2017-11-02

Differential Impact of Serial Measurement of Nonplatelet Thromboxane Generation on Long-Term Outcome After Cardiac Surgery

Nikolaos Kakouros
University of Massachusetts Medical School

Et al.

ORIGINAL RESEARCH

Differential Impact of Serial Measurement of Nonplatelet Thromboxane Generation on Long-Term Outcome After Cardiac Surgery

Nikolaos Kakouros, MBBS, PhD; Tyler J. Gluckman, MD; John V. Conte, MD; Thomas S. Kickler, MD; Katherine Laws, RN; Bruce A. Barton, PhD; Jeffrey J. Rade, MD

***Background*—**Systemic thromboxane generation, not suppressible by standard aspirin therapy and likely arising from nonplatelet sources, increases the risk of atherothrombosis and death in patients with cardiovascular disease. In the RIGOR (Reduction in Graft Occlusion Rates) study, greater nonplatelet thromboxane generation occurred early compared with late after coronary artery bypass graft surgery, although only the latter correlated with graft failure. We hypothesize that a similar differential association exists between nonplatelet thromboxane generation and long-term clinical outcome.

***Methods and Results*—**Five-year outcome data were analyzed for 290 RIGOR subjects taking aspirin with suppressed platelet thromboxane generation. Multivariable modeling was performed to define the relative predictive value of the urine thromboxane metabolite, 11-dehydrothromboxane B_2 (11-dhTXB$_2$), measured 3 days versus 6 months after surgery on the composite end point of death, myocardial infarction, revascularization or stroke, and death alone. 11-dhTXB$_2$ measured 3 days after surgery did not independently predict outcome, whereas 11-dhTXB$_2$ >450 pg/mg creatinine measured 6 months after surgery predicted the composite end point (adjusted hazard ratio, 1.79; P=0.02) and death (adjusted hazard ratio, 2.90; P=0.01) at 5 years compared with lower values. Additional modeling revealed 11-dhTXB$_2$ measured early after surgery associated with several markers of inflammation, in contrast to 11-dhTXB$_2$ measured 6 months later, which highly associated with oxidative stress.

***Conclusions*—**Long-term nonplatelet thromboxane generation after coronary artery bypass graft surgery is a novel risk factor for 5-year adverse outcome, including death. In contrast, nonplatelet thromboxane generation in the early postoperative period appears to be driven predominantly by inflammation and did not independently predict long-term clinical outcome. **(*J Am Heart Assoc*. 2017;6:e007486. DOI: 10.1161/JAHA.117.007486.)**

Key Words: aspirin • inflammation • oxidative stress • thrombosis • thromboxane

Thromboxane A_2 (TXA_2) is a signal-activated eicosanoid generated from the metabolism of arachidonic acid via the actions of cyclooxygenase (COX) and downstream thromboxane synthase enzymes.[1] In healthy adults, TXA_2 is produced predominantly by platelets in response to physiologic agonists, where it potentiates the activation of the platelet in which it is formed. TXA_2 is also released, causing local amplification of thrombotic stimuli by directly activating adjacent platelets and vasoconstriction via binding to cellular thromboxane-prostanoid receptors.[2] The cardioprotective effect of aspirin is principally mediated by the irreversible inhibition of the platelet COX-1 enzyme, which suppresses platelet TXA_2 generation and platelet activation.[3]

A substantial percentage of patients with cardiovascular disease continue to generate significant amounts of TXA_2 despite aspirin therapy, which places them at risk for adverse cardiovascular events.[4–8] Although initially thought to represent a failure of aspirin to inhibit platelet TXA_2 generation,

From the University of Massachusetts Medical School, Worcester, MA (N.K., B.A.B., J.J.R.); and Johns Hopkins School of Medicine, Baltimore, MD (T.J.G., J.V.C., T.S.K., K.L., J.J.R.).

Accompanying Tables S1 through S4 are available at http://jaha.ahajournals.org/content/6/11/e007486/DC1/embed/inline-supplementary-material-1.pdf

Correspondence to: Jeffrey J. Rade, MD, Division of Cardiovascular Medicine, University of Massachusetts Medical School, 55 Lake Ave N, Worcester, MA 01655. E-mail: jeffrey.rade@umassmed.edu

Received August 24, 2017; accepted September 19, 2017.

Clinical Perspective

What Is New?

- Despite being greater in magnitude, nonplatelet thromboxane generation occurring immediately after cardiac surgery does not have the same long-term prognostic value as that occurring following recovery and return to a baseline state.
- Inflammation appears to be a major stimulus for nonplatelet thromboxane generation in the immediate postoperative state, whereas oxidative stress is the predominant stimulus in the long-term baseline state.
- These findings suggest that the source, and not necessarily only the overall magnitude, of nonplatelet thromboxane generation is an important determinant of its impact on cardiovascular risk.

What Are the Clinical Implications?

- Although nonplatelet thromboxane generation is emerging as a novel cardiovascular risk factor, the context in which it is assessed is important for its prognostic significance.
- Identifying the sources of nonplatelet thromboxane generation will facilitate a better understanding of the pathobiological characteristics of nonplatelet thromboxane generation and aid in the development of therapies to mollify its effects.

emerging evidence suggests that residual TXA_2 generation derives principally from nonplatelet sources not suppressible by once-daily dosing regimens.[6,9–13]

In the RIGOR (Reduction in Graft Occlusion Rates) study, nonplatelet TXA_2 generation was identified as a novel risk factor for saphenous vein graft thrombotic occlusion after first-time coronary artery bypass graft (CABG) surgery.[6] Little is known about how extreme physiologic stress affects nonplatelet TXA_2 generation, how it changes over time in a given patient, or how predictive it is of clinical outcome when measured in acute versus chronic conditions. To address these questions, we compared nonplatelet TXA_2 generation in the RIGOR study population measured 3 days after surgery with that measured 6 months later and determined how predictive each was of 5-year outcome. To gain insight into possible sources of nonplatelet TXA_2 generation, we also investigated if variables associated with nonplatelet TXA_2 generation in the early postoperative period differed from those after a return to long-term baseline state.

Methods

Patient Population

The RIGOR study was a multicenter observational study of 368 subjects undergoing first-time CABG surgery between October, 2003 and October, 2006, designed to investigate the association between thrombotic risk factors and early saphenous vein graft occlusion. Detailed descriptions of the study design, patient characteristics, and principal findings have previously been published.[6,14,15] Institutional review board approval was obtained at each participating site, and written consent was obtained from all subjects. Any patient ≥18 years old undergoing first-time CABG surgery with implantation of at least 1 saphenous vein graft was eligible for enrollment. Those with an anticipated requirement for postoperative oral anticoagulation or antiplatelet therapy other than aspirin were excluded, although subjects given these agents for unforeseen postoperative conditions (eg, atrial fibrillation) continued in the study. All patients were administered aspirin (300–325 mg) within 24 hours of surgery. At hospital discharge, patients were given a supply of 325-mg enteric-coated aspirin and instructed to take 1 tablet daily for 6 months, unless directed otherwise by their physician. Pill counts were performed at each postoperative encounter. Demographic, historical, procedural, clinical, and laboratory data were recorded for all patients. Subjects were contacted yearly around the surgical anniversary date for up to 5 years unless known to have expired. Intercurrent clinical end points were verified by review of medical records, and deaths were confirmed by medical and/or Social Security death records.

Laboratory Studies

Details of the laboratory methods used have been described previously.[16] Platelet function studies could only be performed 3 days after CABG surgery in subjects enrolled at the Johns Hopkins Hospital (Baltimore, MD) but were performed in all subjects 6 months after surgery, at the time of graft patency assessment. Platelet-rich plasma was prepared from citrated blood, and the platelet count was adjusted to 180 000/mm^3 by the addition of platelet-poor plasma. Undiluted samples with a platelet count of <100 000/mm^3 were excluded from analysis. Impedance platelet aggregometry was performed on platelet-rich plasma by stimulation with a panel of agonists, including 0.5 mmol/L arachidonic acid, using an aggregometer, and the maximum aggregation response within 5 minutes was recorded (in ohms). Platelet COX-1 activity and TXA_2 generation were considered to be fully suppressed by aspirin if arachidonic acid–induced platelet aggregation was ≤1 Ω (normal range in our laboratory for aspirin-naïve subjects, 5–17 Ω) on the basis of data demonstrating that suppression of platelet TXA_2 generation >99% is required to suppress arachidonic acid–induced platelet aggregation by 95%.[17] Systemic TXA_2 generation was quantified by measuring the concentration of its stable metabolite, 11-dehydrothromboxane B_2 (11-dhTXB_2), in urine

by ELISA and expressed as pg/mg of urine creatinine (coefficients of variance, ≤10%; lower limit of detection, 25 pg/mL).

