

From the Western Vascular Society

# A comparison of recombinant thrombin to bovine thrombin as a hemostatic ancillary in patients undergoing peripheral arterial bypass and arteriovenous graft procedures

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**Objectives:** Recombinant thrombin (rThrombin) is a potential hemostatic alternative to bovine and human plasma-derived thrombin. This report examines the clinical results for the vascular surgery subgroup of patients enrolled in a larger double-blind, randomized, multicenter trial, which evaluated the comparative safety and efficacy of rThrombin and bovine plasma-derived thrombin (bThrombin) when used as adjuncts to surgical hemostasis.

**Methods:** Data from the 164 vascular patients who underwent either a peripheral arterial bypass (PAB) or arteriovenous graft (AV) procedure are included in this analysis. Time to hemostasis at proximal and distal anastomotic sites at 1.5-, 3-, 6-, and 10-minute intervals was determined by procedure (PAB or AV) and overall (PAB + AV). Baseline and day 29 immunologic sera were analyzed. The incidences of postoperative adverse events were compared between treatment groups. Categorical adverse events were evaluated in relation to thrombin product antibody formation.

**Results:** Patients were randomized to either bThrombin (n = 82) or rThrombin (n = 82). Procedures included PAB (n = 88) and AV (n = 76). The bThrombin and rThrombin groups were well matched for demographics and baseline characteristics. A comparable incidence of anastomotic hemostasis was observed in both treatment groups at 10 minutes (94% bThrombin, 91% rThrombin). The incidence of hemostasis was lower at all time points for PAB procedures compared with AV procedures. In the PAB group, a significantly greater proportion of patients receiving rThrombin (55%) achieved hemostasis at 3 minutes compared with bThrombin (39%;  $P < .05$ ). Adverse event profiles and laboratory findings were similar between groups. No patients in the rThrombin group developed anti-rThrombin product antibodies at day 29, whereas 27% of patients in the bThrombin group developed antibodies to bThrombin product ( $P < .0001$ ).

**Conclusions:** rThrombin or bThrombin used as a hemostatic ancillary for anastomotic bleeding was equally effective at 10 minutes; however, rThrombin compared with bThrombin may provide a more rapid onset of hemostasis at 3 minutes in PAB procedures. Adverse events were similar between the two thrombins. In patients undergoing vascular surgery, both treatments were similarly well tolerated, although rThrombin demonstrated a superior immunogenicity profile. (*J Vasc Surg* 2008;47:1266-73.)

Topically applied thrombin is frequently used in reconstructive vascular and vascular access procedures. In the United States, an estimated \$250 million are spent annually on the use of topical thrombin in over 1 million surgical procedures.<sup>1</sup> Currently available sources of thrombin used

in surgical procedures include bovine and human pooled plasma-derived thrombin. Though some clinical trials of bovine thrombin as a component of fibrin sealants and other combination products have been reported, the hemostatic efficacy of bovine thrombin (bThrombin) alone has not been extensively studied in prospective, randomized comparative studies. Nevertheless, bThrombin has gained widespread acceptance as an adjunct to achieving hemostasis in many types of surgical procedures, including vascular, orthopedic, and skin graft procedures. However, bThrombin has been associated with anaphylaxis and the development of antibodies which can cross react with human factor V or human thrombin, sometimes leading to life-threatening coagulopathy and considerable morbidity.<sup>1-10</sup> Human pooled plasma-derived thrombin has recently become commercially available as a stand-alone topical hemostat; this product carries the risk of viral transmission, and the degree of safety conferred by the best processing techniques must be continuously evaluated.<sup>11</sup>

Recombinant thrombin (rThrombin) is a potential alternative to plasma-derived thrombins. Theoretically, a re-

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combinant human thrombin would provide a homologous protein that is safe and effective, but without the attendant concerns of antibody production or viral transmission. To investigate this hypothesis, rThrombin (ZymoGenetics, Seattle, Wash) was compared with bThrombin (Thrombin-JMI, GenTrac, Inc, Middleton, Wis) in a recently completed phase 3, prospective, double-blind, randomized trial. That trial included patients undergoing vascular, liver, and spine procedures, and the overall results were recently published.<sup>12</sup>

