Effects of Aspirin Responsiveness and Platelet Reactivity on Early Vein Graft Thrombosis After Coronary Artery Bypass Graft Surgery

Tyler J. Gluckman, MD,* Rhondalyn C. McLean, MD,* Steven P. Schulman, MD,* Thomas S. Kickler, MD,* Edward P. Shapiro, MD,* John V. Conte, MD,† Kathleen W. McNicholas, MD;‡ Jodi B. Segal, MD,* Jeffrey J. Rade, MD*

Baltimore, Maryland; and Christiana, Delaware

Objectives

The purpose of this study was to determine if an incomplete response to or inadequate antiplatelet effect of aspirin, or both, contribute to saphenous vein graft (SVG) occlusion after coronary artery bypass graft (CABG) surgery.

Background

Thrombosis is the predominant cause of early SVG occlusion. Aspirin, which inhibits cyclooxygenase-1 activity and thromboxane generation in platelets, reduces early SVG occlusion by one-half.

Methods

Aspirin responsiveness and platelet reactivity were characterized 3 days and 6 months after coronary artery bypass graft surgery in 229 subjects receiving aspirin monotherapy by platelet aggregation to arachidonic acid, adenosine diphosphate, collagen and epinephrine, Platelet Function Analyzer-100 (Siemens Healthcare Diagnostics, Newark, Delaware) closure time (CT) using collagen/epinephrine agonist cartridge and collagen/adenosine diphosphate (CADP) agonist cartridge, VerifyNow Aspirin assay (Accumetrics, Inc., San Diego, California), and urine levels of 11-dehydro-thromboxane B2 (UTXB2). SVG patency was determined 6 months after surgery by computed tomography coronary angiography.

Results

Inhibited arachidonic acid-induced platelet aggregation, indicative of aspirin-mediated cyclooxygenase-1 suppression, occurred in 95% and >99% of subjects 3 days and 6 months after surgery, respectively. Despite this, 73% and 31% of subjects at these times had elevated UTXB2. Among tested parameters, only UTXB2 and CADP CT measured 6 months after surgery correlated with outcome. By multivariate analysis, CADP CT of >88 s (odds ratio: 2.85, p = 0.006), target vessel diameter of ≤1.5 mm (odds ratio: 2.38, p = 0.01), and UTXB2 of ≥450 pg/mg creatinine (odds ratio: 2.59, p = 0.015) correlated with SVG occlusion. CADP CT and UTXB2 in combination further identified subjects at particularly high and low risk for SVG occlusion.

Conclusions

Aspirin-insensitive thromboxane generation measured by UTXB2 and shear-dependent platelet hyper-reactivity measured by Platelet Function Analyzer-100 CADP CT are novel independent risk factors for early SVG thrombosis after coronary artery bypass graft surgery. (J Am Coll Cardiol 2011;57:1069–77) © 2011 by the American College of Cardiology Foundation

Coronary artery bypass graft (CABG) surgery is a commonly used revascularization strategy in patients with severe coronary artery disease. Saphenous vein grafts (SVGs) are the most frequently used conduits for this procedure. Unlike arterial grafts, SVGs are particularly susceptible to occlusive thrombosis during the first post-operative year, which exposes patients to increased risks of death, myocardial infarction, and repeat revascularization (1–3).

Aspirin (ASA) is the pharmacologic agent most convincingly shown to prevent SVG thrombosis, reducing its incidence during the first postoperative year by nearly 50% (4). The principal effect of ASA is to suppress agonist-induced platelet activation by irreversibly inhibiting platelet cyclooxygenase (COX)-1, a critical enzyme regulating the

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Abbreviations and Acronyms

<table>
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<th>Acronym</th>
<th>Definition</th>
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<tr>
<td>ASA</td>
<td>aspirin</td>
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<tr>
<td>CABG</td>
<td>coronary artery bypass graft</td>
</tr>
<tr>
<td>CADP</td>
<td>collagen/adenosine diphosphate</td>
</tr>
<tr>
<td>CEPI</td>
<td>collagen/epinephrine</td>
</tr>
<tr>
<td>COX</td>
<td>cyclooxygenase</td>
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<tr>
<td>CT</td>
<td>closure time</td>
</tr>
<tr>
<td>IQR</td>
<td>interquartile range</td>
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<tr>
<td>SVG</td>
<td>saphenous vein graft</td>
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<tr>
<td>TXA₂</td>
<td>thromboxane A₂</td>
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<tr>
<td>UTXB₂</td>
<td>urinary 11-dehydro-thromboxane B₂</td>
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Methods