Assessment of Graft Patency and Left Ventricular Ejection Fraction

The patency of venous and arterial grafts was assessed 6 months after CABG surgery by computed tomographic angiography, as previously described.[14,15] 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 graft patency before a clinical end point. Multisegmented grafts were statistically considered as separate grafts, according to the Society of Thoracic Surgeons criteria. Two blinded reviewers analyzed reconstructed 3-dimensional images to classify grafts as patent (containing stenoses of 0%–75%), significantly diseased (containing stenoses of 76%–99%), or occluded (containing a 100% stenosis). In cases of discordance, a third reviewer adjudicated all grafts in that patient. Left ventricular ejection fractions were calculated from reconstructed computed tomographic angiography images using computer software.

Statistical Analysis

Data from patients who survived the index hospitalization and underwent graft patency assessment were included in the present analysis. Baseline characteristics are presented as mean±SD or median (interquartile range) for continuous variables and as proportions for categorical variables. Proportions were compared using a Fisher exact test, and continuous variables were compared by Student *t* test or Wilcoxon rank-sum test, as appropriate. For graft patency analysis, the small proportion of grafts classified as severely diseased was considered as occluded. Nonnormally distributed variables were transformed using natural log or negative inverse square, as indicated. Median, upper quartile, or upper tertile splits were used to categorize continuous variables that could not be normalized, as indicated. Differences were considered significant when $P<0.05$. A proportional hazard Cox survival model was used to evaluate the independent determinants of the predefined composite outcome of death, myocardial infarction, revascularization (both surgical and percutaneous), and stroke, as well as death alone (because it was a major determinant of the composite outcome). The time origins for the survival analyses were set at the time of surgery and the time of graft patency assessment when urine 11-dhTXB$_2$ was measured 6 months after surgery. For analyses involving the latter, events occurring before the 6-month landmark were excluded. Cox modeling was performed using clustering by patient; for the composite end point, stratification by individual end point was used. Kaplan-Meier survival plots comparing various predictors were constructed. Predictors with unadjusted $P\leq0.15$ were included in the initial multivariable Cox model. The explained relative risk by the models was assessed using the Royston modified version of the Nagelkerke R^2, including a bootstrap confidence interval.[18] Backward stepwise modeling with bootstrapping was performed, and predictors appearing in >50% of resulting models were considered in the final model, with selection guided by the Bayesian information criterion and R^2. Multivariable fractional polynomial interaction analysis was used to assess for interactions between covariates. The assumption of proportionality was evaluated by introducing natural log-time–dependent covariates for predictors in the Cox model and testing for significance. The final optimized multivariable Cox proportional hazard models were used to derive adjusted hazard ratios. Robust univariate regression was performed for predictors of 11-dhTXB$_2$ using variables deemed biologically plausible or supported by the literature. Highly collinear covariates were identified using the Fisher exact test and Pearson correlation for categorical and continuous variables, respectively, and were eliminated on the basis of clinical significance. Variables with $P\leq0.15$ on the initial univariate analysis were included in the initial multivariate model. The Furnival-Wilson leaps-and-bounds algorithm was used for variable subset selection, and the model was optimized on the basis of the Akaike information criteria. The coefficient estimates are reported standardized to a variance of 1 (β coefficients), to facilitate comparison of the predicted magnitude of change in the dependent variable per unit change in the predictor. The relative importance of each variable in the multivariable model was assessed by dominance analysis.[13,19] Analyses were performed using Stata 13.0 for Windows.

Results

Study Population Characteristics

Of the 368 subjects enrolled in the RIGOR study at the 4 participating sites, 293 survived the index hospitalization, were receiving long-term aspirin therapy, and had measurement of both urine 11-dhTXB$_2$ and arachidonic acid–induced platelet aggregation at the time of graft patency assessment at 6 months (median, 189 days; interquartile range, 182–202 days) after CABG surgery. Because 3 subjects were excluded from analysis because of persistent arachidonic acid–induced platelet aggregation, despite aspirin therapy at this time point, 290 subjects formed the study cohort. Of these subjects, 271 also had measurement of urine 11-

Table 1. Baseline, Operative, and Postoperative Characteristics of Subjects Stratified by 11-dhTXB$_2$ Measured at 3 Days and 6 Months After CABG Surgery

Characteristic	Cohort (n=288)	3 Days			6 Months		
		<891 pg/mg Creatinine (n=214)	≥891 pg/mg Creatinine (n=74)	*P* Value	<450 pg/mg Creatinine (n=212)	≥450 pg/mg Creatinine (n=76)	*P* Value
Age, mean±SD, y	63.3±10.0	62.8±10.1	64.8±9.9	0.12	62.4±9.7	65.9±10.7	**0.01**
Male sex	229 (80)	175 (82)	39 (18)	0.13	178 (84)	51 (67)	**0.003**
White race	250 (87)	187 (87)	63 (85)	0.69	192 (91)	58 (76)	**0.003**
Body mass index, median (interquartile range), kg/m^2	30 (26–33)	29 (26–33)	28 (25–33)	0.55	30 (26–33)	29 (25–33)	0.21
Medical history							
Hypertension	236 (82)	173 (81)	63 (85)	0.49	174 (83)	62 (82)	0.86
Dyslipidemia	241 (84)	178 (84)	63 (85)	0.86	177 (84)	64 (84)	1.0
Diabetes mellitus	99 (35)	68 (32)	31 (42)	0.12	63 (30)	36 (48)	**0.007**
Heart failure	35 (12)	18 (8)	17 (23)	**0.002**	20 (9)	15 (20)	**0.024**
Peripheral/cerebral-vascular disease	50 (17)	31 (14)	19 (26)	**0.03**	33 (16)	17 (22)	0.216
Atrial fibrillation	10 (3)	5 (2)	5 (7)	0.13	6 (3)	4 (5)	0.30
Tobacco use at surgery	68 (24)	42 (20)	26 (35)	**0.01**	45 (21)	23 (30)	0.12
Myocardial infarction	118 (41)	78 (36)	40 (54)	**0.009**	81 (38)	37 (49)	0.14
Prior PCI	59 (20)	40 (19)	19 (26)	0.24	45 (21)	14 (18)	0.74
Preoperative LVEF, %				**0.004**			0.61
≤30	24 (8)	11 (5)	13 (18)		16 (8)	8 (11)	
30–50	97 (34)	75 (35)	22 (30)		70 (33)	27 (36)	
>50	167 (58)	128 (60)	39 (53)		126 (59)	41 (54)	
Urgent/emergent surgery	178 (62)	120 (56)	58 (78)	**0.001**	128 (60)	50 (66)	0.41
EuroScore, median (interquartile range)	4 (2–5)	3 (2–5)	4 (2–7)	**0.01**	3 (1–5)	5 (3–6)	**0.005**
Arterial graft implanted	280 (97)	208 (97)	72 (97)	0.96	206 (97)	74 (97)	0.93
No. of SVGs per subject				0.64			0.25
1	78 (27)	55 (26)	23 (31)		57 (27)	21 (28)	
2	121 (42)	90 (42)	31 (42)		85 (40)	36 (47)	
≥3	89 (31)	69 (32)	20 (27)		70 (33)	19 (25)	
Medications at urine 11-dhTXB$_2$ measurement							
Aspirin	288 (100)	214 (100)	74 (100)	1.0	212 (100)	76 (100)	1.0
Dose, <325 mg/d	30 (10)	0 (0)	0 (0)	1.0	17 (8)	13 (17)	**0.046**
Nonaspirin antiplatelet agent	32 (11)	27 (13)	10 (14)	0.84	20 (9)	12 (16)	0.14
Oral anticoagulation	14 (5)	17 (8)	17 (23)	**0.001**	7 (3)	7 (9)	0.06
β Blocker	240 (83)	210 (98)	74 (100)	0.58	181 (85)	59 (78)	0.15
ACE inhibitor/ARB	178 (62)	107 (50)	42 (57)	0.35	133 (63)	45 (59)	0.59
Lipid-lowering agent	255 (89)	207 (97)	74 (100)	0.48	192 (91)	63 (83)	0.09

Data are given as number (percentage) unless otherwise indicated. ACE indicates angiotensin-converting enzyme; ARB, angiotensin receptor blocker; CABG, coronary artery bypass graft; 11-dhTXB$_2$, 11-dehydrothromboxane B$_2$; LVEF, left ventricular ejection fraction; PCI, percutaneous coronary intervention; and SVG, saphenous vein graft.
All statistcally significant P values were in bold.

dhTXB$_2$ a median of 3 days (interquartile range, 3–4 days) after CABG surgery. Only 198 subjects also had verified suppression of arachidonic acid–induced platelet aggregation at this time point, given that platelet function testing was only available at only 1 enrolling site. The median duration of clinical follow-up in the study cohort was 1828 days (interquartile range, 1482–1846 days) after surgery. The characteristics of the study cohort as a whole and in subjects

stratified by urine 11-dhTXB$_2$ values of 891 pg/mg creatinine, measured 3 days after surgery (upper-tertile threshold), and 450 pg/mg creatinine, measured 6 months after surgery (upper-quartile threshold previously shown to independently correlate with vein graft thrombotic occlusion[6]), are shown in Table 1. Similar to other studies, older age, female sex, and nonwhite race were demographic characteristics associated with higher 11-dhTXB$_2$ levels when measured 6 months after surgery.[5,20] In contrast, no demographic characteristics were associated with higher 11-dhTXB$_2$ levels when measured 3 days after surgery.

