Preoperative thromboxane A_2/prostaglandin H_2 receptor activity predicts early graft thrombosis

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Purpose: **This study was carried out to determine whether early failure of infrainguinal bypass grafts is associated with increased expression of platelet thromboxane A_2/prostaglandin H_2 (TXA_2/PGH_2) receptors. A prospective correlation of preoperative platelet TXA_2/PGH_2 receptor-mediated activity with lower extremity graft patency was sought.**

Methods: **Twenty-five patients who underwent infrainguinal bypass surgery for limb salvage were studied at an inpatient academic tertiary referral center and Department of Veterans Affairs Medical Center. Outcome measures were primary graft patency rate at 3 months, platelet TXA_2/PGH_2 receptor activity by equilibrium binding with ^{125}I-BOP, and aggregation to the TXA_2-mimetic U46619.**

Results: **Preoperative platelet TXA_2/PGH_2 receptor density was higher (B_{max}, 3100 ± 1300 vs 1500 ± 1100 sites/platelet [mean ± SD]; $p = 0.004$) in the five patients who had graft thrombosis within 3 months. The EC_{50} for U46619 was lower (26 ± 6 nmol/L vs 57 ± 30 nmol/L; $p < 0.05$) in these patients as well, confirming the functional effect of the increased receptor density. Early graft thrombosis was more likely in patients with a platelet TXA_2/PGH_2 receptor density greater than 3000 sites/platelet (odds ratio, 76; 95% confidence interval, 3.9 to 1500) or an EC_{50} for U46619 less than 30 nmol/L (odds ratio, 16; 95% confidence interval, 1.4 to 180).**

Conclusions: **Elevated platelet TXA_2/PGH_2 receptor levels and enhanced sensitivity of platelet aggregation to TXA_2 predict early arterial graft thrombosis. Specific TXA_2/PGH_2 receptor antagonism may prevent one of the mechanisms that contributes to early graft occlusion. (J Vasc Surg 1998;27:317-28.)**

Platelets play an important role in the early patency of lower extremity revascularization procedures performed for peripheral vascular occlusive disease.[1,2] Interference with normal platelet function by use of various antiplatelet agents, such as aspirin and ticlopidine, appears to prevent graft thrombosis in the early postoperative period.[3-12] Patients with evidence of platelet hyperactivity as determined by relatively nonspecific tests of platelet function, such as bleeding time and aggregation to thrombin, have been reported to be at increased risk for early graft thrombosis.[13,14] The presence of increased circulating von Willebrand factor has similarly been described to increase the risk of a poor operative outcome.[15] The underlying mechanism responsible for this platelet hyperactivity has not been well characterized.

Thromboxane A_2 (TXA_2) and its precursor, prostaglandin H_2 (PGH_2), are potent, ubiquitous promoters of platelet aggregation and vascular smooth muscle contraction that share the same membrane receptor on the surface of platelets and vascular smooth muscle cells.[16-19] Various TXA_2/PGH_2 receptor antagonists, alone and in combination with TXA_2 synthase inhibitors, have been reported to reduce platelet deposition on the luminal surface of implanted vascular grafts.[20-23] Iloprost, a synthetic prostacyclin analog with effects that are antagonistic to those of TXA_2, has also

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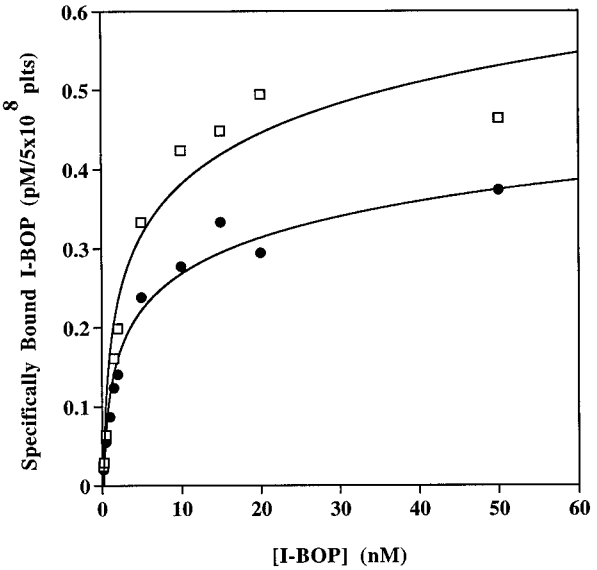

Fig. 1. Binding saturation isotherms of platelets derived before operation according to subsequent graft patency status. *Open squares* indicate thrombosed grafts; *filled circles* indicate patent grafts. Individual patient data have been grouped according to either occluded or patent graft status for each concentration of unlabeled ligand in this figure for ease of illustration.

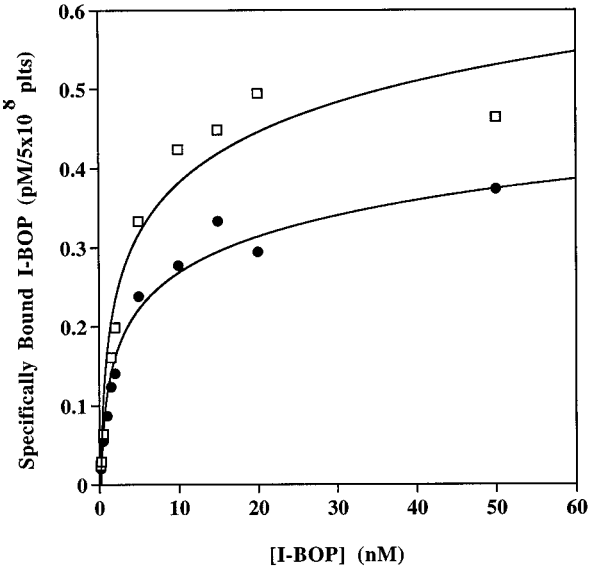

Fig. 2. Scatchard analysis of equilibrium binding data for [^{125}I]-BOP from platelets derived before operation. Nonlinear analysis (LIGAND program) of individual data from patients with thrombosed grafts *(open squares)* yielded an average dissociation constant (Kd) of 8.8 nmol/L and a receptor number (B_{max}) of 3100 sites/platelet, and analysis of patients with patent grafts *(filled circles)* yielded a Kd of 7.9 nmol/L and a receptor number (B_{max}) of 1500 sites/platelet. Individual patient data have been grouped according to either occluded or patent graft status for each concentration of unlabeled ligand in this figure for ease of illustration.

demonstrated the ability to increase early graft patency.[24] Because of these observations, we hypothesized that increases in TXA_2/PGH_2 receptor density, affinity, or both might contribute to the increased platelet activity observed in patients who subsequently have early graft thrombosis. This study was undertaken to test this hypothesis.

METHODS

Patient population. Twenty-five consecutive patients who were scheduled to undergo infrainguinal revascularization surgery for limb salvage at the Medical University of South Carolina and the Ralph Henry Johnson Department of Veterans Affairs Medical Center were enrolled into the study. Before initiation, the study was approved by the Medical University of South Carolina Institutional Review Board for Human Research. All patients approached for enrollment consented to participate in the study.