Patient population. The Reduction in Graft Occlusion Rates (RIGOR) study is a multicenter study of patients undergoing first-time CABG surgery designed to investigate the effects of antiplatelet factor 4/heparin antibody induction on SVG occlusion. A detailed description of the study and its principal findings have been reported previously (12). A second prospectively defined objective of the study was to determine whether ASA resistance, platelet hyper-reactivity, or both, contribute to early SVG occlusion after CABG surgery.

Patients 18 years of age or older undergoing first-time CABG surgery with implantation of 1 or more SVGs were eligible for enrollment. Although patients with an anticipated requirement for post-operative oral anticoagulation or non-ASA antiplatelet therapy were excluded, those administered such agents for unforeseen post-operative conditions remained in the study. All subjects were administered ASA at each postoperative encounter. ASA responsiveness and platelet reactivity were measured in subjects enrolled at the Johns Hopkins Hospital 2 to 4 days after CABG surgery and in all subjects 6 months after CABG surgery at the time SVG patency was assessed by multidetector computed tomography coronary angiography.

Measurement of platelet reactivity and ASA responsiveness.

PLATELET AGGREGOMETRY. Platelet-rich plasma was prepared from blood collected in 3.2% citrate by centrifugation at 100 rpm for 10 min, and the platelet count was adjusted to 180,000/mm³ by the addition of platelet-poor plasma. Undiluted samples with a platelet count of <100,000/mm³ were excluded from analysis. Impedance platelet aggregometry was performed by stimulation with arachidonic acid (0.5 mM), adenosine diphosphate (5, 10, and 20 μM), epinephrine (50 μM), and collagen (1 μg/ml) using a Chrono-Log Model 560CA aggregometer (Chrono-Log, Havertown, Pennsylvania). The maximum aggregation response within 5 minutes was recorded in Ω. Subjects were considered ASA responsive if arachidonic acid-induced platelet aggregation was ≤1 Ω (normal range in our laboratory for ASA-naïve subjects: 5 to 17 Ω).

SHEAR-DEPENDENT PLATELET ACTIVATION. The closure time (CT) of whole blood collected in 3.8% citrate under shear was measured by the Platelet Function Analyzer-100 (PFA-100) device (Siemens Healthcare Diagnostics, Newark, Delaware), as previously described (13). Samples were tested with the collagen/epinephrine (CEPI) agonist cartridge, which is sensitive to the effects of ASA, and the collagen/adenosine diphosphate (CADP) agonist cartridge, which assesses global platelet reactivity, but is not affected by ASA. Samples from subjects with a platelet count <50,000/m³ were excluded from analysis. Samples with nonclosure were assigned a CT value of 300 s, the maximum measurable by the device. ASA responsiveness by the PFA-100 assay was defined as a CEPI CT >193 s, the upper limit of the normal range in our laboratory for ASA-naïve patients (13).

VERIFYNOW ASPIRIN ASSAY. The VerifyNow Aspirin assay (Accumetrics, Inc., San Diego, California) measures arachidonic acid-induced platelet aggregation in whole blood containing fibrinogen-coated beads (14). Samples from subjects with a platelet count <50,000/m³ were excluded from analysis. ASA responsiveness by this assay was defined as an aspirin reaction unit (ARU) value <550 according to the manufacturer’s instruction.

MEASUREMENT OF TXA₂ GENERATION. TXA₂ generation was quantified by measuring the concentration of its stable metabolite 11-dehydro-thromboxane B₂ (UTXB₂) in urine by enzyme-linked immunosorbent assay and expressed as a ratio to urinary creatinine as previously described (13). ASA responsiveness by this assay was defined as UTXB₂ <400 pg/mg creatinine according to established criteria (15).
Assessment of SVG patency. SVG patency was assessed 6 months after CABG surgery by multidetector computed tomography coronary angiography as previously described (12). Data from clinically driven invasive coronary angiograms could be used for the primary end point analysis if performed within 6 weeks of the anticipated 6-month follow-up visit or if it was the only assessment of SVG patency before an adverse clinical end point. Each segment of Y-grafts and skip grafts were considered as separate SVGs according to the Society of Thoracic Surgeons criteria. Reconstructed images were analyzed by 2 blinded reviewers (T.J.G., E.P.S., or J.J.R.) and were classified as patent (containing stenoses of 0% to 75%), significantly diseased (containing stenoses of 76% to 99%), or occluded (containing a 100% stenosis). There was 98% concordance in assessment of SVG patency between reviewers. In cases of discordance, a third reviewer adjudicated all SVGs in that patient.