In contrast to most patients undergoing spine and liver procedures, obstacles to hemostasis such as uremia, chronic renal dialysis, concomitant antiplatelet/anticoagulant medications, vasculopathy, and hypertension are common in the vascular patient population. In addition, patients with vascular disease or in need of vascular access often undergo multiple vascular procedures which may increase thrombin exposure and potentiate an immunologic response to thrombin-based product. It was with the unique aspects of the vascular patient population in mind that a more detailed analysis of the phase 3 vascular cohort was performed. This report details the results of this analysis and characterizes the relative safety and efficacy of topical rThrombin and bThrombin as a hemostatic ancillary in patients undergoing peripheral arterial bypass and arteriovenous graft procedures.

## METHODS

A detailed comparative analysis was performed on the vascular cohort of patients from a larger double-blind, randomized, prospective trial conducted at 34 centers in the United States. Exploratory statistical analyses not specified a priori were conducted to evaluate the safety and efficacy of bThrombin (Thrombin-JMI) versus rThrombin in a subset of patients undergoing vascular surgery. Sixteen centers participating in the prospective trial enrolled patients undergoing peripheral artery bypass (PAB) or arteriovenous (AV) graft formation procedures.

Details regarding the design of the prospective trial have been reported previously.<sup>12</sup> Eligible patients undergoing PAB or AV graft procedures with a polytetrafluoroethylene (PTFE) graft or revision procedures with PTFE graft-graft anastomoses were randomized in a 1:1 ratio prior to surgery to receive either bThrombin (1000 U/mL) or rThrombin (1000 U/mL). Dynamic allocation was used (real-time, computerized method) to attain an approximately equal number of patients randomized to rThrombin and bThrombin by surgeon and within each surgery type. Surgeons were blinded to treatment assignment and study drug was delivered to the operating room from the pharmacy as a clear, colorless solution in identical syringes. The study drugs were applied topically to anastomotic bleeding site(s) in combination with an absorbable gelatin sponge (Surgifoam, Johnson & Johnson Wound Management, Somerville, NJ). Time to hemostasis (TTH), summarized as the incidence of hemostasis within 10 minutes, was determined for four prespecified anastomotic sites: PAB proximal anastomosis, PAB distal anastomosis, AV arterial

anastomosis, and AV venous anastomosis. Administration of coagulation impeding medications was recorded.

Adverse events and clinical laboratory abnormalities were recorded up to day 29 ( $\pm 5$  days). Adverse events of potential clinical interest were prospectively categorized as hemorrhagic, cardiac, hypersensitivity, or thromboembolic based upon clinical importance (cardiac) or whether they were known or possible complications from administration of topical thrombin; a listing of events included in each category is shown in Table I. Adverse events were coded according to the Medical Dictionary for Regulatory Activities (MedDRA) version 9. Standard clinical laboratory data and prothrombin time (PT), activated partial thromboplastin time (aPTT), and international normalized ratio (INR) were measured, and perioperative transfusion was recorded as a surrogate marker for blood loss. Antibodies to either bThrombin or rThrombin product were evaluated in plasma samples collected at baseline and at day 29 ( $\pm 5$  days) using assays developed by ZymoGenetics, Inc (Seattle, Wash).

The differences in the incidence of hemostasis between treatment groups at the timepoints of 1.5, 3, 6, and 10 minutes were evaluated using generalized estimating equations (GEE) to account for potential correlation between measurements from the same patient. Fisher exact test was used to compare the incidence of antiproduct antibodies between treatment groups, and exact binomial confidence intervals were created for an analysis of the potential relationship between adverse clinical outcomes and the presence of antiproduct antibodies after treatment with study drug.