Experimental protocol. After obtaining informed consent and before operation, 60 ml of blood was collected by venipuncture for platelet count, platelet aggregometry, and equilibrium binding studies for TXA_2/PGH_2 receptor activity as described below. Clinical data retrieved included medications, diagnosis, operation, age, sex, race, height, weight, anesthesia type, operative time, operative blood loss, and other medical illnesses, including renal failure. All infrapopliteal operations were performed to the distal tibial, peroneal, or pedal vessels. All patients underwent intraoperative completion angiography or on-table duplex scanning to assess the condition of the graft. In all patients, lower molecular weight Dextran (Rheomacrodex, Medisan Pharmaceuticals, Parsippany, N.J.) was given intravenously at 20 ml/hr for 72 hours after operation. Antiplatelet therapy, typically with aspirin, is normally given to patients who undergo lower extremity arterial surgery, and this practice was not altered. No patients were receiving warfarin or ticlopidine before operation. After discharge from the hospital, patients were seen at 1, 3, 6, and 12 months after the operation and then annually. Primary graft patency was

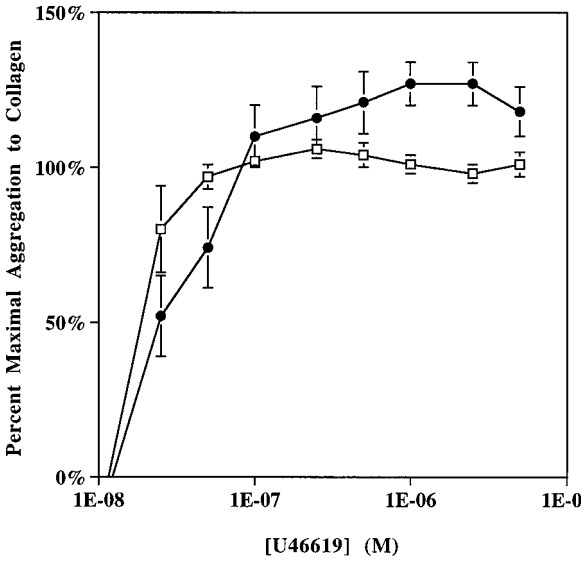

Fig. 3. Combined concentration-response curves for aggregation induced by U46619. *Open squares* indicate thrombosed grafts; *filled circles* indicate patent grafts. *Error bars* represent standard error. Individual patient data have been grouped according to either occluded or patent graft status for each concentration of unlabeled ligand in this figure for ease of illustration.

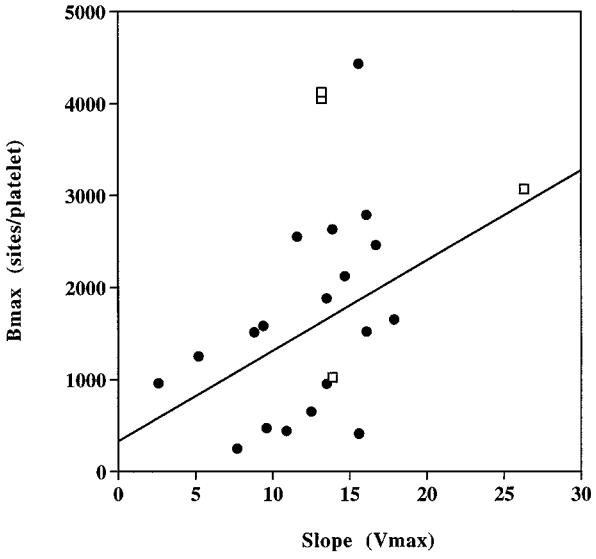

Fig. 4. Correlation of platelet density (B_{max}) with maximal slope (V_{max}) of platelet aggregation to 5 μmol/L U46619. *Open squares* indicate thrombosed grafts; *filled circles* indicate patent grafts. Each data point represents an individual patient ($r = 0.36$; $p < 0.01$).

determined by clinical examination and was confirmed by duplex ultrasound examination.

Platelet TXA_2/PGH_2 receptor assay. The assay for determination of TXA_2/PGH_2 receptor density has previously been described.[25] Briefly, peripheral blood was collected into syringes containing indomethacin (10 μmol/L) and ethylenediamine tetraacetic acid (5 mmol/L; final concentration). Platelet pellets were obtained by differential centrifugation, followed by resuspension in the assay buffer of HEPES (25 mmol/L), NaCl (100 mmol/L), dextrose (5 mmol/L), and indomethacin (10 μmol/L), pH 6.5, to approximately 5×10^8 platelets/ml. A 100 μl aliquot of resuspended platelets was added to 0.2 nmol/L (approximately 10^5 cpm) of [^{125}I]-[1S-1α, 2β(5Z), 3α(1E,3S*), 4α]-7-[3-(3-hydroxy-4-(4′-iodophenoxy)-1-butenyl)7-oxabicyclo-[2.2.1]heptan-2-yl]-5-heptanoic acid (I-BOP) and graded concentrations of [^{127}I]-BOP (0.01 nmol/L to 1.0 μmol/L) to a total volume of 200 μl. After incubation at 37° C for 30 minutes, bound ligand was separated from free ligand by vacuum filtration using Whatman GF/C glass fiber filters and counted in a gamma counter (75% efficiency for ^{125}I). Nonspecific binding was defined as the binding that remained in the presence of the TXA_2/PGH_2 receptor antagonist L657925 (10 μmol/L; Merck Frosst, Quebec). Analysis of equilibrium binding data consisted of Scatchard analysis with the LIGAND program (Release 2.0, Biosoft, Milltown, N. J.) for a one-site model. Platelet protein was determined by the Lowry method.

Platelet aggregometry. Peripheral blood was withdrawn into syringes containing indomethacin (10 μmol/L) and ethylenediamine tetraacetic acid (5 mmol/L; final concentration). The blood was centrifuged at 175*g* for 20 minutes at 24° C, and the resulting platelet-rich plasma was pipetted off and placed into polyurethane centrifuge tubes. The platelet-rich plasma was centrifuged at 800*g* for 20 minutes at 24° C, and the platelet pellet was resuspended in Tris-buffer (Tris-HCl [50 mmol/L], NaCl [100 mmol/L], dextrose [5 mmol/L], and indomethacin [10 μmol/L], pH 7.4) to a concentration of approximately 2.5×10^8 platelets/ml. Before aggregation, $CaCl_2$ was added to the washed platelets (final concentration, 250 μmol/L). Platelet aggregation was performed in a Chronolog model 300 aggregometer (Chronolog, Havertown, Pa.). Washed platelets (450 ml) were added to individual silanized glass cuvettes. The TXA_2 mimetic U46619 (Upjohn, Kalamazoo, Mich.) was added in final con-

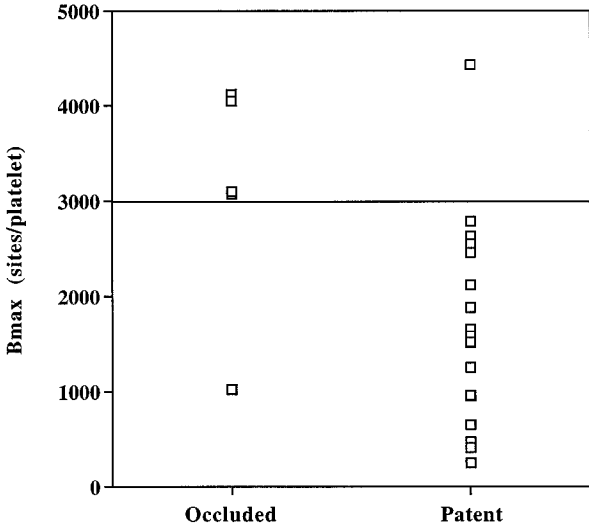

Fig. 5. Distribution of receptor density (B_{max}) according to study group.