Statistical analysis. Data from subjects who survived the index hospitalization, underwent angiographic assessment of SVG patency, and were maintained throughout the study period on ASA as the only antiplatelet agent were analyzed for the outcome of SVG occlusion versus patency. For statistical purposes, the small number of SVGs classified as significantly diseased were classified as patent. Demographic differences between patients with and without occluded SVGs were evaluated using a Student t test for mean values or Wilcoxon rank-sum test for medians as appropriate for the data distribution. Proportions were compared using a chi-square or Fisher exact test and comparisons among groups were carried out with an analysis of variance, the McNemar test, or the Kruskal-Wallis test, as appropriate.

Univariate analyses were performed on a per graft basis for the odds of occlusion versus patency using those variables deemed biologically plausible or supported by the literature. Categorical covariates were tested for collinearity using chi-square or Fisher exact tests, whereas continuous variables were tested using Pearson’s correlation coefficients. Highly correlated covariates (e.g., rho ≥0.7) were eliminated based on clinical significance. Variables with statistical significance of p ≤ 0.15 in the univariate analysis or those supported by the literature were entered into a multilevel random effects model. A 4-level model was explored initially: analyzing SVG outcome with random intercepts for patient, surgeon, hospital, and multisegmented SVGs (i.e., skip grafts and Y-grafts) considered as single grafts. Because random intercepts on surgeon, hospital, and multisegmented SVGs were not significant, the analysis thereafter included clustering on patient alone. Stepwise backwards elimination of variables was performed to generate several potential models. Akaica Information Criterion was used for model comparison.

Analyses were performed using Stata/MP version 10.0 for Windows software (Stata, Inc., College Station, Texas). Differences were considered significant when p < 0.05.

Results

Study population characteristics. The RIGOR study enrolled 368 subjects undergoing first-time CABG surgery with implantation of ≥1 SVG. The study population was representative of patients undergoing isolated CABG surgery based on comparison with the Society of Thoracic Surgeons National Database (12). Two hundred twenty-nine subjects who survived the index hospitalization, underwent angiographic assessment of SVG patency and were maintained on ASA throughout the study period as the only antiplatelet agent were included in the present analysis (Fig. 1). In addition to major differences in antiplatelet regimens and angiographic follow-up, subjects included in the analysis were more likely to be men, to be referred electively for CABG surgery, to have a slightly lower median euroSCORE and prevalences of current tobacco use, and to have a history of atrial fibrillation than excluded subjects (Supplemental Table 1). The baseline, operative, and post-operative characteristics of subjects with at least 1 occluded SVG were very similar to those with patent SVGs, except that the former group underwent implantation of ≥4 SVGs more frequently (Table 1). Of the 497 SVGs implanted in these subjects (median: 2, range: 1 to 6 SVGs/subject), 94 (19%) were totally occluded when assessed a median of 189 days (interquartile range [IQR]: 182 to 203 days) after CABG surgery. Only 9 SVGs (2%) contained high-grade (>75%) stenoses, consistent with the concept that thrombosis and neither atherosclerosis nor neointima hyperplasia is the predominant cause of early

Figure 1 Schematic of RIGOR Study Enrollment and Analysis Criteria

ASA = aspirin; CTA = multidetector computed tomography coronary angiography; MR = magnetic resonance; RIGOR = Reduction in Graft Occlusion Rates; SVG = saphenous vein graft.
SVG failure. Seventy subjects (31%) had occlusion of ≥1 SVG and 21 subjects (9%) had occlusion of all implanted SVGs.

