6	0.91	1.0 (0.8–1.2)	
Creatinine > 2.0 mg/dl	6 (9)	12 (9)	0.99	1.0 (0.4–2.8)	
Preoperative β blockers	32 (49)	49 (37)	0.11	1.6 (0.9–3.0)	
Preoperative aspirin	23 (35)	60 (46)	0.16	0.6 (0.4–1.2)	0.5 (0.2–1.1)
Preoperative statin	19 (29)	49 (37)	0.26	0.7 (0.4–1.3)	
Type of surgery					
Infrainguinal	19 (29)	69 (53)	< 0.01	1.0	
Abdominal aortic	22 (34)	35 (26)		2.3 (1.1–4.8)	4.1 (1.7–14.4)
Thoracic aortic	24 (37)	27 (21)		3.2 (1.5–6.8)	6.4 (2.7–23.8)
Postoperative β blockers	54 (83)	96 (73)	0.13	1.8 (0.8–3.8)	
Postoperative aspirin	27 (42)	64 (49)	0.33	0.7 (0.4–1.4)	
Pro33+	24 (37)	27 (21)	0.01	2.3 (1.2–4.4)	2.4 (1.2–6.2)
Met145+	20 (31)	20 (15)	0.01	2.5 (1.2–5.0)	3.4 (1.4–9.3)

Multivariate odds ratios (ORs) are adjusted for the potential confounding influence of preoperative aspirin use.

* By chi-square or *t* test.

CABG = coronary artery bypass grafting; CHF = congestive heart failure; CI = confidence interval; F = female; M = male; Met145+ = Met145/Met145 and Thr145/Met145 genotypes; MI = myocardial infarction; NW = nonwhite; Pro33+ = Pro33/Pro33 and Leu33/Pro33 genotypes; W = white.

Patients with a heterozygous or homozygous genotype for the Pro33 or Met145 polymorphisms were more likely to experience the main composite ischemic outcome (table 2). Allele frequencies for Pro33 (0.19 *vs.* 0.11; $P = 0.03$) and Met145 (0.15 *vs.* 0.09; $P = 0.07$) were also greater in patients who experienced the main ischemic outcome. Alleles for both genes were in Hardy-Weinberg equilibrium. Among patients who experienced the secondary ischemic outcome, 15 of 41 (36%) possessed a genotype with the Pro33 allele compared with 36 of 155 patients (23%) who did not experience the secondary ischemic outcome (OR 1.9 [0.9–4.0]; $P = 0.09$). Similarly, 12 of 41 patients with ischemia (29%) possessed a genotype with the Met145 allele compared with 28 of 155 patients (18%) who did not experience the secondary outcome (OR 1.9 [0.8–4.1]; $P = 0.12$). Four subjects had cardiovascular death from MI, but there was no relation between genotype and death.

Multivariate regression identified five factors that were independently associated with the main ischemic outcome measure (table 2, multivariate OR): DM, AAS, TAS, Pro33 genotype, and Met145 genotype. The greatest risk was associated with the operative procedure (TAS > AAS > IIS). In addition, there was a trend toward a relation between ischemic outcome and a history of angina or MI, and these two factors were colinear. There was also a trend toward a protective effect of preoperative aspirin use. DM (OR 5.8 [2.2–22.2]; $P < 0.01$), TAS (OR 8.8 [2.8–41.3]; $P < 0.01$), and Met145 genotype (OR 2.5 [1.1–7.0]; $P = 0.04$) were also independently associated with the secondary outcome measure by multivariate analysis. Angina (OR 3.0 [0.8–11.5]; $P = 0.02$) and AAS (OR 2.9 [0.9–11.2]; $P = 0.05$) showed a strong trend toward a relation with the secondary outcome. The relation between Pro33 genotype and the secondary ischemic outcome did not reach statistical significance (OR 1.8 [0.7–4.3]; $P = 0.18$).