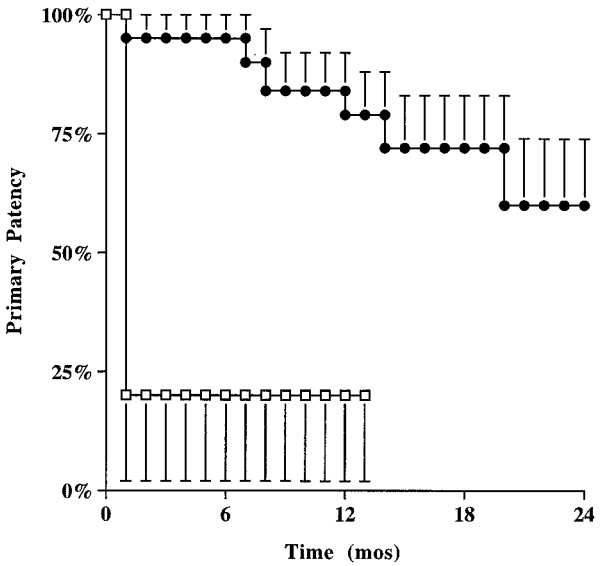

Fig. 6. Life table analysis of cumulative primary graft patency data for patients according to TXA_2/PGH_2 receptor density >3000 sites/platelet (*open squares;* n = 5) or <3000 sites/platelet (*filled circles*; n = 20). *Error bars* represent standard error. Z (Yates correction) = 2.97; $p < 0.005$ between groups.

Table I. Demographic and preoperative risk factors of patients with thrombosed and occluded grafts at 3 months

	Thrombosed (%) n=5	*Patent (%) n=20*
Mean age (range)	68 yr (51 to 75)	69 yr (54 to 88)
Male/female	5/0 (100/0)	12/8 (60/40)
Black/white	3/2 (60/40)	13/7 (65/35)
Diabetes/no diabetes	5/0 (100/0)	13/7 (65/35)
Preoperative ASA/no ASA	3/2 (60/40)	12/8 (60/40)
Tissue loss/rest pain	4/1 (80/20)	15/5 (75/25)
Vein conduit/ePTFE	4/1 (80/20)	16/4 (80/20)
Popliteal/infrapopliteal	1/4 (20/80)	6/14 (30/70)

NS, Not significant; *ASA,* acetylsalicylic acid (aspirin); *ePTFE,* expanded polytetrafluoroethylene. *Popliteal/infrapopliteal* indicates distal anastomosis target vessel.

centrations of 20 nmol/L to 5 μmol/L. The aggregation response was observed for 2 minutes and was expressed as a percentage of the maximal response observed to collagen (1 μg/ml). Dose-response curves were constructed for U46619. The EC_{50} values, defined as the concentration required to produce 50% of the maximum aggregation response within 1 minute, were derived from the dose-response curves. Maximal velocity of aggregation (V_{max}) was defined as the initial slope of the aggregation response to 5 μmol/L U46619.

Data management. Statistical analysis for individual patient measurements consisted of two-tailed, unpaired Student's *t* test, Fisher's exact test, and the χ^2 test where appropriate. A p level less than 0.05 was considered significant to reject the null hypothesis. All values in the text are expressed as mean ± SD.

RESULTS

Twenty-five patients were enrolled in the study. Five of the 25 patients (20%) had occlusion of their graft within the first 3 months. All five occluded grafts were comprised of autogenous vein. Four expanded polytetrafluoroethylene grafts and 16 autogenous vein grafts remained patent during the first 3 months. A missed valve was the cause for graft occlusion in one patient. In the other patients, no cause for occlusion could be definitively identified. Preoperative patient demographic and risk factors are presented in Table I according to graft patency status and in Table II according to TXA_2/PGH_2 receptor density.

The specific binding of [^{125}I]-BOP was significantly greater before operation in the platelets of patients who subsequently had graft thrombosis (Fig. 1). Preoperative platelet TXA_2/PGH_2 receptor density was significantly higher in the five patients who had graft thrombosis within 3 months of operation (B_{max} [platelet mass], 2.6 ± 1.0 pmol/mg protein vs 1.3 ± 0.9 pmol/mg protein, $p = 0.001$; B_{max} [platelet number], 3100 ± 1300 vs 1500 ± 1100

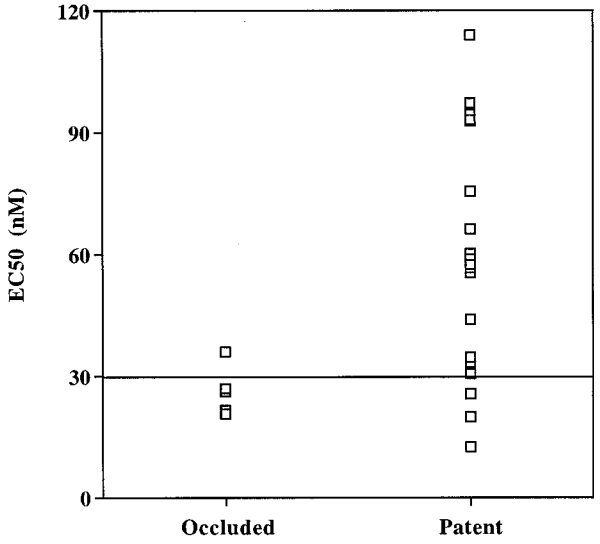

Fig. 7. Distribution of platelet EC_{50} according to study group.