## RESULTS

A total of 183 vascular patients were enrolled and randomized between October 2005 and July 2006; 19 patients were not treated with study drug due to lack of an appropriate TTH evaluation site or other reasons (Fig). One hundred sixty-four patients received blinded study drug (bThrombin,  $n = 82$ ; rThrombin,  $n = 82$ ), and 161 completed the study. Eighty-eight and 76 patients underwent PAB and AV graft procedures, respectively, and were randomized and treated. The majority of patients (90% bThrombin, 85% rThrombin) were treated with blinded study drug at more than one anastomotic site. Fifty-eight percent were male, and the median age was 64 years. Baseline demographics, baseline characteristics, and type of surgical procedure performed were similar between treatment groups (Table II). Overall, the use of anticoagulants/antiplatelet medications was similar between treatment groups (66% bThrombin, 62% rThrombin). Patients undergoing PAB (89% bThrombin, 84% rThrombin) more often received preoperative coagulation-impeding medications than patients undergoing AV graft procedures (39% bThrombin, 37% rThrombin).

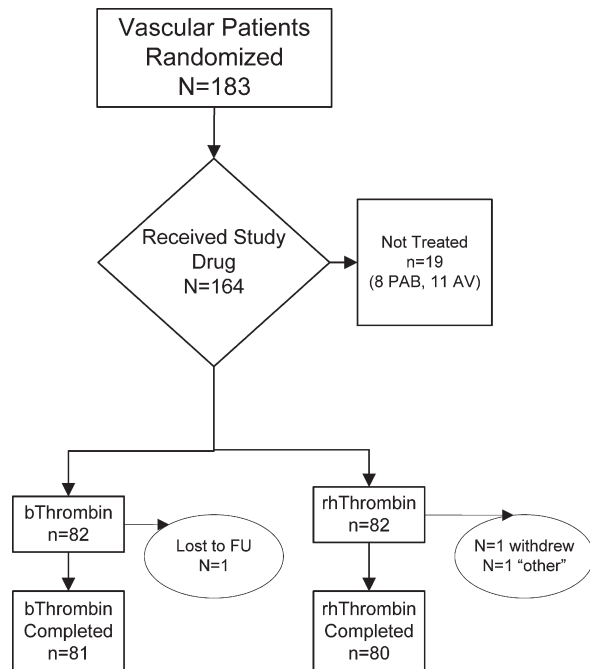
The incidence of hemostasis within 10 minutes at all hemostasis evaluation sites (PAB + AV graft) was comparable between groups (94% bThrombin, 91% rThrombin) (Table III). In patients undergoing PAB surgery, the overall incidence of hemostasis within 10 minutes was 88% (90%

**Table I.** Adverse events of potential clinical interest by category

<b>Hemorrhagic</b>	Arteriovenous graft site hematoma Arteriovenous graft site hemorrhage Blood urine present Catheter site hemorrhage Cerebrovascular accident Contusion	Ecchymosis Epistaxis Genital hemorrhage Gingival bleeding Hematuria Hemiparesis <sup>a</sup>	Incision site hematoma Incision site hemorrhage Postprocedural hematoma Postprocedural hemorrhage Wound hemorrhage
<b>Cardiac</b>	Acute myocardial infarction Angina pectoris Aortic valve sclerosis <sup>a</sup> Atrial fibrillation Atrial thrombosis Cardiac failure congestive Cardiac hypertrophy <sup>a</sup> Cardiac murmur Diastolic dysfunction Dilatation atrial <sup>a</sup> Heart valve insufficiency <sup>a</sup>	Ischemic cardiomyopathy <sup>a</sup> Mitral valve incompetence <sup>a</sup> Mitral valve sclerosis <sup>a</sup> Palpitations Pulmonary edema Sinus arrhythmia Sinus bradycardia Sinus tachycardia Tachycardia	Tricuspid valve incompetence <sup>a</sup> Troponin increased Ventricular extrasystoles Ventricular hypertrophy <sup>a</sup> Ventricular tachycardia
<b>Hypersensitivity</b>	Dermatitis Erythema Incision site erythema	Pruritus Pruritus generalized Rash macular	Urticaria
<b>Thromboembolic</b>	Arteriovenous graft thrombosis Atrial thrombolysis Cerebrovascular accident Graft thrombosis	Hemiparesis Septic embolus Thrombophlebitis Superficial	Thrombotic Thrombocytopenic purpura Vascular graft occlusion

Coded with MedDRA version 9.0.