Six clinical factors met criteria ($P < 0.10$, LRT) for inclusion in the model to predict the main composite ischemic outcome: DM, angina, MI, preoperative aspirin use, AAS, and TAS. The addition of platelet genetic factors to the clinical model, separately (Pro33 chi-square = 3.01; $P = 0.08$, and Met145 chi-square = 7.34; $P < 0.01$) or together (chi-square = 13.5; $P < 0.001$), improved fit

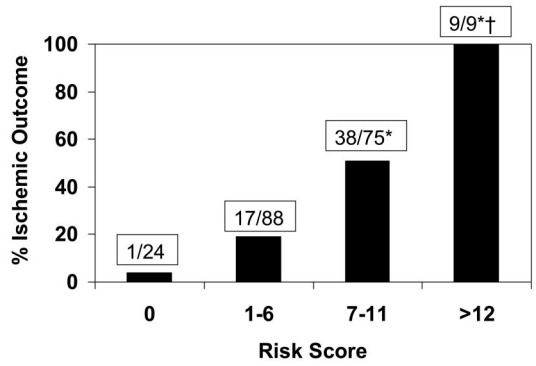

Fig. 1. The risk of postoperative myocardial ischemia increases with summary risk score. Risk factors were added to derive a summary risk score, and subjects were classified into four categories based on the summary score. The incidence of composite ischemic outcome in these categories was compared by Fisher exact test. * $P < 0.001$ *versus* risk score = 0 or 1–6. † $P < 0.01$ *versus* risk score = 7–11.

of the ischemia prediction model. None of the other variables improved model fit, including age, sex, and race. Four clinical factors contributed to prediction of the secondary ischemic outcome: DM, angina, AAS, and TAS (MI did not contribute). The addition of GPIbα genotype met criteria for model improvement (chi-square = 3.51; $P = 0.06$); however, addition of GPIIIa genotype did not (chi-square = 1.34; $P = 0.25$).

A final model was constructed to predict the main ischemic outcome measure. Aspirin use was excluded from the final model to limit the ratio of variables:outcomes to 1:10. Each of the seven remaining variables was assigned an integer score weighted in proportion to its OR derived from multivariate logistic regression, *i.e.*, DM = 4, angina = 2, MI = 2, AAS = 4, TAS = 6, Pro33+ = 2, and Met145+ = 3. These seven variables were used to create an additive risk model, and a summary risk score was calculated for each subject. The likelihood of developing postoperative ischemia increased with increasing summary risk score (fig. 1). Discrimination of the additive risk model, by area under the ROC curve, was 0.73 [0.65–0.81], and positive predictive values were 37, 56, and 100% at risk score cutoffs of 1, 7, and 12, respectively (table 3). Without platelet genotype in the model, the area under the ROC curve was 0.64 [0.57–0.74] ($P = 0.04$ *vs.* model including genetic factors). A separate model was created to predict the secondary outcome measure, and it included the following variables: DM, angina, AAS, TAS, and GPIIIa and GPIbα genotypes. The area under the ROC curve for this model was 0.73 [0.63–0.83], which was similar to the model to predict the primary outcome. Without platelet genotype in the model, the ROC was 0.67 [0.57–0.75] ($P = 0.12$ *vs.* model including genotype).

Table 3. Accuracy of Model to Predict Main Composite Ischemic Outcome Measure at Different Summary Score Cutoffs

	Summary Risk Score Cutoff Value		
	1	7	12
% Sensitivity (95% CI)	98 (95–100)	72 (61–82)	14 (6–23)
% Specificity (95% CI)	18 (11–24)	72 (63–79)	100 (100–100)
% Positive predictive value (95% CI)	37 (30–44)	56 (45–67)	100 (100–100)
% Negative predictive value (95% CI)	96 (86–100)	84 (76–90)	70 (63–76)

CI = confidence interval.