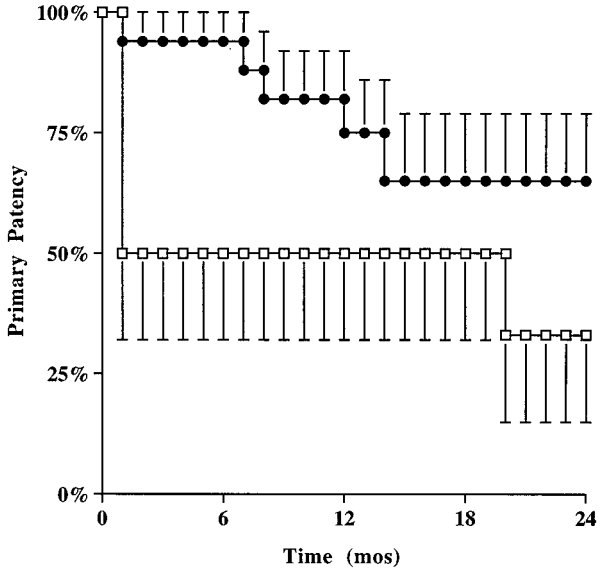

Fig. 8. Life table analysis of cumulative primary graft patency data for patients according to EC_{50} for platelet aggregation to U46619 <30 nmol/L (*open squares*; n = 8) or >30 nmol/L (*filled circles*; n = 17). *Error bars* represent standard error. Z (Yates correction) = 1.10; p = NS between groups.

sites/platelet, $p = 0.004$). A single I-BOP binding site was observed in the platelets of all patients (Fig. 2). No significant differences in TXA_2/PGH_2 receptor affinity for I-BOP (K_d 8.8 ± 3.6 nmol/L vs 7.9 ± 4.5 nmol/L; $p > 0.05$) or platelet number (320,000 ± 190,000/min^3 vs 300,000 ± 80,000/mm^3; $p > 0.05$) were noted between groups.

Aggregometry revealed that the EC_{50} for U46619 was lower (26 ± 6 nmol/L vs 57 ± 30 nmol/L; $p < 0.05$) in patients with early thrombosis, indicating functionally increased sensitivity to TXA_2 mimetics (Fig. 3). Differences in V_{max} between patients with occluded and patent grafts did not attain statistical significance (16 ± 6 vs 12 ± 4; $p = 0.12$), although the power to detect a 25% difference in this measurement was less than 0.50, allowing the possibility of a type II statistical error. Platelet receptor density (B_{max}) did correlate with V_{max} ($r = 0.36$; Fig. 4).

Early graft thrombosis was more likely in patients with a platelet TXA_2/PGH_2 receptor density greater than 3000 sites per platelet (odds ratio, 76; 95% confidence interval, 3.9 to 1500; Fig. 5). Life table analysis of patients grouped according to a preoperative B_{max} threshold of greater than 3000 sites per platelet confirmed the primary patency rate to be significantly worse in this group ($p < 0.005$; Fig. 6; Appendix A). Early graft thrombosis was also more likely in patients with an EC_{50} for U46619 less than 30 nmol/L (odds ratio, 16; 95% confidence interval, 1.4 to 180; Fig. 7). These early differences in the patency rate were not sustained and did not reach statistical significance by life table analysis (Fig. 8; Appendix B). The potential value of B_{max} and EC_{50} as preoperative predictors of graft occlusion is illustrated in Table III.

Preoperative use of aspirin did correlate with platelet aggregation to U46619 (EC_{50}, 40 ± 19 nmol/L with aspirin vs 68 ± 35 nmol/L without aspirin; $p = 0.02$) but not platelet TXA_2/PGH_2 receptor density (B_{max}, 1600 ± 1000 sites per platelet with aspirin vs 1500 sites per platelet without aspirin; $p > 0.05$) or graft patency (80% for both groups). No correlations were observed between diabetes mellitus and platelet TXA_2/PGH_2 receptor density (B_{max}, 1900 ± 1400 sites per platelet with diabetes vs 1700 ± 900 sites per platelet without diabetes; $p > 0.05$), platelet aggregation (EC_{50}, 52 ± 31 nmol/L with diabetes vs 48 ± 27 nmol/L without diabetes; $p > 0.05$), or graft patency rate (13 of 18 [72%] with diabetes vs 7 of 7 [100%] without diabetes; $p > 0.05$).

DISCUSSION

This study has detected an increase in sensitivity to TXA_2 in the platelets of patients who subsequently have graft thrombosis early after lower extremity bypass surgery. This heightened sensitivity appears to result from increased expression of TXA_2/PGH_2 receptors on the platelet surface. These findings support earlier reports of increased

Table II. Demographic and preoperative risk factors of patients according to TXA_2/PGH_2 receptor density

	B_{max} > 3000 sites/platelet n=5	B_{max} < 3000 sites/platelet n=20
Mean age (range)	67 yr (51 to 75)	69 yr (54 to 88)
Male/female	5/0 (100/0)	12/8 (60/40)
Black/white	3/2 (60/40)	13/7 (65/35)
Diabetes/no diabetes	5/0 (100/0)	13/7 (65/35)
Preoperative ASA/no ASA	2/3 (40/60)	13/7 (65/35)
Tissue loss/rest pain	4/1 (80/20)	15/5 (75/25)
Vein conduit/ePTFE	4/1 (80/20)	16/4 (80/20)
Popliteal/infrapopliteal	1/4 (20/80)	6/14 (30/70)

NS, Not significant; *ASA,* acetylsalicylic acid (aspirin); *ePTFE,* expanded polytetrafluoroethylene. *Popliteal/infrapopliteal* indicates distal anastomosis target vessel.

Table III. Value of TXA_2/PGH_2 receptor density (B_{max}) and sensitivity of platelet aggregation to TXA_2 mimetics (EC_{50}) for predicting early graft occlusion

	B_{max} > 3000 sites/platelet	EC_{50} < 30 nmol/L
Sensitivity	0.8	0.8
Specificity	0.95	0.8
Likelihood ratio positive	16.0	4.0
Likelihood ratio negative	0.21	0.25

platelet activity in patients who are prone to graft thrombosis.[13,14] Platelet aggregometry to adenosine diphosphate, a weak platelet agonist, has been studied as a predictor of failure for knitted Dacron femoropopliteal grafts in human patients.[13] An aggregation score was derived from a combination of the magnitude of the initial reversible wave of aggregation, the overall magnitude of the second wave of irreversible aggregation, and the patient's platelet count. This complex scoring system was able to identify a subgroup of patients with increased platelet aggregability who subsequently had an extremely high rate of graft failure. Aggregation to thromboxane A_2 was not evaluated in that study.

Bleeding time as a measure of in vivo platelet function has been reported to accurately predict subsequent graft occlusion in patients who undergo femoropopliteal bypass grafting with polytetrafluoroethylene grafts.[14] Six grafts that were patent longer than 5 years were associated with a bleeding time of at least 6.5 minutes, whereas none of the six patients whose grafts occluded within 6 months of implantation had bleeding times longer than 5.5 minutes. Other parameters of coagulation, including prothrombin time and partial thromboplastin time, were not associated with the graft patency rate. As we also observed, this predictive ability was independent of the concomitant use of aspirin. It may at first seem surprising that the increases in platelet sensitivity as a result of TXA_2/PGH_2 receptor density might occur as a result of concurrent aspirin use. Although this has not yet been observed in humans, aspirin does appear to increase TXA_2/PGH_2 receptor density in a porcine model of carotid bypass.[26] The mechanism for this effect would presumably relate to upregulation of TXA_2/PGH_2 receptors as a result of a chronic suppression of the TXA_2 ligand.