<sup>a</sup>Treatment emergent or new diagnosis during study period sorted by etiologic grouping.



**Fig.** Patient disposition.

**Table II.** Demographic and baseline characteristics

Characteristic	bThrombin (N = 82)	rThrombin (N = 82)
Median age, y (range)	63.5 (30, 85)	65.0 (28, 88)
Gender, n (%)		
Female	34 (41)	35 (43)
Male	48 (59)	47 (57)
Surgery, n (%)		
PAB – total	44 (54)	44 (54)
Proximal and distal	39 (89)	38 (86)
Proximal only	3 (7)	2 (5)
Distal only	2 (5)	4 (9)
AV graft – total	38 (46)	38 (46)
Arterial and venous	35 (92)	32 (84)
Arterial only	2 (5)	3 (8)
Venous only	1 (3)	3 (8)
Preoperative coagulation impeding medications	54 (66)	51 (62)
Comorbidity, n (%)		
Hypertension	73 (89)	70 (85)
Heart failure	16 (20)	15 (18)
Coronary artery disease	28 (34)	33 (40)
COPD/pulmonary	17 (21)	17 (21)
Diabetes	42 (51)	42 (51)
Renal insufficiency	41 (50)	42 (51)

PAB, Peripheral artery bypass; AV, arteriovenous; COPD, chronic obstructive pulmonary disease.

bThrombin, 87% rThrombin), while in patients having AV graft surgery, the overall incidence of hemostasis within 10 minutes was 97% (99% bThrombin, 96% rThrombin) (Tables IV and V). The cumulative incidence of hemostasis at

all timepoints was lower in PAB patients than in AV patients, independent of treatment assignment. When examined at the clinically relevant timepoint of 3 minutes, a statistically significantly greater incidence of hemostasis was

**Table III.** Cumulative incidence of hemostasis over time by treatment group at all (PAB + AV graft) anastomotic sites

	<i>bThrombin</i> (82 patients)	<i>rThrombin</i> (82 patients)	P value
Number of bleeding sites	156	152	
Time to hemostasis, n (%) within:			
1.5 min	56 (36)	62 (41)	0.44
3 min	90 (58)	103 (68)	0.11
6 min	124 (79)	128 (84)	0.33
10 min	147 (94)	138 (91)	0.28

PAB, Peripheral artery bypass; AV, arteriovenous.  
Bleeding sites: PAB, proximal and distal; AV, arterial and venous.  
General Estimating Equation analysis for nonindependent variables.

**Table IV.** Cumulative incidence of hemostasis over time by treatment group at AV graft anastomotic sites

	<i>bThrombin</i> (82 patients)	<i>rThrombin</i> (82 patients)	P value
Number of bleeding sites/ number of patients	73/38	70/38	
Time to hemostasis, n (%) within:			
1.5 min	39 (53)	42 (60)	0.49
3 min	58 (79)	58 (83)	0.66
6 min	68 (93)	66 (94)	0.77
10 min	72 (99)	67 (96)	0.29

AV, Arteriovenous.  
Bleeding sites: AV, arterial and venous.  
General Estimating Equation analysis for nonindependent variables.

**Table V.** Cumulative incidence of hemostasis over time by treatment group at PAB anastomotic sites

	<i>bThrombin</i> (82 patients)	<i>rThrombin</i> (82 patients)	P value
Number of bleeding sites/ number of patients	83/44	82/44	
Time to hemostasis, n (%) within:			
1.5 min	17 (20)	20 (24)	0.58
3 min	32 (39)	45 (55)	0.046
6 min	56 (67)	62 (76)	0.28
10 min	75 (90)	71 (87)	0.47

PAB, Peripheral artery bypass.  
Bleeding sites: PAB, proximal and distal.  
General estimating equation analysis for nonindependent variables.

observed with rThrombin compared with bThrombin (55% rThrombin, 39% bThrombin,  $P < .05$ ) in PAB procedures.