Prevalence of ASA nonresponsiveness after CABG surgery. Platelet function testing was performed on 168 subjects a median of 3 days (IQR: 3 to 4 days) and on all 229 subjects a median of 189 days (IQR: 182 to 203 days) after CABG surgery. UTXB2 was measured in 219 and 228 subjects at these same respective time points. Of the assays performed, 4 have definable thresholds limits for ASA responsiveness. The prevalence of ASA nonresponsiveness varied widely by assay and by time after CABG surgery (Fig. 2A). By arachidonic acid-induced platelet aggregation, a specific measure of platelet COX-1 activity, ASA nonresponsiveness was present in 5% of subjects (median aggregation: 5 Ω, IQR:
2 to 10 Ω) 3 days after surgery and in <1% of subjects (median aggregation: 12 Ω, range 8 to 15 Ω) 6 months after surgery. Concurrent nonsteroidal anti-inflammatory drug use did not correlate with the presence of ASA nonresponsiveness defined by this method at either time point (data not shown). ASA nonresponsiveness also was significantly more prevalent immediately after CABC surgery when defined by PFA-100 CT using the CEPI agonist cartridge, but remained relatively constant over time when assessed by the VerifyNow Aspirin assay. Despite a high degree of platelet COX-1 inhibition by ASA, persistent TXA2 generation was observed in 73% and 31% of subjects 3 days and 6 months after CABC surgery, respectively, as evidenced by often significantly elevated levels of UTXB2 (Fig. 2B).

Association with early SVG occlusion. No laboratory parameter measured 3 days after CABC surgery could differentiate occluded from nonoccluded SVGs or subjects with occluded versus patent SVGs (Table 2). When assessed 6 months after surgery, however, occluded SVGs were associated with a higher mean level of UTXB2 and lower median PFA-100 CADP CT (Table 3). Subjects with ≥1 occluded SVG also had a lower median PFA-100 CADP CT than subjects with patent SVGs. Although SVG occlusion was inversely proportional to PFA-100 CADP CT, it was most prevalent in subjects with UTXB2 ≥450 pg/mg creatinine (Fig. 3).

The associations of PFA-100 CADP CT and UTXB2 to early SVG occlusion then were determined in relationship

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**Table 2** Relationship Between Platelet Reactivity and ASA Responsiveness, Measured 3 Days After CABC Surgery, and 6-Month SVG Patency

<table>
<thead>
<tr>
<th>Assay</th>
<th>Per SVG</th>
<th>Per Subject</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Occluded SVG</td>
<td>Patent SVG</td>
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<tr>
<td>Arachidonic acid, 0.5 mM</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
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<tr>
<td>ADP, 5 μM</td>
<td>17.1 ± 5.6</td>
<td>18.8 ± 4.9</td>
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<tr>
<td>ADP, 10 μM</td>
<td>18.0 ± 4.8</td>
<td>18.6 ± 5.1</td>
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<tr>
<td>ADP, 20 μM</td>
<td>16.5 ± 4.6</td>
<td>16.6 ± 4.4</td>
</tr>
<tr>
<td>Epinephrine, 50 μM</td>
<td>10.7 ± 6.5</td>
<td>9.94 ± 6.56</td>
</tr>
<tr>
<td>Collagen, 1 μg/ml</td>
<td>14.1 ± 5.4</td>
<td>12.7 ± 6.3</td>
</tr>
<tr>
<td>PFA-100 CT (s)</td>
<td>CEP</td>
<td>121 (94–203)</td>
</tr>
<tr>
<td></td>
<td>CADP</td>
<td>96 (82–137)</td>
</tr>
<tr>
<td></td>
<td>UTXB2 (ln pg/mg creatinine)</td>
<td>6.42 ± 0.71</td>
</tr>
<tr>
<td></td>
<td>VerifyNow Aspirin (ARU)</td>
<td>475 ± 59</td>
</tr>
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</table>

Values are median (interquartile range) or mean ± SD.
ADP = adenosine diphosphate; ARU = aspirin reaction unit; CADP = collagen/ADP agonist cartridge; CEP = collagen/epinephrine agonist cartridge; NS = not significant; PFA-100 = Platelet Function Analyzer 100; UTXB2 = urine 11-dehydro-thromboxane B2; other abbreviations as in Table 1.
to a comprehensive array of known and potential risk factors. In addition to several patient- and graft-specific factors, PFA-100 CADP CT and UTXB₂ measured 6 months after CABG surgery correlated with early SVG occlusion by univariate analysis (Supplemental Tables 2 to 5) when expressed both as continuous variables and as binary variables based on respective median (88 s) and upper quartile values (450 pg/mg creatinine). When analyzed either as continuous (model 1) or binary (model 2) variables in multivariate analyses (Table 4), PFA-100 CADP CT and UTXB₂ remained significantly associated with SVG outcome along with target vessel diameter \( \leq 1.5 \) mm, historically recognized as one of the strongest risk factor for early SVG occlusion (16–18). SVG occlusion also was more prevalent in subjects with both CADP CT \( \leq 88 \) s and UTXB₂ \( \geq 450 \) pg/mg creatinine than in subjects with CADP CT >88 s and UTXB₂ <450 pg/mg creatinine (32% vs. 10%, respectively, \( p < 0.0001 \)). By multivariate analysis, this equated to a 6.9-fold increased odds of SVG occlusion (95% confidence interval: 2.16 to 21.7, \( p = 0.001 \)) between groups.