Response to platelet aggregometry has long been recognized as a predictor of graft patency in a canine model, with nonaggregators much more likely to enjoy long-term graft patency.[27,28] It has been conjectured that the superior predictive value of arachidonic acid–induced aggregation compared with adenosine diphosphate might indicate a difference in sensitivity of the receptor to TXA_2. Such a theory is further supported by the failure to detect concomitant increases in TXB_2, the stable metabolite of TXA_2, which would imply that no additional TXA_2 is necessary in the presence of increased platelet TXA_2/PGH_2 receptor density.[29]

Instead of assuming that increases in TXA_2/PGH_2 receptor density before graft implantation may predispose patients to early graft thrombosis, it is equally possible that such changes are merely indicative of more advanced systemic disease. It has been observed that patients with acute myocardial infarction, presumably associated with coronary thrombosis, also exhibit increased platelet TXA_2/PGH_2 receptor density compared with patients with unstable angina or with noncoronary causes for chest pain.[30] It is intriguing that once these patients began to convalesce from their myocardial infarctions, the B_{max} of their platelets returned toward normal regardless of aspirin therapy, suggesting that the original cause might involve a stress response. It is unfortunate that convalescent samples were not available in the present study for comparison to see whether this phenomenon occurs with peripheral vascular reconstruction, as well.

Despite these observations, the actual mechanism for the observed changes in platelet receptor density remains unclear. One explanation that has been offered is that a greater proportion of the platelets are younger and larger in patients with advanced atherosclerotic disease because of increased platelet turnover.[30-34] Such presenescent platelets might be expected to demonstrate a greater

TXA_2/PGH_2 receptor density. Others have observed a decrease in the density of platelet prostacyclin receptors[35] and an increase in the affinity and density of α2-adrenergic receptors in patients with advanced atherosclerotic disease, which might synergistically lead to platelet hypersensitivity to TXA_2.[36]

Certainly, increased platelet sensitivity to TXA_2 would seem to be particularly hazardous immediately after bypass surgery. Locally increased TXB_2 production has been detected after arterial bypass grafting.[37-40] The luminal surface of a normal upstream artery spontaneously secretes both prostacyclin and TXA_2, whereas the arterial wall distal to a prosthetic graft may produce increased levels of these prostanoids with an overabundance of TXA_2 relative to prostacyclin substances at different proportions. Even gentle use of a valvulotome during in situ autogenous vein bypass grafting appears to provoke increased platelet release of TXA_2.[41]

Although patients with diabetes are known to experience increased platelet turnover, enhanced sensitivity to various aggregating agents, and increased platelet TXA_2 production,[30,42] this group of patients was not excluded from the study. In fact, reflecting the risk factors of patients who are typically treated at the authors' institutions, the vast majority of patients had diabetes. No significant difference in the incidence of diabetes was observed between the two groups (Table I), and the presence of diabetes did not correlate with any of the other measured factors. It is possible that the effect of diabetes on graft patency rates or TXA_2/PGH_2 receptor density may have been missed because of the small sample size. To prove that the observed differences in graft patency rates and TXA_2/PGH_2 receptor density were statistically significant, a total of 38 and 376 patients, respectively, would have been required, assuming an alpha error of 0.05 and a power of 0.80.

Elevated platelet TXA_2 receptor levels and enhanced sensitivity of platelet aggregation to TXA_2 appear to predict early arterial graft thrombosis independent of aspirin use. This preliminary study comprised only 25 patients, so that a number of possibilities for the introduction of a type II error exist. Clinical use of TXA_2/PGH_2 receptor density to predict early graft thrombosis must await larger, more comprehensive studies, including independent confirmation of our results. However, although many of the implications of our observations remain to be investigated, they would appear to suggest an advantage for the use of specific TXA_2/PGH_2 receptor antagonism to prevent one of the mechanisms that contributes to early graft occlusion.

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REFERENCES

1. Clowes AW, Gown AM, Hanson SR, Reidy MA. Mechanisms of arterial graft failure. I. Role of cellular proliferation in early healing of PTFE prostheses. Am J Pathol 1985;118:43-54.
2. Clowes AW. The role of aspirin in enhancing arterial graft patency. J Vasc Surg 1986;3:381-5.
3. Hagen PO, Wang ZG, Mikat EM, Hackel DB. Antiplatelet therapy reduces aortic intimal hyperplasia distal to small diameter vascular prostheses (PTFE) in nonhuman primates. Ann Surg 1982;195:328-39.
4. Oblath RW, Buckley FO, Green RM, Schwartz SI, DeWeese JA. Prevention of platelet aggregation and adherence to prosthetic vascular grafts by aspirin and dipyridamole. Surgery 1978;84:37-44.
5. Brothers TE, Vincent CK, Darvishian D, Harrell KA, Burkel WE, Stanley JC, et al. Effects of duration of acetylsalicylic acid administration on patency and anastomotic hyperplasia of ePTFE grafts. ASAIO Trans 1989;35:558-60.
6. Clowes AW, Kirkman TR, Clowes MM. Mechanisms of arterial graft failure. II. Chronic endothelial and smooth muscle cell proliferation in healing polytetrafluoroethylene prostheses. J Vasc Surg 1986;3:877-84.
7. McDaniel MD, Huntsman WT, Miett TO, Cronenwett JL. Effect of a selective thromboxane synthase inhibitor on arterial graft patency and platelet deposition in dogs. Arch Surg 1987;122:887-92.
8. Graham LM, Vincent CK, Brothers TE, Harrell KA, Darvishian D, Sell R, et al. Efficacy of antiplatelet agents in promoting patency and reducing anastomotic hyperplasia of endothelial cell seeded and unseeded ePTFE grafts. Surg Forum 1988;39:348-50.
9. Hansen KJ, Howe HR, Edgerton TA, Faust KB, Kon ND, Geisinger KR, Meredith JH. Ticlopidine versus aspirin and dipyridamole: influence on platelet deposition and three-month patency of polytetrafluoroethylene grafts. J Vasc Surg 1986;4:174-8.
10. Sharp WV, Schmidt SP, Donovan DL. Prostaglandin biochemistry of seeded endothelial cells on Dacron prostheses. J Vasc Surg 1986;3:256-63.
11. McCollum C, Alexander C, Kenchington G, Franks PJ, Greenhalgh R. Antiplatelet drugs in femoropopliteal vein bypasses: a multicenter trial. J Vasc Surg 1991;13:150-62.
12. Goldman S, Copeland J, Moritz T, Henderson W, Zadina K, Ovitt T, et al. Improvement in early saphenous vein graft patency after coronary artery bypass surgery with antiplatelet therapy: results of a Veterans Administration Cooperative Study. Circulation 1988;77:1324-32.
13. Saad EM, Kaplan S, El-Massry S, Kaplan A, Marcoe KF, Zammit M, et al. Platelet aggregometry can accurately predict failure of externally supported knitted Dacron femoropopliteal bypass grafts. J Vasc Surg 1993;18:587-95.
14. Fowl RJ, Palascak JE, Ose KJ, Blebea J, Kempczinski RR. Bleeding time as a predictor of early PTFE graft failure [abstract]. J Vasc Surg 1993;18:532-3.
15. Woodburn KR, Rumley A, Lowe GDO, Love JG, Murray GD, Pollock JG. Clinical, biochemical, and rheologic factors affecting the outcome of infrainguinal bypass grafting. J Vasc Surg 1996;24:639-46.
16. Hanson SR, Harker LA. Effect of dazoxiben on arterial graft