Of the primary hemostasis evaluation sites (proximal anastomoses), 14 sites (7 bThrombin, 7 rThrombin) failed to achieve hemostasis within 10 minutes. Blinded study drug was the most frequently administered treatment following hemostatic failure, and was successful in achieving hemostasis at all but one site in a patient receiving rThrom-

**Table VI.** Incidence of adverse events of potential clinical interest by category and treatment group<sup>a</sup>

	<i>bThrombin</i> N = 82	<i>rThrombin</i> N = 82
Hemorrhagic, n (%)	17 (21)	16 (20)
Cardiac, n (%)	11 (13)	12 (15)
Hypersensitivity, n (%)	9 (11)	9 (11)
Thromboembolic, n (%)	7 (9)	7 (9)

<sup>a</sup>Categories grouped by MedDRA coded events; thromboembolic category includes thrombotic and embolic adverse events.

**Table VII.** Development of anti-product antibodies

	<i>bThrombin</i>	<i>rThrombin</i>	P value
Detectable at baseline	6/81 (7.4%)	3/80 (3.8%)	
Antibody positive <sup>a</sup>	22/81 (27%)	0/80 (0.0)	<.0001
Seroconversion <sup>b</sup>	17/75 (23%)	0 (0.0)	
$\geq 1.0$ titer unit change <sup>c</sup>	5/6 (83%)	0 (0.0)	

<sup>a</sup>Seroconversion or  $\geq 1$  unit increase in titer postbaseline.

<sup>b</sup>Denominators include only patients who had a postbaseline observation and no detectable anti-product antibodies at baseline.

<sup>c</sup>Denominators include only patients who had a postbaseline observation and had detectable anti-product antibodies at baseline.

bin. There were no clinically meaningful differences in the rate of perioperative transfusion between treatment groups (data not shown). There were no cross-over thrombin applications between treatment groups and no potentially confounding thrombin product administration during the 29-day study period.

No deaths occurred during the study in either group. Six patients in the bThrombin group experienced graft complications or thrombosis (two PAB, four AV) while seven patients in the rThrombin group developed graft occlusion (two PAB, five AV). Serious adverse events occurred in 22% of those receiving bThrombin vs 17% in those receiving rThrombin. Serious adverse events were evenly distributed among organ system class and were consistent with underlying subject comorbidities and known surgical complications. Prospectively categorized adverse events (hemorrhagic, cardiac, hypersensitivity, and thromboembolic) are listed by treatment group in Table VI. The incidence of adverse events within these categories was similar between treatment groups. Peri- and postoperative bleeding complications were experienced by approximately 20% of patients in both treatment groups, and thromboembolic complications occurred in 9% of patients in each group. No postoperative cardiac events were attributed to study drug in either treatment group. No meaningful differences were found between treatment groups at baseline or day 29 for clinical laboratory results, PT, aPTT, or INR.

A total of 161 patients were evaluated for the presence of antibodies to either the bThrombin or the rThrombin products at baseline and postexposure (Table VII). Anti-product antibodies were detectable at baseline in six pa-

**Table VIII.** Association of postexposure anti-bThrombin product antibodies with adverse clinical outcomes

	<i>Anti-bThrombin product antibody</i>	
	<i>Negative n/N (%) 95% CI</i>	<i>Positive n/N (%) 95% CI</i>
Patients with thromboembolic events	6/59 (10) 4%-21%	1/22 (5) 0%-23%
Patients with hypersensitivity events	4/59 (7) 2%-16%	5/22 (23) 8%-45%
Patients with elevated aPTT <sup>a</sup>	12/59 (20) 11%-33%	8/22 (36) 17%-59%
Patients with hemorrhagic events	14/59 (24) 14%-37%	3/22 (14) 3%-35%
Patients with cardiac events	8/59 (14) 6%-25%	3/22 (14) 3%-35%

aPTT, Activated partial thromboplastin time.