Median 11-dhTXB$_2$ in the study cohort was significantly higher 3 days (648 pg/mg creatinine; interquartile range, 398–1171 pg/mg creatinine) compared with 6 months after surgery (331 pg/mg creatinine; interquartile range, 232–462 pg/mg creatinine; $P<0.0001$) (Figure 1A). In the 198 subjects with paired samples and verified suppression of platelet thromboxane generation, the change in urine 11-dhTXB$_2$ over time was linearly related with 151 subjects (76%) manifesting a net decrease in 11-dhTXB$_2$ after 6 months (median change, 323 pg/mg creatinine; interquartile range, 173–668 pg/mg creatinine) and only 48 subjects (24%) manifesting a net increase (median change, 119 pg/mg creatinine; interquartile range, 48–201 pg/mg creatinine) (Figure 1B).

Urine 11-dhTXB$_2$ as a Predictor of Outcome

Univariate analyses were used to explore the association of urine 11-dhTXB$_2$, along with a wide array of demographic, clinical, and laboratory variables, to the primary composite outcome of death, myocardial infarction, revascularization, or stroke at 6 months (Table S1) and both the composite outcome and death alone at 5 years (Table S2). Urine 11-dhTXB$_2$, measured 3 days after surgery and expressed as a dichotomous (using the upper-tertile threshold of 891 pg/mg creatinine) but not as a continuous variable, was significantly associated with the composite outcome end point at 6 months. Urine 11-dhTXB$_2$ measurements at both 3 days and 6 months were significantly associated with the 5-year composite outcome end point and death, a major determinant of the composite end point (Figure 2). Interestingly, there was no association with revascularization, the other major driver of the composite end point (Table S3). To determine the relative predictive value of 11-dhTXB$_2$ measured at both time points, multivariable models were constructed to identify independent predictors of outcome. Urine 11-dhTXB$_2$ measured 3 days after CABG surgery was not an independent predictor of the composite outcome at 6 months (Table S4) or at 5 years (Table 2). In contrast, urine 11-dhTXB$_2$ measured 6 months after surgery was an independent predictor of both the composite outcome and death at 5 years (Table 2).

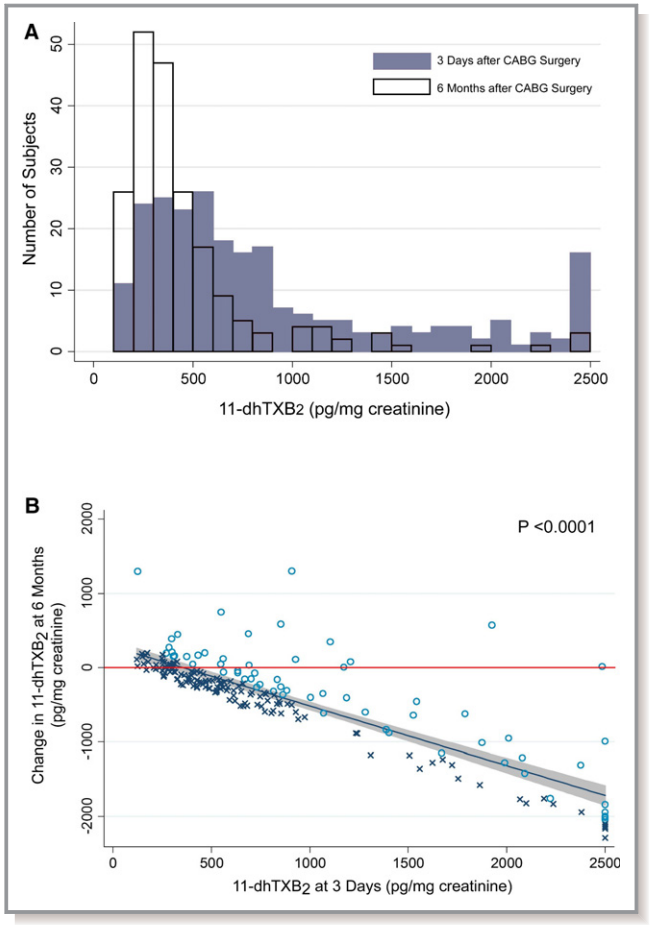

Figure 1. A, Distribution of urine 11-dehydrothromboxane B$_2$ (11-dhTXB$_2$) 3 days and 6 months after coronary artery bypass graft (CABG) surgery. B, Change in urine 11-dhTXB$_2$ from 3 days to 6 months after CABG surgery in individual subjects.

Occlusion of left internal mammary grafts, but not vein grafts, 6 months after CABG surgery predicted the composite end point, which was predominantly driven by revascularization. Not unexpectedly, concurrent peripheral or cerebrovascular disease, postoperative renal insufficiency, and reduced ejection fraction independently predicted higher long-term mortality; statin therapy predicted lower long-term mortality.

Variables Associated With Nonplatelet TXA$_2$ Generation Early After Surgery

In a prior analysis of this study cohort,[13] oxidative stress, as measured by urine 8-iso-prostaglandin 2α, was identified as the strongest variable associated with urine 11-dhTXB$_2$, measured 6 months after surgery, accounting for approximately half of the modeled effect and consistent with data from other study populations.[20,21] Age, race, statin use, and aspirin dose were also associated, but to lesser degrees. Given the observed differences in subject characteristics associated with high 11-dhTXB$_2$ levels when measured at