thrombosis in the baboon. Br J Clin Pharmacol 1983;15(suppl 1):57S-60S.
17. Halushka PV, Mais DE, Saussy DL Jr. Platelet and vascular smooth muscle thromboxane A_2/prostaglandin H_2 receptors. Fed Proc 1987;46:149-53.
18. Mais DE, Saussy DL Jr, Chaikhouni A, Kochel PJ, Knapp DR, Hamanak N, et al. Pharmacologic characterization of human and canine thromboxane A_2/prostaglandin H_2 receptors in platelets and blood vessels: evidence for different receptors. J Pharmacol Exp Ther 1985;233:418-24.
19. Saussy DL Jr, Mais DE, Burch RM, Halushka PV. Identification of a putative thromboxane A_2/prostaglandin H_2 receptor in human platelet membranes. J Biol Chem 1986;261:3025-9.
20. Lundella, Bergquist D, Leide S, Lindblad B, Ljungberg J. The effect of a combined thromboxane receptor and synthesis-antagonist on platelet, fibrinogen, and leucocyte uptake in ePTFE grafts: an experimental study in sheep. Eur J Vasc Surg 1992;6:276-81.
21. Foster MR, Hornby EJ, Stratton LE. Effect of GR32191 and other thromboxane receptor blocking drugs on human platelet deposition onto deendothelialized arteries. Thromb Res 1992;65:769-84.
22. Bech FR, Cronenwett JL, McDaniel MD, Ogletree ML, Freeman DH Jr. The effect of thromboxane receptor blockade versus thromboxane synthase inhibition on canine arterial graft patency. J Vasc Surg 1990;12:119-25.
23. Lane IF, Lumley P, Michael MF, Peters AM, McCollum CN. A specific thromboxane receptor blocking drug, AH23848, reduces platelet deposition on vascular grafts in man. Thromb Haemost 1990;64:369-73.
24. Iloprost Bypass International Study Group. Effects of perioperative iloprost on patency of femorodistal bypass grafts. Eur J Endovasc Surg 1996;12:363-71.
25. Morinelli TA, Oatis JE Jr, Okwu AK, Mais DE, Mayeux PR, Masuda A, et al. Characterization of an ^{125}I-labeled thromboxane A_2/prostaglandin H_2 receptor agonist. J Pharmacol Exp Ther 1989;251:557-62.
26. Brothers TE, Robison JG, Elliott BM, Boggs JM, Halushka PV. Thromboxane A_2 receptor density increases during chronic exposure to thromboxane A_2 receptor antagonists after porcine carotid bypass. Cardiovasc Surg 1997;5:92-8.
27. Greisler HP, McGurrin JF, Klosak JJ, Endean ED, Ellinger J, Pozar JD, et al. The validity of canine platelet aggregometry in predicting vascular graft patency. J Cardiovasc Surg (Torino) 1990;31:712-8.
28. Freeman MB, Sicard GA, Valentin LI, Neuberger S, Mangino MJ, Anderson CB. The association of in vitro arachidonic acid responsiveness and plasma thromboxane levels with early platelet deposition on the luminal surface of small-diameter grafts. J Vasc Surg 1988;7:554-61.
29. McGoff MA, Allen BT, Der T, Sicard GA, Santoro SA. Mechanisms of vascular graft thrombosis: role of altered canine platelet sensitivity to thromboxane. Thromb Res 1989;55:695-707.
30. Dorn GW III, Liel N, Trask JL, Mais DE, Assey ME, Halushka PV. Increased platelet thromboxane A_2/prostaglandin H_2 receptors in patients with acute myocardial infarction. Circulation 1990;81:212-8.
31. Davies MJ, Thomas AC, Knapman PA, Hangarter JR. Intramyocardial platelet aggregation in patients with unstable angina suffering sudden ischemic cardiac death. Circulation 1986;73:418-27.
32. Schwartz MB, Hawiger J, Timmons S, Friesinger GC. Platelet aggregates in ischemic heart disease. Thromb Haemost 1980;43:185-8.
33. Wu KK, Hoak JC. Spontaneous platelet aggregation in arterial insufficiency: mechanisms and implications. Thromb Haemost 1976;35:702-11.
34. Ward AS, Porter N, Preston FE, Morris-Jones WI. Platelet aggregation in patients with peripheral vascular disease. Atherosclerosis 1978;29:63-8.
35. Jaschonek K, Karsch KR, Weisenberger H, Tidow S, Faul C, Renn W. Platelet prostacyclin binding in coronary artery disease. J Am Coll Cardiol 1986;8:259-66.
36. Weiss RJ, Smith CB. Altered platelet alpha 2-adrenoreceptors in patients with angina pectoris. J Am Coll Cardiol 1983;2:631-7.
37. Bush HL Jr, Jakubowski JA, Hong SL, McCabe M, Deykin D, Nabseth DC. Luminal release of prostacyclin and thromboxane A_2 by arteries distal to small-caliber prosthetic grafts. Circulation 1984;70:111-5.
38. Merhi Y, Guidoin R, Forest JC. Fate of polyester arterial prostheses implanted as thoracoabdominal bypasses in dogs: haematology, pathology, and biochemistry. Clin Invest Med 1988;11:403-16.
39. Lewin J, Swedenborg J, Egberg N, Vesterqvist O, Green K. Effect of acetylsalicylic acid on increased production of thromboxane after aortic graft surgery. Eur J Vasc Surg 1989;3:213-8.
40. Vesterqvist O, Green K, Lewin J, Swedenborg J. Synthetic arterial grafts cause prolonged increases in the in vivo formation of thromboxane and prostacyclin in humans. Res Exp Med (Berl) 1987;187:175-84.
41. Barman SA, Chaudhry SS, Moideen AS, Iljas J, Barman AA. Measurement of thromboxane and prostacyclin in valvulotomized human saphenous veins. Am Surg 1997;63:291-3.
42. Halushka PV, Rogers RC, Loadholt CB, Colwell A. Increased platelet thromboxane synthesis in diabetes mellitus. J Lab Clin Med 1981;97:87-96.