### Table 3

<table>
<thead>
<tr>
<th>Assay</th>
<th>Per SVG</th>
<th>Per Subject</th>
<th>n</th>
<th>p Value</th>
<th>≥1 Occluded SVG</th>
<th>No Occluded SVG</th>
<th>n</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Arachidonic acid, 0.5 mM</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>495</td>
<td>NS</td>
<td>0 (0–0)</td>
<td>0 (0–0)</td>
<td>228</td>
<td>NS</td>
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<tr>
<td>ADP, 5 ( \mu )M</td>
<td>17.5 ± 5.2</td>
<td>17.7 ± 4.7</td>
<td>463</td>
<td>NS</td>
<td>17.7 ± 5.5</td>
<td>17.8 ± 4.8</td>
<td>212</td>
<td>NS</td>
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<tr>
<td>ADP, 10 ( \mu )M</td>
<td>17.5 ± 4.7</td>
<td>18.0 ± 4.9</td>
<td>457</td>
<td>NS</td>
<td>17.8 ± 4.8</td>
<td>17.7 ± 5.1</td>
<td>209</td>
<td>NS</td>
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<tr>
<td>ADP, 20 ( \mu )M</td>
<td>16.1 ± 5.3</td>
<td>17.1 ± 4.7</td>
<td>483</td>
<td>NS</td>
<td>16.4 ± 5.3</td>
<td>17.1 ± 4.4</td>
<td>224</td>
<td>NS</td>
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<tr>
<td>Epinephrine, 50 ( \mu )M</td>
<td>5.47 ± 5.59</td>
<td>5.23 ± 6.33</td>
<td>490</td>
<td>NS</td>
<td>5.20 ± 5.76</td>
<td>5.50 ± 6.45</td>
<td>227</td>
<td>NS</td>
</tr>
<tr>
<td>Collagen, 1 ( \mu )g/ml</td>
<td>14.3 ± 5.6</td>
<td>14.9 ± 5.2</td>
<td>495</td>
<td>NS</td>
<td>15.1 ± 5.1</td>
<td>14.8 ± 5.2</td>
<td>228</td>
<td>NS</td>
</tr>
<tr>
<td>PFA-100 CT (s)</td>
<td>CEPI</td>
<td>288 (245–300)</td>
<td>295 (260–300)</td>
<td>491</td>
<td>NS</td>
<td>288 (245–300)</td>
<td>300 (257–300)</td>
<td>226</td>
</tr>
<tr>
<td>PFA-100 CADP CT</td>
<td>80 (72–98)</td>
<td>88 (77–106)</td>
<td>492</td>
<td>&lt;0.001</td>
<td>80 (71–95)</td>
<td>92 (77–110)</td>
<td>228</td>
<td>0.001</td>
</tr>
<tr>
<td>UTXB₂ (ln pg/mg creatinine)</td>
<td>5.98 ± 0.63</td>
<td>5.76 ± 0.55</td>
<td>495</td>
<td>&lt;0.001</td>
<td>5.90 ± 0.62</td>
<td>5.77 ± 0.52</td>
<td>228</td>
<td>NS</td>
</tr>
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</table>

Values are median (interquartile range) or mean ± SD. Abbreviations as in Tables 1 and 2.