Discussion

Postoperative myocardial ischemia was common in our cohort of 196 infrainguinal and aortic vascular surgery patients. A 2% incidence of cardiac death and a 5% incidence of MI are comparable to previous reports.[3,25–28] Increased surveillance troponin I and prolonged-duration electrocardiographic ischemia occurred frequently and were related to clinical MI and cardiac death. These data confirm previous reports that demonstrate that a large number of postoperative ischemic episodes are not clinically detected but are nonetheless strongly related to overt morbidity and mortality.[17,18,29–31]

Among traditional clinical cardiac risk factors, only DM, MI, and angina were associated with postoperative myocardial ischemia. These factors have been consistently identified as perioperative cardiac risk factors by other investigators[1–3] and are classified as intermediate clinical predictors by current American College of Cardiology/American Heart Association guidelines.[5] The strongest predictor of cardiac risk was the specific surgical procedure. One of the novel findings of this study was the differential cardiac risk associated with different vascular surgical procedures (TAS > AAS > IIS), which, according to current risk assessment strategies, are all categorized as having an equivalently high risk. Our findings contrast with those of L'Italien *et al.*,[4] who reported greater risk in infrainguinal than aortic procedures, and those of Boersma *et al.*,[28] who reported equivalent cardiac risk in infrainguinal and aortic procedures. Discordant results among these studies may be attributable to differences in the use of β blockers: More than three fourths of the patients in the current study received perioperative β blockade compared with less than one third in the study by Boersma *et al.*, and the study by L'Italien *et al.* evaluated data before the era of perioperative β blocker therapy. Differential risk in our study may be related to the magnitude of stress imposed by aortic *versus* peripheral vascular procedures and the ability of β blockers to mitigate those different stresses.

Gene polymorphisms in platelet GPIIIa (Pro33) and GPIbα (Met145) were independent predictors of the main ischemic outcome after major vascular surgery. This finding is consistent with numerous reports that

describe an association between these polymorphisms and myocardial ischemic morbidity in nonsurgical settings,[13–16,32] although some authors have refuted this relation.[33–35] A significant relation between the GPIbα polymorphism and the harder endpoint of cardiac death or myocardial injury was also present on multivariate analysis, but the relation between the GPIIIa polymorphism and the secondary outcome measure was not significant. Whether this reflects a pathophysiologic difference between the primary and secondary outcome measures or reflects inadequate statistical power from a smaller number of secondary outcomes is unclear.

Glycoprotein IIIa is one of the integrin proteins that comprise the platelet fibrinogen receptor (GPIIb–IIIa), and the Pro33 polymorphism is reported to enhance platelet reactivity.[36,37] GPIbα is one of four proteins that form a complex that functions as the platelet von Willebrand factor receptor, and the Met145 polymorphism is reported to enhance von Willebrand factor binding.[38] However, the functional consequences of these platelet polymorphisms remain controversial, and a mechanistic link between these genetic polymorphisms and clinical outcome remains unproved. Stress-related adrenergic stimulation with increased myocardial oxygen consumption is the mechanism traditionally attributed to perioperative myocardial ischemia.[8,9] Our data suggest that platelet-mediated coronary occlusion may contribute to postoperative myocardial ischemia more frequently than previously recognized. The reduction in cardiac risk we observed in association with preoperative aspirin use supports this possibility. Further support comes from autopsy series, where plaque rupture and acute coronary thrombosis seem to complicate fatal perioperative MI as frequently as in nonoperative settings.[10]

The addition of platelet GPIIIa and GPIbα genotypes to historic and surgical risk factors improved prediction of the main ischemic outcome measure in this population of infrainguinal and aortic vascular surgery patients. This effect was demonstrated both by improvement in model fit (by likelihood ratio testing) and by comparison of ROC curves for models inclusive and exclusive of genetic factors. The area under the ROC curve of 0.73 indicates moderate predictive power and suggests that additional factors not measured in this study probably contribute to ischemic outcome. However, the discriminative ability of the derived model was comparable to other risk assessment tools[1–3] and provided better positive predictive accuracy than reported for those other models or noninvasive cardiac testing.[6,7] In addition, whereas other risk assessment tools have proved ineffective in patients undergoing aortic surgery,[3] our model performed well in this population. The addition of platelet polymorphisms to clinical factors provided more modest improvement in prediction of the secondary ischemic outcome measure, and this benefit was derived mainly from the GPIbα polymorphism. The weaker relation between platelet genes and the secondary outcome measure could reflect inadequate statistical power or a true difference in the biologic relation between the polymorphisms and the two outcomes.