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APPENDIX A. Life table analysis of cumulative primary graft patency data according to TXA_2/PGH_2 receptor density

Interval end (mo)	*No. of grafts at risk*	*No. of grafts failed*	*No. of grafts withdrawn*	*Interval patency rate (%)*	*Cumulative patency rate (%)*	*Standard error (%)*
B_{max} > 3000 sites/platelet						
0-2	5	4	0	20	20	18
2-4	1	0	0	100	20	18
4-6	1	0	0	100	20	18
6-8	1	0	0	100	20	18
8-10	1	0	0	100	20	18
10-12	1	0	0	100	20	18
12-14	1	0	1	100	20	18
B_{max} < 3000 sites/platelet						
0-2	20	1	0	95	95	5
2-4	19	0	1	100	95	5
4-6	18	0	0	100	95	5
6-8	18	2	0	89	84	8
8-10	16	0	0	100	84	8
10-12	16	1	0	94	79	9
12-14	15	1	4	93	74	10
14-16	10	0	3	100	74	10
16-18	7	0	1	100	74	10
18-20	6	1	1	83	62	14
20-22	4	0	1	100	62	14
22-24	3	0	0	100	62	14

APPENDIX B. Life table analysis of cumulative primary graft patency data according to EC_{50} for platelet aggregation to U46619

Interval end (mo)	*No. of grafts at risk*	*No. of grafts failed*	*No. of grafts withdrawn*	*Interval patency rate (%)*	*Cumulative patency rate (%)*	*Standard error*
EC_{50} < 30 nmol/L						
0-2	8	4	0	50	50	18
2-4	4	0	0	100	50	18
4-6	4	0	0	100	50	18
6-8	4	0	0	100	50	18
8-10	4	0	0	100	50	18
10-12	4	0	0	100	50	18
12-14	4	0	0	100	50	18
14-16	4	0	0	100	50	18
16-18	4	0	1	100	50	18
18-20	3	1	0	67	33	18
20-22	2	0	0	100	33	18
22-24	2	0	0	100	33	18
EC_{50} > 30 nmol/L						
0-2	17	1	0	94	94	6
2-4	16	0	1	100	94	6
4-6	15	0	0	100	94	6
6-8	15	2	0	87	82	10
8-10	13	0	0	100	82	10
10-12	13	1	0	92	75	11
12-14	12	1	5	92	69	12
14-16	6	0	3	100	69	12
16-18	3	0	0	100	69	12
18-20	3	0	1	100	69	12
20-22	2	0	1	100	69	12
22-24	1	0	0	100	69	12

DISCUSSION

Dr. William C. Krupski (Denver, Colo.). First, I would like to compliment Dr. Brothers and his associates for a nicely performed study and an excellent presentation this morning. The authors have shown increased thromboxane receptor density in the platelets of patients whose graft thrombosed within 3 months of undergoing bypass grafting. Early graft thromboses were more likely to occur in patients with thromboxane receptor density greater than 3000 sites per platelet. In addition, platelets from patients who had early graft occlusion required less thromboxane mimetic to aggregate. Thus patients with early graft occlusion presumably have enhanced sensitivity to thromboxane.

Vascular surgeons have often said in jest—or occasionally truly believed—that all of their graft occlusions occurred in patients with hypercoagulable states. Dr. Brothers' presentation would lend support to this contention. I wish it were so simple.

Four years ago at these meetings, Dr. Mills and colleagues described serial duplex surveillance in 227 consecutive infrainguinal bypass grafts. Of note, only 8% of the grafts thrombosed in the 5-year follow-up period, in contrast to a 20% 3-month thrombosis rate in the current study. Technical problems were far and away the most common cause of failed or failing grafts. In the Mills study, corroborating data were reported from groups from Northwestern and the New England Medical Center. In the Mills report, only 4.3% of failures were attributed to hypercoagulable states, a far cry from the 100% described this morning. Although I applaud the authors for their honesty in reporting this high early failure rate, how do they justify a 20% 3-month occlusion rate when others report 3% to 5%? Did the authors use intraoperative angioscopy, duplex ultrasound, or arteriography to rule out technical or anatomic problems that might explain early graft thromboses? What was the postoperative surveillance protocol?

A second concern I have with the manuscript relates to hematologic considerations. It is clear that absolute platelet counts play an important role in thromboembolic events such as graft occlusions. The authors at the very least must provide data regarding platelet counts in patients who had early graft occlusion versus those with early patency. Do you have any in vitro data investigating the other two pathways for platelet aggregation, namely thrombin and ADP-mediated aggregation?

Although aspirin treatment reportedly conferred no protection from thrombosis, it would be important to determine compliance by measuring salicylate levels. I am also concerned that the relatively small number of patients in this study may produce a type II statistical error with respect to the efficacy of aspirin treatment.

Finally, because this is, in a sense, a study of hypercoagulability, did the authors measure other factors that contribute to thrombosis, such as antithrombin III, protein C, protein S, or factor V (Leiden)?

Several additional factors warrant analysis by the authors. I would be interested to know the correlation with graft thrombosis of at least the following variables: active cigarette smoking, concomitant procedures that may have contributed to the complexity of the case, the target vessels for bypass grafting, and more detailed information regarding runoff.

In summary, the study reported this morning does have great potential, particularly if specific thromboxane receptor inhibitors are developed and are shown to prevent early graft thrombosis. However, despite our desire to blame platelets for our graft thromboses, I fear we must continue to accept responsibility and attribute most failures to technical problems.

Dr. Thomas E. Brothers. Thank you, Dr. Krupski. I very much appreciate your initial gracious comments followed by your pointed yet insightful criticism. I would like to emphasize that the overall purpose of this study and our presentation is not to blame hypercoagulable states for our failures, nor is it necessarily to report to you our overall experience with lower extremity bypass grafting, but rather to describe our attempts to elicit the nature of the platelet hyperactivity that has been described by others. However, since the point is raised, I am forced to respond to it. In fact, I would agree that the 20% early thrombosis rate is quite high. However, it is a very small sample that does not really accurately reflect our overall experience, which, as has previously been published, is much closer to 10% or below.

When we actually went back and looked at the results of why these grafts thrombosed, we could only identify one out of the five occlusions as being a truly technical error, and the rest were of uncertain cause. This observation mirrors our overall experience that in many of these patients we cannot quite find out the cause for the failure despite aggressive searching.

In terms of completion studies, practices varied among the members of our group, but all of the patients in this study did undergo completion angiography or on-table duplex scanning. We certainly agree with that practice as well as the concept of postoperative surveillance. Our postoperative surveillance protocol involves getting a study at 3 weeks, 3 months, 6 months, and 12 months during the first year after bypass grafting.

I would agree very much that it is important to include the platelet numbers, and I will certainly add platelet numbers to the manuscript. But for the moment you're just going to have to take my word for it that there were no differences in the mean and a range of the platelet numbers between the patient groups.