### Figure 3

A

**Prevalence of SVG Occlusion by PFA-100 CADP CT and UTXB₂**

Percentage of occluded SVGs in subjects stratified by quartiles of (A) PFA-100 collagen/adenosine diphosphate (CADP) CT and (B) UTXB₂ measured 6 months after CABG surgery. Abbreviations as in Figures 1 and 2.
Discussion

Despite advances in surgical technique and postoperative management, including routine administration of ASA, the incidence of early SVG occlusion after CABG surgery remains substantial in a contemporary patient population. The rate of SVG occlusion 6 months after CABG surgery observed in the RIGOR study is consistent with results from several recent studies, including the Project of Ex-vivo Vein Graft Engineering via Transfection 4 trial, which reported an overall SVG occlusion rate of 26% in 2400 patients 12 to 18 months after CABG surgery (19). Among patient- and graft-specific variables associated with SVG occlusion, bypass of small-diameter (≤1.5 mm) target vessels historically has been considered one of the strongest (17,18). In addition to this traditional risk factor, we identified shear-dependent platelet activation, measured by PFA-100 CADP CT, and ASA-insensitive TXA2 generation, measured by UTXB2, as potent novel independent risk factors for early SVG thrombosis. The combination of PFA-100 CADP CT and UTXB2 further was able to stratify subjects into those at particularly high- and low-risk for SVG thrombosis.

In normal individuals, the overwhelming majority of TXA2 generated in the body is produced in platelets by metabolism of arachidonic acid by the COX-1 enzyme. Because ASA efficiently and irreversibly inhibits platelet COX-1 activity, it markedly suppresses TXA2 generation as measured by UTXB2 and blunts platelet reactivity to several agonists (15,20). Growing evidence suggests that some patients with cardiovascular disease have persistent TXA2 generation despite ASA therapy and consequently are at increased risk for adverse clinical events. This association first was noted in a subgroup analysis from the Heart Outcomes Prevention Evaluation study and later confirmed in the larger Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance trial, where subjects receiving ASA with the highest compared with the lowest quartile of UTXB2 at study entry had an approximate 1.7-fold increased risk (p ≤ 0.03 for both studies) of myocardial infarction, stroke, or cardiovascular death over the subsequent 2 to 5 years (8,9). Our findings add to the body of evidence suggesting that ASA-insensitive TXA2 generation is a significant cardiovascular risk factor by demonstrating that it is associated independently with early SVG occlusion after CABG surgery. Follow-up data on RIGOR subjects currently are being collected to determine if ASA-insensitive TXA2 generation after CABG surgery also adversely affects long-term clinical outcome.

A fundamental question arising from the above data is whether ASA-insensitive TXA2 generation in patients with cardiovascular disease represents a failure of ASA to inhibit platelet COX-1–mediated TXA2 biosynthesis, the strictest and most accurate definition of ASA resistance (5), or the presence of alternate TXA2 production pathways that are not effectively inhibited by ASA. Zimmerman et al. (11) found that platelets from patients taking 100 mg/day ASA generate more TXA2 in the first week after CABG surgery than platelets from healthy ASA-treated controls. TXA2 generation was suppressed only partially by the further exposure of platelets in vitro to high concentrations of ASA, suggesting both incomplete COX-1 inhibition in vivo by low-dose ASA, as well as the presence of COX-1–independent TXA2 synthesis (10). Others subsequently confirmed that platelets from ASA-treated subjects are capable of generating TXA2 via non–COX-1–mediated pathways, although the amounts are relatively small in comparison with those produced by platelets from ASA-naive patients (21). Although we did not measure platelet TXA2 generation directly in our study, arachidonic acid-induced platelet aggregation is known to be inhibited when platelet TXA2 generation is suppressed by >90% (22). The suppression of arachidonic acid-induced platelet aggregation in ≥95% of subjects both early and late after CABG surgery argues for a high degree of platelet COX-1 inhibition by ASA, and therefore a very low prevalence of true ASA resistance in our study population.

Despite evidence for effective platelet COX-1 suppression by ASA, UTXB2 was elevated markedly in a substantial number of subjects both early and late after CABG surgery. Because UTXB2 reflects TXA2 generation in the entire body, not only from platelets, our data suggest that significant TXA2 production may originate from nonplatelet sources that are not inhibited effectively by ASA. Potential sources include inflammatory and endothelial cells that are capable of producing TXA2 via both COX-1– and inducible COX-2–dependent pathways. ASA is a relatively weak inhibitor of COX-2 at standard doses (20), and unlike platelets, both cell types have the capacity to regenerate COX-1 that is irreversibly inhibited by ASA. Another potential source is from nonenzymatic conversion of arachidonic acid to F2-isoprostanes under conditions of oxidative stress. F2-isoprostanes are capable of activating platelet and cellular TXA2 receptors and also can stimulate TXA2 production directly in endothelial cells (23,24). Additional studies are needed to identify the sources of this ASA-insensitive TXA2 generation and the mechanism by which its production adversely affects clinical outcome.