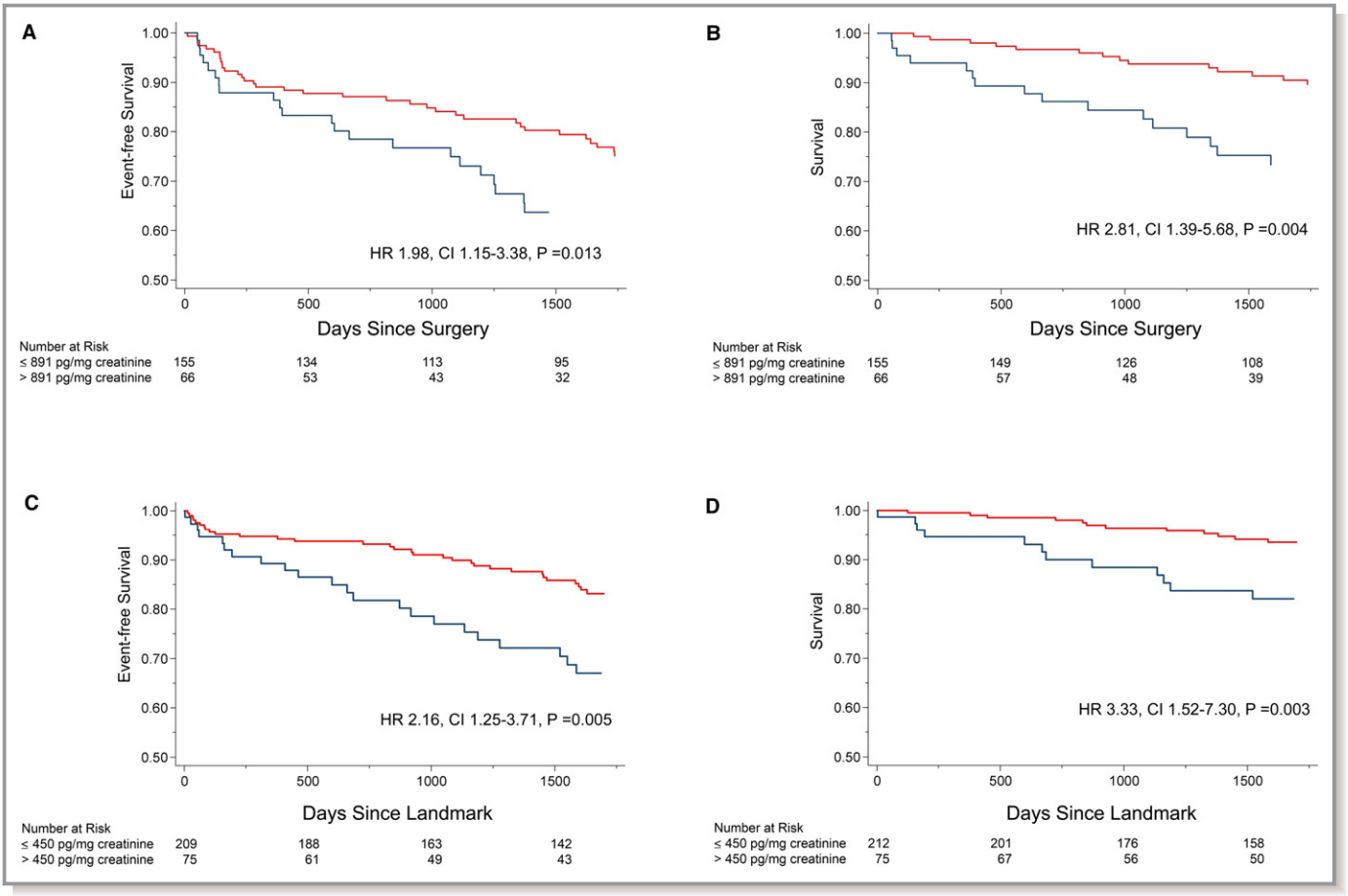

Figure 2. Kaplan-Meier plots for: survival free of myocardial infarction, revascularization, or stroke (A) and survival alone (B) in subjects stratified by urine 11-dehydrothromboxane B_2 (11-dhTXB_2) ≤891 (red) or >891 (blue) pg/mg creatinine measured 3 days after coronary artery bypass graft (CABG) surgery; survival free of myocardial infarction, revascularization, or stroke (C) and survival alone (D) in subjects stratified by urine 11-dhTXB_2 ≤450 (red) or >450 (blue) pg/mg creatinine measured 6 months after CABG surgery (landmark). CI indicates confidence interval; and HR, hazard ratio.

different time points (Table 1), we sought to understand if stimuli for nonplatelet TXA_2 generation during the early perioperative period, when subjects were under acute physiologic stress, differed from those 6 months later, when subjects would more likely to be at their physiologic baseline. Modeling of variables associated with 11-dhTXB_2 measured 3 days after surgery revealed that red cell distribution width, a novel marker of inflammation and cardiovascular risk,[22–25] was the strongest associated variable, accounting for nearly a third of the modeled effect (Table 3). Other markers and modulators of inflammation, such as C-reactive protein and statin use, were also associated to lesser degrees, as was oxidative stress, as measured by urine 8-iso-prostaglandin$_{2\alpha}$.

Discussion

The major findings of this study were as follows: (1) in patients undergoing CABG surgery, nonplatelet TXA_2 generation is markedly higher in the early postoperative period than 6 months later; (2) nonplatelet TXA_2 generation measured 6 months after CABG surgery, but not that measured in the early postoperative period, independently predicted long-term outcome, particularly mortality; and (3) although oxidative stress is a major stimulus for nonplatelet TXA_2 generation 6 months after CABG surgery, inflammation is a stronger stimulus for nonplatelet TXA_2 generation in the early postoperative period.

Studies have emerged in recent years demonstrating that persistent TXA_2 generation in patients with cardiovascular disease undergoing aspirin therapy predicts an increased risk of atherothrombosis and death.[4,7,8] Although initially assumed to be attributable to the failure of aspirin to inhibit platelet COX-1–mediated thromboxane generation and consequent platelet activation, it is now recognized that aspirin is efficient at inhibiting platelet COX-1 and that much of the residual TXA_2 generation in patients taking aspirin originates from nonplatelet sources.[26–28] A unique feature of our analysis was that it only included subjects in whom inhibition of platelet thromboxane generation was verified, conclusively demonstrating that TXA_2 originating from nonplatelet tissue adversely affects clinical outcome and survival.

Table 2. Multivariable Cox Proportional Hazard Models for 5-Year Outcomes

Variable	Death, Myocardial Infarction, Revascularization, and Stroke			Death		
	Adjusted HR	95% CI	*P* Value	Adjusted HR	95% CI	*P* Value
Model 1*						
Peripheral/cerebrovascular disease	2.47	1.42–4.32	0.001	3.34	1.52–7.32	0.003
Insulin therapy	2.31	1.31–4.08	0.004			
Lipid-lowering agent				0.34	0.15–0.77	0.01
eGFR, mL/min per 1.73 m^2				0.98	0.95–1.0	0.04
Urine 11-dhTXB$_2$ at 6 mo (ln pg/mg creatinine)	1.59	1.0–2.54	0.05	2.36	1.24–4.50	0.009
LIMA occlusion	2.95	1.50–5.81	0.002			
Postoperative LVEF, %						
≤30	2.58	1.10–6.03	0.03	5.45	0.94–14.85	0.06
30–50	2.67	1.54–5.63	<0.001	3.67	1.50–8.14	0.004
>50	Reference			Reference		
Model 2†						
Peripheral/cerebrovascular disease	2.50	1.41–4.42	0.002	3.58	1.63–7.89	0.002
Insulin therapy	2.21	1.22–3.98	0.009			
Lipid-lowering agent				0.34	0.16–0.75	0.007
eGFR, mL/min per 1.73 m^2				0.97	0.95–1.0	0.03
Urine 11-dhTXB$_2$ at 6 mo (>450 pg/mg creatinine)	1.79	1.08–2.96	0.02	2.90	1.29–6.50	0.01
LIMA graft occlusion	3.20	1.62–6.34	0.001			
Postoperative LVEF, %						
≤30	2.80	1.14–6.90	0.025	3.51	0.71–17.33	0.12
30–50	2.66	1.53–4.62	0.001	3.43	1.53–7.67	0.003
>50	Reference			Reference		

CI indicates confidence interval; 11-dhTXB$_2$, 11-dehydrothromboxane B$_2$; eGFR, estimated glomerular filtration rate; HR, hazard ratio; LIMA, left internal mammary artery; and LVEF, left ventricular ejection fraction.

*For death, myocardial infarction, revascularization, and stroke: pseudo R^2 0.47 (95% CI: 0.31–0.69). For death: pseudo R^2 0.71 (95% CI: 0.49–0.93).

†For death, myocardial infarction, revascularization, and stroke: pseudo R^2 0.47 (95% CI: 0.31–0.70). For death: pseudo R^2 0.70 (95% CI: 0.49–0.92).

Sources of nonplatelet TXA$_2$ generation have not been conclusively identified and may conceivably vary depending on the patient population and conditions under study. Many tissues and cell types produce TXA$_2$, which is quickly degraded to TXB$_2$, a relatively stable metabolite that circulates widely. TXB$_2$ itself is extensively metabolized via enzyme-mediated β oxidation and dehydrogenation to dozens of different stable end-order metabolites, including 11-dhTXB$_2$, that are concentrated in the urine.[29] Because TXB$_2$ produced from TXA$_2$ generated in the kidney does not circulate and is, therefore, not subjected to extensive further metabolism, urine levels of TXB$_2$ are considered to predominantly represent renal TXA$_2$ generation; those of 11-dhTXB$_2$ are considered to predominantly represent extrarenal systemic TXA$_2$ generation.[30] Measurement of urine 11-dhTXB$_2$ in our study was performed using an ELISA with high specificity for this metabolite and strong correlation with values measured by mass spectrometry.[31] Two candidate sources for significant nonrenal, nonplatelet TXA$_2$ generation in our study population include vascular endothelial and inflammatory cells based on convincing in vitro data showing that each is capable of producing TXA$_2$.[13,32–34]