We did not investigate the activity of the GP IIb/IIIa receptors, nor did we duplicate the studies of the group

from Seattle in looking at the response to ADP. Although certainly from a mechanistic standpoint it occurs to me that either the change in thromboxane density may either be somewhat supplemental to the increased aggregation to ADP or that there are other things going on that affect both of these findings at the same time. I am not prepared to invoke thromboxane receptor activity as the sole cause of the platelet hyperactivity.

You certainly make a very important point regarding the compliance with aspirin. We were not wise enough to think to measure salicylate levels. I will say that all patients did receive aspirin after operation, and, at least while they were in the hospital, that could be monitored.

Because of our previous work and interest, this study was focused on the involvement of the platelet and the thromboxane receptor, not on the presence or absence of hypercoagulable states, and we do not routinely check for those conditions in all of our patients. Certainly the presence of a hypercoagulable state is greater in patients with early disease, especially among younger patients, as has been well described by our colleagues in Columbia, South Carolina. Neither the thromboxane density nor the graft patency data correlated with active tobacco use, concomitant operations, the target vessel, or the arterial runoff.

In summary, I would agree absolutely that we do need to accept responsibility for our failures and not try to pass them off as something out of our control. However, it is hard for me to see how a technical failure that occurs, such as a missed valve, could be predicted by a preoperative elevation in thromboxane receptor density. And I would certainly point out, in fact, that these elevations were associated with our subsequent results and that we obviously have quite a bit of work ahead of us trying to sort this out further.

Dr. William M. Abbott (Boston, Mass.). We hear about test after test after test looking for the hypercoagulable state, and we are continuously left with results and confounded about what we are supposed to do about them. This adds a lot of time and expense. I am curious from a practical standpoint how difficult this test is to perform. Can it be done in the operating room, for example? How expensive would it be?

Dr. Brothers. These tests are not for routine clinical use. They are quite expensive. They require at least 8 hours of time. Again, these assays are not intended as routine preoperative screening tests. They are receptor-ligand assays and can be somewhat difficult to do. This is not something that I personally see as something that we should expect to use in the near future, nor would I recommend doing so on the basis of these preliminary studies.

Dr. Richard J. Fowl (Scottsdale, Ariz.). I was in Cincinnati, and you referred to our study there, so I rise to support some of your conclusions. The platelets, I think, are probably a very important parameter in causing occlusion, and yet it's a very difficult thing to prove. In response to one of Dr. Krupski's comments, we limited our study to polytetrafluoroethylene only. And we did perform all the coagulation parameter assays, including protein S, protein C, antithrombin III, a fibrinolytic profile, and D-dimers, and just about anything else you could think of. However, factor V (Leiden) assay wasn't available at the time.

We compared two groups of people. One group of people had 5-year patency with polytetrafluoroethylene and no problems, and the grafts in the other occluded within 6 months. The only thing that was different was the bleeding time, and it was markedly short, although all of the patients were taking aspirin. I think that one of the conclusions I'm coming to from your work as well as our own is that aspirin is probably not such a good antiplatelet agent in many people. When I visited his laboratory in Seattle several years ago, Dr. Sauvage observed, and this isn't published anywhere, that he found that about half his population does not respond to aspirin therapy as an antiplatelet agent. So I wonder whether you have thought about trying any other antiplatelet agents and rechecking your thromboxane assays? I think that might be a good thing to try, using perhaps dipyridamole or even ibuprofen or any of the other agents that are known to be antiplatelet agents.

Dr. Brothers. I appreciate the comments. We actually have not specifically done that at this time. In an animal model that was previously presented, however, we looked at the results of chronic aspirin use or chronic use of a thromboxane receptor antagonist and actually found, at least in the porcine model, that chronic use of one of these agents is actually associated with an increase in the density. So as you might expect, if there is less of the ligand around, one of the ways that the cell responds is to increase expression of the receptor. From a functional standpoint, I'm not entirely sure what this all means, but we have not actually looked at other antiplatelet agents at this point.

Dr. Michael Sobel (Richmond, Va.). I compliment you on a very elegant scientific study and would just like to focus my question on the question of chicken versus egg. And this is a real problem with all of the studies we perform regarding platelets, that is, when platelets are activated for any reason their receptors are upregulated. Because what you and all of us may be looking at is a symptom or manifestation of a state of activated platelets, it really doesn't get to the issue of causation. So that while inhibition or blockade of thromboxane receptors might be effective, it also might be just treating the symptom rather than getting to the issue of why certain patients have activated platelets in their circulation.

Dr. Brothers. I couldn't agree with you more. And again, I'm not claiming to prove that there is a cause and effect relationship here. Certainly, in the other study from our institution, the suggestion was that either the presence of increased stress associated with chest pain and hemodynamic instability might perhaps contribute to the increase in receptor density or perhaps just that that was a marker of more diffuse atherosclerotic disease.

As a way to kind of check that in our group of patients, we thought that if there was an increase in either gangrene or ongoing ulceration or pain in the extremities, such stressful situations might be associated with increased density. It turned out not to be the case. Also, trying to look at the degree of outflow or the overall condition of the outflow as an indicator of the extent of atherosclerosis, also did not seem to correlate. But I agree, we have reported an association. At this point, I would not say that this represents a cause and effect.

Dr. Robert W. Hopkins (Providence, R.I.). I enjoyed the paper very much, but I do have a question about your rejection of the hypothesis that diabetes might be related to this. If I understand your data correctly, all of the patients whose grafts occluded had diabetes. There are a lot of patients with diabetes in the study group, and in the nonoccluded group about two thirds had diabetes. But not all diabetes is the same. I have two questions for you. First, was there a difference in the characteristics of the diabetes between the occluded and nonoccluded groups? Second, in the previous study with coronary disease, was there any particular relationship between the patients with diabetes and those without diabetes?

Dr. Brothers. You are very perceptive. In fact, more than 70% of our patients overall have diabetes. That's just the population that we see. Those differences were not significant. Perhaps that's a type II error, perhaps not. Whether it was type I or type II diabetes did not seem to correlate. I will say that the same group at our institution that published the previous results looking at coronary disease actually looked at the expression of the receptor in patients with diabetes, and in fact there is increased receptor density in patients with type I diabetes. This is probably related to some degree to the fact that patients with type I diabetes are known to have larger platelets and perhaps that in some way allows for more receptors to be present. We actually have an active proposal to try to correlate the degree of thromboxane receptor density in patients with type I diabetes with subsequent progression of atherosclerotic or lower extremity arteriosclerotic disease.

Dr. Edmund J. Harris, Jr. (Stanford, Calif.). Did you repeat the thromboxane assay at the time of the first graft thrombosis and also at your first follow-up with the patent grafts? From what I understand from your presentation, you did the thromboxane assay only once before operation. How do we know that that value is valid in the perioperative period when you're observing the grafts failing?

Dr. Brothers. These assays were all performed before the patient was anesthetized, before they had any intervention, usually the day before operation. Sadly, we did not get follow-up studies to look at, especially in light of the data with coronary disease that suggested that once the acute myocardial infarction was over then the receptor density went back to normal. That is definitely something we ought to do, but unfortunately we don't have those data.