Various aspects of platelet function, including ASA responsiveness, can be characterized by a wide array of ex vivo assays. One of the most physiologic is the PFA-100 device, which measures agonist-induced platelet aggregation under conditions of high shear by quantifying time to closure of a membrane aperture by the formation of a platelet plug (25). The PFA-100 was designed primarily to screen for defects in primary hemostasis, with the CEPI agonist cartridge detecting the antiplatelet effects of ASA and the CADP agonist cartridge identifying patients with congenital and acquired platelet disorders (26). Among patients with cardiovascular disease, a CEPI CT in the normal range (94 to 193 s) despite ASA therapy is associated with an increased risk of recurrent cardiovascular events.
(27,28). However, the specificity and ultimate usefulness of the PFA-100 at detecting true ASA resistance has been called into question by the observation that CEPI CTs are highly influenced by plasma levels of von Willebrand factor (29). Our results not only confirm the poor correlation between ASA responsiveness defined by CEPI CT compared with arachidonic acid-induced platelet aggregometry, but also reveal no association between CEPI CT and SVG outcome.

A major finding of our study was the strong association between low CADP CT and early SVG occlusion. Unlike CEPI CT, CADP CT is not influenced by ASA or thienopyridine use and therefore is considered an indicator of global platelet reactivity. Similar to CEPI CT, we found a correlation between low CADP CT and high plasma von Willebrand factor levels (13), but did not find an independent association between von Willebrand factor levels and early SVG occlusion (data not shown). A CADP CT below the normal range (71 to 118 s) suggests platelet hyper-reactivity and has been observed in patients with acute coronary syndromes, where it correlated with the degree of myocardial necrosis and recurrent ischemic events (30–32). In a recent study of 700 patients undergoing percutaneous coronary intervention, a CADP CT <65 s measured before the procedure was associated with a 3.5-fold increase risk (95% confidence interval: 1.2 to 10.4, p < 0.03) of major adverse cardiac events at 24 months (21). These studies, along with our own, suggest the potential of measuring shear-dependent platelet activation as a means to risk stratify patients with cardiovascular disease.

Study limitations. A limitation of the RIGOR study was that ASA responsiveness and platelet reactivity were not assessed before surgery. This was by design because we anticipated that not all eligible subjects would have been receiving chronic ASA therapy before CABG surgery and that some would have been receiving concurrent non-ASA antiplatelet agents that would confound interpretation of many of the assays. It was reasoned that values obtained 6 months after CABG surgery, after resolution of the associated inflammatory response and hematologic changes, would be indicative of the subject’s baseline state. Now that ASA-insensitive thromboxane generation and shear-dependent platelet activation have been identified as important parameters, additional studies will be needed to determine if their preoperative measurement can identify patients at high risk for SVG thrombosis and what therapies might be useful to mitigate such risk.

Conclusions

We have identified ASA-insensitive TXA2 generation, measured by UTXB2, and shear-dependent platelet hyper-reactivity, measured by PFA-100 CADP CT, as novel independent risk factors for early SVG thrombosis. Both entities seem to represent pathways that are independent of platelet COX-1 activity and are not inhibited effectively by ASA. We further found that the prevalence of ASA nonresponsiveness varied widely by several commonly used assays and time relative to CABG surgery, but generally was not associated with graft outcome. Using arachidonic acid-induced platelet aggregometry as a specific indicator of platelet TXA2 generation, however, the overwhelming majority of patients seem to have a high degree of platelet COX-1 inhibition by ASA, and therefore a low incidence of ASA resistance both early and late after CABG surgery.

Reprints requests and correspondence: Dr. Jeffrey J. Rade, Division of Cardiology, Johns Hopkins School of Medicine, Ross 1165, 720 Rutland Avenue, Baltimore, Maryland 21205. E-mail: jjrade@jhmi.edu.

REFERENCES


Key Words: aspirin • platelet • thrombosis • thromboxane • vein graft.

APPENDIX

For supplemental tables, please see the online version of this article.