The findings in this report should be considered preliminary because of a number of study limitations. First, although the relation between platelet gene polymorphisms and the main ischemic outcome measure was strong, the relation was weaker with the harder secondary outcome measure. A study that involves a larger number of hard endpoints is required to verify the relation between platelet genes and postoperative myocardial injury. Second, although this study shows that the addition of platelet polymorphisms to clinical risk factors improves our model to predict composite ischemic outcome, it is inadequately powered to determine whether the derived model has better diagnostic accuracy (*i.e.*, positive and negative predictive value) than a model limited to clinical risk factors alone. Third, additional studies are required to reproduce our findings before the derived model can be considered clinically useful. Nonetheless, the current study demonstrates that genetic testing has the potential to uncover risk factors for perioperative ischemia that are missed by clinical assessment alone.

References

1. Goldman L, Caldera DL, Nussbaum SR, Southwick FS, Krogstad D, Murray B: Multifactorial index of cardiac risk in noncardiac surgical procedures. N Engl J Med 1977; 297:845–50

2. Detsky AS, Abrams HB, Forbath N, Scott JG, Hilliard JR: Cardiac assessment for patients undergoing noncardiac surgery: A multifactorial clinical risk index. Arch Intern Med 1986; 146:2131–4

3. Lee TH, Marcantonio ER, Mangione CM, Thomas EJ, Polanczyk CA, Cook EF, Sugarbaker DJ, Donaldson MC, Poss R, Ho KKL, Ludwig LE, Pedan A, Goldman L: Derivation and prospective validation of a simple index for prediction of cardiac risk in major noncardiac surgery. Circulation 1999; 100:1043–9

4. L'Italien GJ, Paul SD, Hendel RC, Leppo JA, Cohen MC, Fleisher LA, Brown KA, Zarich SW, Cambria RP, Cutler BS, Eagle KA: Development and validation of a Bayesian model for perioperative cardiac risk assessment in a cohort of 1,081 vascular surgical candidates. J Am Coll Cardiol 1996; 27:779–86

5. Eagle KA, Berger PB, Calkins H, Chaitman BR, Ewy GA, Fleischman KE, Fleisher LA, Froehlich JB, Gusberg RJ, Leppo JA, Ryan T, Schlant RC, Winters WL: ACC/AHA guideline update for perioperative cardiovascular evaluation for noncardiac surgery: executive summary: A report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee to Update the 1996 Guidelines on Perioperative Cardiovascular Evaluation for Noncardiac Surgery). Circulation 2002; 105:1257–67

6. Eagle KA, Brundage BH, Chaitman BR, Ewy GA, Fleisher LA, Hertzer NR, Leppo JA, Ryan T, Schlant RC, Spencer WH, Spittell JA, Twiss RD: Guidelines for perioperative cardiovascular evaluation for noncardiac surgery: Report of the American College of Cardiology/American Heart Association Task Force on Practice Guidelines (Committee on Perioperative Cardiovascular Evaluation for Noncardiac Surgery). Circulation 1996; 93:1278–317

7. Grayburn PA, Hillis LD: Cardiac events in patients undergoing noncardiac surgery: Shifting the paradigm from noninvasive risk stratification to therapy. Ann Intern Med 2003; 138:506–11

8. Mangano DT, Layug EL, Wallace A, Tateo I, Investigators. M: Effect of atenolol on mortality and cardiovascular morbidity after noncardiac surgery. N Engl J Med 1996; 335:1713–20

9. Mangano DT, Hollenberg M, Fegert G, Meyer ML, London MJ, Tubau JF, Krupski WC: Perioperative myocardial ischemia in patients undergoing noncardiac surgery: I. Incidence and severity during the 4 day perioperative period. J Am Coll Cardiol 1991; 17:843–50