A second unique feature of our study that helps gain insight into potential sources of nonplatelet TXA$_2$ generation was the serial measurement of urine 11-dhTXB$_2$ in individual subjects during 2 markedly different physiologic states: a period of intense physiologic stress and inflammation induced by cardiac surgery and then 6 months later when subjects would have likely returned to their physiologic baseline. We previously found that urine 11-dhTXB$_2$ levels measured in subjects 6 months after CABG surgery were strongly correlated with evidence of increased oxidative stress and were influenced by age, sex, and race, similar to other studies of patients with stable cardiovascular disease or diabetes

mellitus receiving aspirin therapy.[13,20,35] Our findings that cultured endothelial cells under oxidative stress generate TXA_2 and have the enzymatic capacity to metabolize it to 11-dhTXB$_2$[13] provide evidence that dysfunctional vascular endothelium may be a significant source of in vivo nonplatelet TXA_2 generation.[13] In contrast, urine 11-dhTXB$_2$ measured in the early postoperative period was associated with multiple markers of inflammation, but not with age, race, or sex. This suggested that inflammatory cells, such as monocytes, that produce TXA_2 when activated[34,36,37] may be a significant source of in vivo nonplatelet TXA_2 generation during conditions of intense inflammation. The marked nonplatelet TXA_2 generation that occurred during the immediate postoperative period did not independently predict long-term outcome, whereas the nonplatelet TXA_2 generation that was produced over a long time did independently predict long-term outcome. This raises the possibility, analogous to that observed with C-reactive protein, that the source of nonplatelet TXA_2 generation, rather than its overall magnitude, might be the more important determinant of its impact on clinical outcome.

How nonplatelet TXA_2 generation modifies cardiovascular risk and mortality is also unknown. The lack of relationship between urine 11-dhTXB$_2$ and platelet function in the RIGOR study[6,13] and the inability of dual antiplatelet therapy to alter the associated hazard of 11-dhTXB$_2$ to outcome in the Clopidogrel for High Atherothrombotic Risk and Ischemic Stabilization, Management and Avoidance (CHARISMA) study[5] suggest that increased platelet reactivity is not the predominate mechanism by which nonplatelet TXA_2 generation causes atherothrombosis and death. We previously found that nonplatelet TXA_2 generation is a novel risk factor for early vein graft failure, which is predominantly attributable to thrombotic occlusion.[6,15,38] TXA_2 has been shown to be capable of stimulating expression of tissue factor and adhesion molecules in endothelial cells, suggesting that alteration in endothelial thromboresistance may be one mechanism by which it mediates thrombosis.[39–41] Nonplatelet TXA_2 generation may also promote the progression of atherosclerosis. This is supported by studies in apolipoprotein E–deficient mice that revealed that atherogenesis was suppressed by deletion or pharmacologic inhibition of the thromboxane-prostanoid receptor but not by the administration of aspirin.[42,43] Whether nonplatelet TXA_2 generation is a marker or mediator of atherothrombosis in humans will need to be determined by clinical trials aimed at reducing its production or blocking its effects on the thromboxane-prostanoid receptor.

Table 3. Risk Factors for Urine 11-dhTXB$_2$ (ln pg/mg Creatinine) Measured Early After CABG Surgery After Adjustment of Other Variables by Multivariable Regression Analysis

Variable	Standardized Coefficient	*P* Value	Dominance Weight	Dominance Ranking
RDW ($-\%^{-3}$)	−0.34	<0.001	0.33	1
Heparin use before surgery	0.26	<0.001	0.18	2
FFP transfusion (<2 vs ≥2 U)	0.21	<0.001	0.17	3
Urine 8-iso-PGF$_{2\alpha}$ (ln pg/mg creatinine)	0.19	0.002	0.17	4
C-reactive protein (mg/L$^{-1/2}$)	0.15	0.017	0.08	5
Lipid-lowering therapy	−0.12	0.002	0.07	6

Pseudo R^2 (95% confidence interval), 0.45 (0.34–0.56). CABG indicates coronary artery bypass graft; 11-dhTXB$_2$, 11-dehydrothromboxane B$_2$; FFP, fresh-frozen plasma; 8-iso-PGF$_{2\alpha}$, 8-iso-prostaglandin 2α; and RDW, red cell distribution width.

There are several limitations to the present study. Although the RIGOR study cohort was extremely well phenotyped, thus permitting exclusion of subjects with persistent platelet TXA_2 generation, it was of moderate size and composed only of subjects with established cardiovascular disease who, by definition, had undergone surgical revascularization. The impact of nonplatelet TXA_2 generation on outcome in other larger and nonselected study populations will need to be determined. Because the cause of death could not be ascertained for all expired subjects, the end point analysis was performed for all-cause mortality, although most deaths were known to be cardiovascular in origin. Although compliance with aspirin therapy was verified during the 6-month active phase of the study by pill counts, it could not be verified during the 5-year follow-up phase of the study and was dependent on subject self-reporting. Given that standard aspirin therapy does not suppress nonplatelet TXA_2 generation but is only critical for its identification, the effects on outcome of noncompliance during the follow-up period would be expected to be distributed between groups and not likely affect the results of the study. Finally, because platelet function testing was only available at 1 of the enrolling sites, not all subjects in whom urine 11-dhTXB$_2$ was measured early after surgery were included in the analysis. Thus, this could have conceivably biased the results. However, additional analyses (data not shown) with inclusion of these subjects, 95% of whom would be expected to have aspirin-induced suppression of platelet thromboxane generation at this time point,[6] did not alter findings that nonplatelet thromboxane generation measured in the early perioperative period does not independently predict outcome.

In summary, nonplatelet TXA_2 generation is a predictor of early graft failure after CABG surgery and of long-term outcome, including mortality. The source of nonplatelet TXA_2 generation, which can vary in individual patients under

different physiologic conditions, may be a more important determinant of outcome than its overall magnitude.

Sources of Funding

This study was supported by the Johns Hopkins Institute for Clinical and Translational Research (funded by UL1 RR025005 from the National Center for Research Resources, National Institutes of Health, Bethesda, MD); grants from AstraZeneca Pharmaceuticals, Sanofi-BMS, and the Flight Attendant Medical Research Foundation; and material support from Siemens Healthcare Diagnostics, Inc, and GlaxoSmithKline. The authors had sole control of the design of the study, collection, analysis, and dissemination of the data.

Disclosures

None.