10. Dawood MM, Gutpa DK, Southern J, Walia A, Atkinson JB, Eagle KA: Pathology of fatal perioperative myocardial infarction: Implications regarding pathophysiology and prevention. Int J Cardiol 1996; 57:37–44

11. Trip MD, Cats VM, van Capelle FJ, Vreeken J: Platelet hyperreactivity and prognosis in survivors of myocardial infarction. N Engl J Med 1990; 322:1549–54

12. Antiplatelet Trialists' Collaboration: Collaborative overview of randomised trials of antiplatelet therapy: I. Prevention of death, myocardial infarction and stroke by prolonged antiplatelet therapy in various categories of patients. BMJ 1994; 308: 81–106

13. Weiss EJ, Bray PF, Tayback M, Schulman SP, Kickler TS, Becker LC, Weiss JL, Gerstenblith G, Goldschmidt-Clermont PJ: A polymorphism of a platelet glycoprotein receptor as an inherited risk factor for coronary thrombosis. N Engl J Med 1996; 334:1090–4

14. Di Castelnuovo A, de Gaetano G, Donati MB, Iacoviello L: Platelet glycoprotein receptor IIIa polymorphism PI^{A1}/PI^{A2} and coronary risk: A meta-analysis. Thromb Haemost 2001; 85:626–33

15. Gonzalez-Conejero R, Lozano ML, Rivera J, Corral J, Iniesta JA, Moraleda JM, Vicente V: Polymorphisms of platelet membrane glycoprotein Ibα associated with arterial thrombotic disease. Blood 1998; 92:2771–6

16. Mikkelsson J, Perola M, Penttila A, Karhunen PJ: Platelet glycoprotein Ibα HPA-2 Met/VNTR B haplotype as a genetic predictor of myocardial infarction and sudden cardiac death. Circulation 2001; 104:876–80

17. Kim LJ, Martinez EA, Faraday N, Dorman T, Fleisher LA, Perler BA, Williams GM, Chan D, Pronovost PJ: Cardiac troponin I predicts short-term mortality in vascular surgical patients. Circulation 2002; 106:2366–71

18. Landesberg G, Mosseri M, Wolf Y, Vasselov Y, Weissman C: Perioperative myocardial ischemia and infarction: Identification by continuous 12-lead electrocardiogram with online ST-segment monitoring. ANESTHESIOLOGY 2002; 96:264–70

19. Martinez EA, Kim LJ, Faraday N, Rosenfeld B, Bass EB, Perler BA, Williams GM, Dorman T, Pronovost PJ: Sensitivity of routine intensive care unit surveillance for detecting myocardial ischemia. Crit Care Med 2003; 31:2302–8

20. Jin Y, Dietz HC, Nurden A, Bray PF: Single-strand conformation polymorphism analysis is a rapid and effective method for the identification of mutations and polymorphisms in the gene for glycoprotein IIIa. Blood 1993; 82:2281–8

21. Murata M, Matsubara Y, Kawano K, Zama T, Aoki N, Yoshino H, Watanabe G, Ishikawa K, Ikeda Y: Coronary artery disease and polymorphisms in a receptor mediating shear stress-dependent platelet activation. Circulation 1997; 96:3281–6

22. Palomaki P, Miettinen H, Mustaniemi H, Lehto S, Pyorala K, Mahonen M, Tuomilehto J: Diagnosis of acute myocardial infarction by MONICA and FINMONICA diagnostic criteria in comparison with hospital discharge diagnosis. J Clin Epidemiol 1994; 47:659–66

23. Fleisher LA, Nelson AH, Rosenbaum SH: Postoperative myocardial ischemia: Etiology of cardiac morbidity or manifestation of underlying disease. J Clin Anesth 1995; 7:97–102

24. Shao J: Linear model selection by cross-validation. J Am Stat Assoc 1993; 88:486–94

25. Taylor LM, Yeager RA, Moneta GL, McConnell DB, Porter JM: The incidence of perioperative myocardial infarction in general vascular surgery. J Vasc Surg 1991; 15:52–61