References

1. Patrono C, Ciabattoni G, Davi G. Thromboxane biosynthesis in cardiovascular diseases. *Stroke*. 1990;21:IV130–IV133.
2. Capra V, Back M, Angiolillo DJ, Cattaneo M, Sakariassen KS. Impact of vascular thromboxane prostanoid receptor activation on hemostasis, thrombosis, oxidative stress, and inflammation. *J Thromb Haemost*. 2014;12:126–137.
3. Patrono C, Rocca B. Aspirin: promise and resistance in the new millennium. *Arterioscler Thromb Vasc Biol*. 2008;28:s25–s32.
4. Eikelboom JW, Hirsh J, Weitz JI, Johnston M, Yi Q, Yusuf S. Aspirin-resistant thromboxane biosynthesis and the risk of myocardial infarction, stroke, or cardiovascular death in patients at high risk for cardiovascular events. *Circulation*. 2002;105:1650–1655.
5. Eikelboom JW, Hankey GJ, Thom J, Bhatt DL, Steg PG, Montalescot G, Johnston SC, Steinhubl SR, Mak KH, Easton JD, Hamm C, Hu T, Fox KAA, Topol EJ. Incomplete inhibition of thromboxane biosynthesis by acetylsalicylic acid: determinants and effect on cardiovascular risk. *Circulation*. 2008;118:1690.
6. Gluckman TJ, McLean RC, Schulman SP, Kickler TS, Shapiro EP, Conte JV, McNicholas KW, Segal JB, Rade JJ. Effects of aspirin responsiveness and platelet reactivity on early vein graft thrombosis after coronary artery bypass graft surgery. *J Am Coll Cardiol*. 2011;57:1069–1077.
7. McCullough PA, Vasudevan A, Sathyamoorthy M, Schussler JM, Velasco CE, Lopez LR, Swift C, Peterson M, Bennett-Firm J, Schiffmann R, Bottiglieri T. Urinary 11-dehydro-thromboxane B2 and mortality in patients with stable coronary artery disease. *Am J Cardiol*. 2017;119:972–977.
8. Szczeklik W, Stodolkiewicz E, Rzeszutko M, Tomala M, Chrustowicz A, Zmudka K, Sanak M. Urinary 11-dehydro-thromboxane B2 as a predictor of acute myocardial infarction outcomes: results of leukotrienes and thromboxane in myocardial infarction (LTIMI) study. *J Am Heart Assoc*. 2016;5:e003702. DOI: 10.1161/JAHA.116.003702.
9. Patrignani P. Aspirin insensitive eicosanoid biosynthesis in cardiovascular disease. *Thromb Res*. 2003;110:281–286.
10. Patrignani P, Tacconelli S, Piazuelo E, Di FL, Dovizio M, Sostres C, Marcantoni E, Guillem-Llobat P, Del BP, Zucchelli M, Patrono C, Lanas A. Reappraisal of the clinical pharmacology of low-dose aspirin by comparing novel direct and traditional indirect biomarkers of drug action. *J Thromb Haemost*. 2014;12:1320–1330.
11. Patrono C, Rocca B. Drug insight: aspirin resistance–fact or fashion? *Nat Clin Pract Cardiovasc Med*. 2007;4:42–50.
12. Santilli F, Rocca B, De Cristofaro R, Lattanzio S, Pietrangelo L, Habib A, Pettinella C, Recchiuti A, Ferrante E, Ciabattoni G, Davi G, Patrono C. Platelet cyclooxygenase inhibition by low-dose aspirin is not reflected consistently by platelet function assays: implications for aspirin "resistance." *J Am Coll Cardiol*. 2009;53:667–677.
13. Kakouros N, Nazarian SM, Stadler PB, Kickler TS, Rade JJ. Risk factors for nonplatelet thromboxane generation after coronary artery bypass graft surgery. *J Am Heart Assoc*. 2016;5:e002615. DOI: 10.1161/JAHA.115.002615.
14. Gluckman TJ, Segal JB, Schulman SP, Shapiro EP, Kickler TS, Prechel MM, Conte JV, Walenga JM, Shafique I, Rade JJ. Effect of anti-platelet factor-4/heparin antibody induction on early saphenous vein graft occlusion after coronary artery bypass surgery. *J Thromb Haemost*. 2009;7:1457–1464.
15. McLean RC, Nazarian SM, Gluckman TJ, Schulman SP, Thiemann DR, Shapiro EP, Conte JV, Thompson JB, Shafique I, McNicholas KW, Villines TC, Laws KM, Rade JJ. Relative importance of patient, procedural and anatomic risk factors for early vein graft thrombosis after coronary artery bypass graft surgery. *J Cardiovasc Surg (Torino)*. 2011;52:877–885.
16. Nazarian SM, Thompson JB, Gluckman TJ, Laws K, Jani JT, Kickler TS, Rade JJ. Clinical and laboratory factors associated with shear-dependent platelet hyperreactivity in patients on chronic aspirin therapy. *Thromb Res*. 2009;126:379–383.
17. Pulcinelli FM, Riondino S, Celestini A, Pignatelli P, Trifiro E, Di Renzo L, Violi F. Persistent production of platelet thromboxane A2 in patients chronically treated with aspirin. *J Thromb Haemost*. 2005;3:2784–2789.
18. Royston P. Explained variation for survival models. *Stata J*. 2006;6:83–96.
19. Grömping U. Estimators of relative importance in linear regression based on variance decomposition. *Am Stat*. 2007;61:139–147.
20. Ames PR, Batuca JR, Muncy IJ, De La Torre IG, Pascoe-Gonzales S, Guyer K, Matsuura E, Lopez LR. Aspirin insensitive thromboxane generation is associated with oxidative stress in type 2 diabetes mellitus. *Thromb Res*. 2012;130:350–354.
21. McCullough PA, Vasudevan A, Lopez LR, Swift C, Peterson M, Bennett-Firm J, Schiffmann R, Bottiglieri T. Oxidative stress reflected by increased F2-isoprostanes is associated with increasing urinary 11-dehydro thromboxane B2 levels in patients with coronary artery disease. *Thromb Res*. 2016;148:85–88.
22. Al-Kindi SG, Kim CH, Morris SR, Freeman ML, Funderburg NT, Rodriguez B, McComsey GA, Dalton JE, Simon DI, Lederman MM, Longenecker CT, Zidar DA. Brief report: elevated red cell distribution width identifies elevated cardiovascular disease risk in patients with HIV infection. *J Acquir Immune Defic Syndr*. 2017;74:298–302.
23. Felker GM, Allen LA, Pocock SJ, Shaw LK, McMurray JJ, Pfeffer MA, Swedberg K, Wang D, Yusuf S, Michelson EL, Granger CB. Red cell distribution width as a novel prognostic marker in heart failure: data from the CHARM Program and the Duke Databank. *J Am Coll Cardiol*. 2007;50:40–47.
24. Tonelli M, Sacks F, Arnold M, Moye L, Davis B, Pfeffer M. Relation between red blood cell distribution width and cardiovascular event rate in people with coronary disease. *Circulation*. 2008;117:163–168.
25. Danese E, Lippi G, Montagnana M. Red blood cell distribution width and cardiovascular diseases. *J Thorac Dis*. 2015;7:E402–E411.
26. Faraday N, Becker DM, Yanek LR, Herrera-Galeano JE, Segal JB, Moy TF, Bray PF, Becker LC. Relation between atherosclerosis risk factors and aspirin resistance in a primary prevention population. *Am J Cardiol*. 2006;98:774–779.
27. Homorodi N, Kovacs EG, Lee S, Katona E, Shemirani AH, Haramura G, Balogh L, Bereczky Z, Szoke G, Peterfy H, Kiss RG, Edes I, Muszbek L. The lack of aspirin resistance in patients with coronary artery disease. *J Transl Med*. 2016;14:74.
28. Chen CY, Poole EM, Ulrich CM, Kulmacz RJ, Wang LH. Functional analysis of human thromboxane synthase polymorphic variants. *Pharmacogenet Genomics*. 2012;22:653–658.
29. Roberts LJ, Sweetman BJ, Oates JA. Metabolism of thromboxane B2 in man: identification of twenty urinary metabolites. *J Biol Chem*. 1981;256:8384–8393.
30. Catella F, Nowak J, FitzGerald GA. Measurement of renal and non-renal eicosanoid synthesis. *Am J Med*. 1986;81:23–29.
31. Olson MT, Kickler TS, Lawson JA, McLean RC, Jani J, FitzGerald GA, Rade JJ. Effect of assay specificity on the association of urine 11-dehydro thromboxane B2 determination with cardiovascular risk. *J Thromb Haemost*. 2012;10:2462–2469.
32. Hou X, Gobeil F Jr, Peri K, Speranza G, Marrache AM, Lachapelle P, Roberts J, Varma DR, Chemtob S, Ellis EF. Augmented vasoconstriction and thromboxane formation by 15-F(2t)-isoprostane (8-iso-prostaglandin F(2alpha)) in immature pig periventricular brain microvessels. *Stroke*. 2000;31:516–524.
33. Lahaie I, Hardy P, Hou X, Hassessian H, Asselin P, Lachapelle P, Almazan G, Varma DR, Morrow JD, Roberts LJ, Chemtob S. A novel mechanism for vasoconstrictor action of 8-isoprostaglandin F2 alpha on retinal vessels. *Am J Physiol*. 1998;274:R1406–R1416.
34. Penglis PS, Cleland LG, Demasi M, Caughey GE, James MJ. Differential regulation of prostaglandin E2 and thromboxane A2 production in human monocytes: implications for the use of cyclooxygenase inhibitors. *J Immunol*. 2000;165:1605–1611.

35. Cipollone F, Ciabattoni G, Patrignani P, Pasquale M, Di Gregorio D, Bucciarelli T, Davi G, Cuccurullo F, Patrono C. Oxidant stress and aspirin-insensitive thromboxane biosynthesis in severe unstable angina. *Circulation*. 2000;102:1007–1013.

36. Tilley SL, Coffman TM, Koller BH. Mixed messages: modulation of inflammation and immune responses by prostaglandins and thromboxanes. *J Clin Invest*. 2001;108:15–23.

37. Kovarik JJ, Holzl MA, Hofer J, Waidhofer-Sollner P, Sobanov Y, Koeffel R, Saemann MD, Mechtcheriakova D, Zlabinger GJ. Eicosanoid modulation by the short-chain fatty acid n-butyrate in human monocytes. *Immunology*. 2013;139:395–405.

38. Motwani JG, Topol EJ. Aortocoronary saphenous vein graft disease. *Circulation*. 1998;97:916–931.

39. Bode M, Mackman N. Regulation of tissue factor gene expression in monocytes and endothelial cells: thromboxane A2 as a new player. *Vascul Pharmacol*. 2014;62:57–62.

40. Del Turco TS, Basta G, Lazzerini G, Chancharme L, Lerond L, De Caterina CR. Involvement of the TP receptor in TNF-alpha-induced endothelial tissue factor expression. *Vascul Pharmacol*. 2014;62:49–56.

41. Ishizuka T, Kawakami M, Hidaka T, Matsuki Y, Takamizawa M, Suzuki K, Kurita A, Nakamura H. Stimulation with thromboxane A2 (TXA2) receptor agonist enhances ICAM-1, VCAM-1 or ELAM-1 expression by human vascular endothelial cells. *Clin Exp Immunol*. 1998;112:464–470.

42. Kobayashi T, Tahara Y, Matsumoto M, Iguchi M, Sano H, Murayama T, Arai H, Oida H, Yurugi-Kobayashi T, Yamashita JK, Katagiri H, Majima M, Yokode M, Kita T, Narumiya S. Roles of thromboxane A(2) and prostacyclin in the development of atherosclerosis in apoE-deficient mice. *J Clin Invest*. 2004;114:784–794.

43. Cayatte AJ, Du Y, Oliver-Krasinski J, Lavielle G, Verbeuren TJ, Cohen RA. The thromboxane receptor antagonist S18886 but not aspirin inhibits atherogenesis in apo E-deficient mice: evidence that eicosanoids other than thromboxane contribute to atherosclerosis. *Arterioscler Thromb Vasc Biol*. 2000;20:1724–1728.

SUPPLEMENTAL MATERIAL

Table S1. Univariate analyses of variables associated with 6-month composite endpoint (death, myocardial infarction, revascularization and stroke).

	Composite endpoint	
Variable	**HR**	**P-value**
Demographic/pre-operative variables		
Age (years)	1.0	0.98
Male sex	2.01	0.18
White race (vs. non-white race)	0.58	0.39
Body mass index (kg/m2)	0.96	0.46
Medical History of:		
Hypertension	1.75	0.47
Dyslipidemia	1.54	0.51
Diabetes	1.32	0.58
Heart failure	1.71	0.32
Peripheral/cerebrovascular disease	0.95	0.92
Atrial fibrillation	6.47	**0.001**
Preoperative tobacco use	1.73	0.28
Myocardial infarction	1.02	0.97
Prior PCI	1.65	0.35
Pre-operative LVEF		
≤30%	1.35	0.66
30-50%	0.88	0.81
>50%	Reference	
Surgical variables		
Urgent/Emergent surgery	1.64	0.31
Euroscore	1.05	0.48
Arterial graft implanted	0.86	0.88
Number of SVG per subject		
1	Reference	
2	0.22	**0.01**
≥3	0.54	0.35
CABG only (vs concurrent valve surgery)	0.38	**0.04**
Cardiopulmonary bypass time (ln min)	2.15	0.21
Cross clamp	1.0	1.0
Cross clamp time (ln min)	1.2	0.75
Heparin units (ln units)	0.61	0.49
Protamine dose (mg^2)	1.0	0.86
Crystalloid dose ($mL^{1/2}$)	1.03	**0.05**
Colloid dose (mL)	1.0	0.55
Packed RBC transfusion	1.70	0.25
Packed RBC units – None	Reference	
1-2	1.66	0.34
>3	1.73	0.27
Cell saver volume ($ml^{1/2}$)	1.0	0.90
FFP transfusion	1.15	0.74
FFP units: None	Reference	
1-2	4.96	0.12
>2	17.4	**0.008**

Platelet transfusion	1.80	0.12
Platelet units -None	Reference	
1-2	1.55	0.34
>3	2.21	0.10
Post-operative variables		
Medications:		
Non-aspirin antiplatelet	2.89	0.08
Oral anticoagulation	2.51	0.38
Beta-blocker	0.29	0.05
ACE Inhibitor/ARB	1.35	0.54
Insulin	0.64	0.63
Insulin sensitizer	1.30	0.68
Insulin secretagogue	1.68	0.37
eGFR (mL/min/1.73m^2)	1.0	0.27
Leukocyte count (10^3/mm^3)	0.96	0.64
Hematocrit (%)	0.96	0.40
Red cell distribution width (≤14.5 vs >14.5%)	1.46	0.43
Platelet count (<150 vs. ≥150x 10^3/mm^3)	1.16	0.75
Reticulocyte (ln %)	0.67	0.55
Blood Group: O vs. other	0.58	0.28
C-reactive protein (mg/L$^{1/2}$)	0.96	0.67
Fibrinogen (<390 vs. ≥390 mg/dL)	1.08	0.91
vonWillebrand factor (>150 vs. ≤150%)	4.22	0.16
Urine 8-isoPGF$_{2\alpha}$ (ln pg/mg creatinine)	1.72	0.15
Urine 8-isoPGF$_{2\alpha}$ (<1870 vs. ≥1870 pg/mg creatinine)	1.32	0.61
Urine 11-dhTXB$_2$ (ln pg/mg creatinine)	1.72	0.08
Urine 11-dhTXB$_2$ tertiles		
≤ 487 pg/mg creatinine	Reference	
>487 to ≤891pg/mg creatinine	1.98	0.25
> 891 pg/mg creatinine	3.56	**0.03**
Impedance platelet aggregometry (ohms)		
ADP (20μM)	0.90	0.11
ADP (10μM)	0.87	**0.03**
ADP (5μM)	0.90	0.06
Collagen (1 μg/mL)	0.99	0.74
Epinephrine (50 μM)	0.98	0.67
PFA-100 Collagen-ADP (Closure Time in sec^{-1})	1.01	**0.01**
PFA-100 Collagen-ADP (Closure Time ≤88 sec vs. >88 sec)	0.27	**0.03**
PFA-100 Collagen-Epinephrine (Closure Time in sec^{-1})	0.90	0.11

Abbreviations: PCI= percutaneous coronary intervention; LVEF= left ventricular ejection fraction; SVG= saphenous vein graft; RBS= red blood cell; FFP= fresh-frozen plasma; ACE= angiotensin converting enzyme; ARB= angiotensin receptor blocker; 11-dhTXB2= 11-dehydro thromboxane B_2; eGFR= estimated glomerular filtration rate; ADP= adenosine diphosphate; PFA-100= Platelet Function Analyzer-100

Table S2. Univariate analyses of variables associated with 5-year composite endpoint (death, myocardial infarction, revascularization and stroke) and mortality.

	Composite endpoint		**Death**	
Variable	**HR**	**P-value**	**HR**	**P-value**
Demographic/pre-operative variables				
Age (years)	0.99	0.63	1.04	0.07
Male sex	2.07	**0.015**	1.60	0.29
White race (vs. non-white race)	0.48	**0.034**	0.39	**0.046**
Body mass index (kg/m2)	0.99	0.75	0.98	0.63
Medical History of:				
Hypertension	1.43	0.38	1.50	0.51
Dyslipidemia	1.29	0.49	1.01	0.98
Diabetes	2.34	**0.002**	2.30	**0.04**
Heart failure	2.38	**0.013**	2.84	**0.02**
Peripheral/cerebrovascular disease	3.94	**<0.001**	4.11	**0.001**
Atrial fibrillation	1.77	0.32	2.33	0.25
Preoperative tobacco use	1.70	0.08	2.42	**0.03**
Myocardial infarction	1.26	0.40	0.99	0.98
Prior PCI	0.83	0.59	0.17	0.08
Pre-operative LVEF				
>50%	Reference		Reference	
30-50%	1.40	0.25	1.31	0.55
≤30%	1.63	0.39	2.38	0.13
Surgical variables				
Urgent/Emergent surgery	1.1	0.84	0.87	0.73
Euroscore	1.12	**0.02**	1.18	**0.005**
Arterial graft implanted	1.93	0.51	0.81	0.84
Number of SVG per subject				
1	Reference		Reference	
2	1.31	0.42	0.98	0.95
3	0.63	0.29	0.40	0.17
≥4	0.52	0.28	1.0	1.0
CABG only (vs concurrent valve surgery)	0.52	0.11	0.27	**0.01**
Cardiopulmonary bypass time (ln min)	2.97	**0.003**	4.78	**0.005**
Cross clamp	0.48	0.16	0.84	0.87
Cross clamp time (ln min)	2.0	0.08	2.44	0.16
Heparin units (ln units)	0.37	**0.01**	0.14	**0.003**
Protamine dose ($mg^{1/2}$)	1.0	0.14	1.0	0.62
Crystalloid dose ($mL^{1/2}$)	1.0	0.38	1.0	0.56
Colloid dose (mL)	1.0	0.73	1.0	0.85
Packed RBC transfusion	3.5	**<0.001**	4.78	**0.01**
Packed RBC units : None	Reference		Reference	
1-2	2.70	0.012	3.38	0.08
>3	4.16	**<0.001**	5.94	**0.005**
Cell saver volume ($mL^{1/2}$)	1.04	**0.003**	1.06	**0.02**
FFP transfusion	2.18	**0.003**	3.18	**0.005**
FFP units: None	Reference			
1-2	0.63	0.15	0.54	0.24