26. Mamode N, Scott RN, McLaughlin SC, McLelland A, Pollock JG: Perioperative myocardial infarction in peripheral vascular surgery. BMJ 1996; 312:1396–7

27. Poldermans D, Boersma E, Bax JJ, Thomson IR, van de Ven LLM, Blankensteijn JD, Baars HF, Yo T-I, Trocino G, Vigna C, Roelandt JRTC, van Urk H: The effect of bisoprolol on perioperative mortality and myocardial infarction in high-risk patients undergoing vascular surgery. N Engl J Med 1999; 341:1789–94

28. Boersma E, Poldermans D, Bax JJ, Steyerberg EW, Thomson IR, Banga JD, van de Ven LLM, van Urk H, Roelandt JRTC: Predictors of cardiac events after major vascular surgery: Role of clinical characteristics, dobutamine echocardiography, and β-blocker therapy. JAMA 2001; 285:1865–73

29. Mangano DT, Browner WS, Hollenberg M, London MJ, Tubau JF, Tateo IM: Association of perioperative myocardial ischemia with cardiac morbidity and mortality in men undergoing noncardiac surgery. The Study of Perioperative Ischemia Research Group. N Engl J Med 1990; 323:1781–8

30. Raby KE, Barry J, Creager MA, Cook EF, Weisberg MC, Goldman L: Detection and significance of intraoperative and postoperative myocardial ischemia in peripheral vascular surgery. JAMA 1992; 268:222–7

31. Landesberg G, Luria MH, Cotev S, Eidelman LA, Anner H, Mosseri M, Schechter D, Assaf J, Erel J, Berlatzky Y: Importance of long-duration postoperative ST-segment depression in cardiac morbidity after vascular surgery. Lancet 1993; 341:715–9

32. Bray PF, Cannon CP, Goldschmidt-Clermont P, Moye LA, Pfeffer MA, Sacks FM, Braunwald E: The platelet PI^{A2} and angiotensin-converting enzyme (ACE) D allele polymorphisms and the risk of recurrent events after acute myocardial infarction. Am J Cardiol 2001; 88:347–52

33. Ridker PM, Hennekens CH, Schmitz C, Stampfer MJ, Lindpaintner K: PIA1/A2 polymorphism of platelet glycoprotein IIIa and risks of myocardial infarction, stroke, and venous thrombosis. Lancet 1997; 349:385–8

34. Aleksic N, Juneja H, Folsom AR, Ahn C, Boerwinkle E, Chambless LE, Wu KK: Platelet Pl(A2) allele and incidence of coronary heart disease: Results from the Atherosclerosis Risk in Communities (ARIC) study. Circulation 2000; 102: 1901–5

35. Ardissino D, Mannucci PM, Merlini PA, Duca F, Fetiveau R, Tagliabue L, Tubaro M, Galvani M, Ottani F, Ferrario M, Corral J, Margaglione M: Prothrombotic genetic risk factors in young survivors of myocardial infarction. Blood 1999; 94:46–51

36. Feng DL, Lindpaintner K, Larson MG: Increased platelet aggregability associated with platelet GPIIIa PlA2 polymorphism: The Framingham Offspring Study. Arterioscler Thromb Vasc Biol 1999; 19:1142–7

37. Michelson AD, Furman MI, Goldschmidt-Clermont P, Mascelli MA, Hendrix C, Coleman L, Hamlington J, Barnard MR, Kickler T, Christie DJ, Kundu S, Bray PF: Platelet GP IIIa PI^{A} polymorphisms display different sensitivities to agonists. Circulation 2000; 101:1013–8

38. Ulrichts H, Vanhoorelbeke K, Cauwenberghs S, Vauterin S, Kroll H, Santoso S, Deckmyn H: Von Willebrand factor but not alpha-thrombin binding to platelet glycoprotein Ibalpha is influenced by the HPA-2 polymorphism. Arterioscler Thromb Vasc Biol 2003; 23:1302–7