>2	2.26	**0.04**	3.48	**0.03**
Platelet transfusion	1.67	0.045	2.71	**0.01**
Platelet units – None	Reference			
1-2	1.63	0.10	2.49	0.046
>3	1.75	0.13	3.15	**0.03**
Post-operative laboratory variables				
Urine 11-dhTXB$_2$ (ln pg/mg creatinine)	1.59	**0.03**	2.43	**0.001**
Urine 11-dhTXB$_2$ tertiles				
< 487 pg/mg creatinine	Reference			
≥487 to <891pg/mg creatinine	1.25	0.54	2.55	0.13
≥ 891 pg/mg creatinine	2.11	0.06	4.46	**0.01**
6-month follow-up variables				
Medications:				
Non-aspirin antiplatelet	1.85	0.10	1.60	0.39
Aspirin <325 mg daily	0.94	0.88	0.89	0.85
Oral anticoagulation	2.16	0.11	4.02	**0.01**
Beta-blocker	0.82	0.60	0.42	0.06
ACE Inhibitor/ARB	1.04	0.88	1.25	0.60
Lipid-lowering agent	0.59	0.16	0.33	**0.01**
Diuretic	1.21	0.54	1.58	0.26
Insulin	2.67	**0.002**	1.73	0.32
Insulin sensitizer	1.29	0.43	1.23	0.66
Insulin secretagogue	1.50	0.19	1.86	0.16
eGFR (mL/min/1.73m^2)	1.0	0.59	0.98	**0.016**
LVEF at 6 Months				
>50%	Reference		Reference	
30-50%	2.88	**<0.001**	4.55	**<0.001**
≤30%	5.66	**0.001**	9.58	**<0.001**
Graft occlusion				
None	Reference		Reference	
1	0.81	0.54	0.50	0.17
2+	1.88	0.06	0.48	0.34
Percent of grafts occluded	1.0	**0.03**	1.0	0.69
LIMA graft occlusion	3.26	**0.001**	0.90	0.89
SVG occlusion				
None	Reference		Reference	
1	0.89	0.73	0.61	0.33
2+	1.44	0.35	0.38	0.34
Laboratory variables:				
Leukocyte count				
4.5-11x10^3/mm^3	Reference		Reference	
≤4.5x10^3/mm^3	1.02	0.96	1.67	0.36
≥11x10^3/mm^3	4.16	**0.02**	4.73	**0.04**
Hematocrit (%)	0.89	**0.005**	0.85	**0.003**
Red cell distribution width (≤14.5 vs >14.5%)	1.44	0.18	1.46	0.35
Platelet count (<150 vs. ≥150 x10^3/mm^3)	1.44	0.32	6.47	0.07
Reticulocyte (ln %)	1.89	0.27	0.34	0.09
Blood Group: O vs. other	1.23	0.46	1.36	0.44
Rh factor (positive vs. negative)	1.64	0.35	1.64	0.50

C-Reactive Protein (<5 vs. ≥5 mg/l^{-1})	1.87	**0.03**	2.33	**0.04**
Fibrinogen (<390 vs. ≥390 mg/dL)	1.86	**0.03**	1.86	0.15
vonWillebrand factor (>150 vs. ≤150%)	1.50	0.17	1.52	0.34
eGFR (ml/min/1.73m^2)	1.0	0.59	0.98	**0.016**
Urine 8-iso Prostaglandin $F_{2\alpha}$ (ln pg/mg creatinine)	1.53	**0.02**	1.52	0.13
Urine 8-iso Prostaglandin $F_{2\alpha}$ (≥1620 vs. <1620 pg/mg creatinine)	1.96	**0.03**	1.68	0.24
Urine TXB_2 (ln pg/mg creatinine)	1.97	**0.004**	2.94	**<0.001**
Urine TXB_2 (>450 vs. ≤450 pg/mg creatinine)	2.15	**0.006**	3.33	**0.003**
Oxidized LDL (<2.8 vs. ≥2.8 U/mL)	*0.90*	*0.74*	*0.69*	*0.44*
Oxidized LDL				
<1.32 U/mL	Reference		Reference	
1.32 to 5.04 U/mL	1.37	0.41	1.42	0.50
>5.04 U/mL	0.91	0.81	0.63	0.47
Cotinine (≤10 vs. >10 ng/mL)	2.89	**0.004**	2.0	0.22
Impedance platelet aggregometry (ohms)				
ADP (20μM)	0.99	0.80	0.99	0.83
ADP (10μM)	0.98	0.53	0.95	0.19
ADP (5μM)	1.0	0.95	0.98	0.51
Collagen (1 μg.ml^{-1})	1.0	0.94	0.96	0.24
Epinephrine (50 μM)	1.0	0.51	0.99	0.77
PFA-100 Collagen-ADP (Closure Time in sec^{-1})	1.0	0.63	1.0	0.18
PFA-100 Collagen-ADP (Closure Time ≤88 sec vs. >88 sec)	0.87	0.59	1.41	0.40
PFA-100 Collagen-Epinephrine (Closure Time in sec^{-1})	1.0	0.41	1.0	0.62

Abbreviations: PCI= percutaneous coronary intervention; LVEF= left ventricular ejection fraction; SVG= saphenous vein graft; CABG= coronary artery bypass graft; RBS= red blood cell; FFP= fresh-frozen plasma; ACE= angiotensin converting enzyme; ARB= angiotensin receptor blocker; LIMA= left internal mammary; 11-dhTXB2= 11-dehydro thromboxane B_2; LDL= low density lipoprotein; eGFR= estimated glomerular filtration rate; ADP= adenosine diphosphate; PFA-100= Platelet Function Analyzer-100

Table S3. Univariate analyses of the association of urine 11-dhTXB$_2$ with the individual components of the 5-year composite endpoint.

Time of measure	**3 Days (n=198)**					**6 Months (n=288)**				
	11-dhTXB$_2$ (ln pg/mg creatinine)		**11-dhTXB$_2$ (≤ 891 vs. > 891 pg/mg creatinine)**			**11-dhTXB$_2$ (ln pg/mg creatinine)**		**11-dhTXB$_2$ (≤ 450 vs. > 850 pg/mg creatinine)**		
Outcome	**HR**	**P-value**	**HR**	**P-value**	**Event Rate**	**HR**	**P-value**	**HR**	**P-value**	**Event Rate**
Death	2.43	**0.001**	2.66	**0.017**	12/146 vs. 11/52	2.94	**0.001**	3.3	**0.003**	12/213 vs. 13/75
MI	2.68	0.11	5.82	**0.044**	2/146 vs. 4/52	2.30	0.23	2.28	0.28	4/213 vs. 3/75
Revascularization	0.65	0.16	0.58	0.39	14/146 vs. 3/52	0395	0.83	1.06	0.90	17/213 vs. 6/75
Stroke	2.99	**0.035**	3.01	0.26	2/146 vs. 2/52	2.77	**0.027**	3.26	0.092	4/213 vs. 4/75

Table S4. Multivariable Cox proportional hazard models for 6-month composite outcome.

Variable	**Adj HR**	**CI**	**P-value**
Atrial fibrillation	5.73	1.62-20.24	**0.007**
Number of SVG: 1	*Reference*		
2	0.22	0.07-0.68	**0.008**
≥3	0.76	0.19-2.98	0.69
FFP units: None	Reference		
1-2	5.40	0.68-42.9	0.11
>2	14.62	1.62-132.2	**0.017**

Differential Impact of Serial Measurement of Nonplatelet Thromboxane Generation on Long–Term Outcome After Cardiac Surgery

Nikolaos Kakouros, Tyler J. Gluckman, John V. Conte, Thomas S. Kickler, Katherine Laws, Bruce A. Barton and Jeffrey J. Rade

J Am Heart Assoc. 2017;6:e007486; originally published November 2, 2017;
doi: 10.1161/JAHA.117.007486
The *Journal of the American Heart Association* is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Online ISSN: 2047-9980