<sup>a</sup>aPTT elevation as any value greater than upper.

tients (7.4%) receiving bThrombin. In the rThrombin treatment group, three patients (3.8%) had anti-rThrombin antibodies at baseline. At day 29, 27% of patients in the bThrombin group had seroconverted or had a 10-fold increase in detectable antibodies to bThrombin product. No patients receiving rThrombin seroconverted or had an increase in anti-rThrombin product antibody titer. This difference in immunogenicity was highly statistically significant ( $P < .0001$ ). In the bThrombin group, antibody positivity due to seroconversion occurred in 17 patients and an increase in anti-bThrombin product antibody  $>1$  titer unit occurred in five of the six patients with bThrombin antibodies at baseline.

Because of the reported association between antibodies to bThrombin and adverse events, selected adverse clinical outcomes were sorted by presence or absence of postexposure antibodies to bThrombin product (Table VIII). Although patients who developed antibodies to bThrombin product had a numerically increased incidence of hypersensitivity events compared with antibody-negative patients (23% vs 7%) and an elevation in aPTT relative to patients without bThrombin antibodies (36% vs 20%), these differences were not statistically significant.

## DISCUSSION

Thrombin is a central component in the coagulation cascade. Thrombin activates platelets and cleaves fibrinogen to fibrin, leading directly to clot formation. Thrombin also activates factor XIII, leading to fibrin cross-linking and clot stability.<sup>13</sup> The ability of thrombin to bypass the initial enzymatic steps of the coagulation pathway has led to a widespread adoption of thrombin from multiple biologic sources as a topical hemostatic agent. Thrombin is rapidly neutralized by naturally circulating plasma inhibitors, including anti-thrombin III, alpha-2-macroglobulin, and heparin cofactor II, which act to limit its duration of action and prevent the active form from diffusing into the general circulation.<sup>14-17</sup>

Hemostasis is vital to the success of any vascular procedure. When ligation, electrocautery, or simple pressure fails to arrest bleeding, surgeons turn to a number of hemostatic aids. Mechanical hemostats include relatively inert compounds such as collagen gelatins (Surgifoam) and cellulose (Surgicel, Johnson & Johnson Wound Management, Somerville, NJ), which serve as a lattice for clot formation. To accelerate this process, exogenous thrombin has been added by surgeons to these "carrier" products. The effectiveness of this combination therapy has led to the increased use of topical thrombins derived from either bovine or human plasma sources.

Human pooled plasma-derived thrombin, a product collected from up to 60,000 plasma donors per manufacturing lot, is now commercially available as a stand-alone product and used in combination with hemostatic and fibrin sealant products. The main risks associated with human plasma-derived thrombin include possible transmission of blood-borne pathogens such as retrovirus, viral hepatitis, and prions.<sup>11</sup> Concerns regarding the transmission of blood-borne pathogens by human pooled plasma-derived thrombin have been largely reduced but not eliminated by improved processing. These improvements led to approval as a fibrin/hemostatic component.<sup>2</sup>

Bovine plasma-derived thrombin (Thrombin-JMI) is one of the most commonly used topical surgical hemostats in the United States market. Though used in over 1 million surgical cases per year, with an average of 10,000 IU per procedure, there is growing recognition that antibodies to bovine thrombin and bovine factor V can develop from the innate human immune response to the  $\alpha$ -Gal glycosylation moiety unique to lower mammalian species as well as other antigenic impurities in the product.<sup>1</sup> These antibodies can, by cross-reacting with endogenous human coagulation factors and acting as acquired inhibitors of native human thrombin and factor V, cause a variety of complications ranging from laboratory abnormalities to potentially life-threatening bleeding or thrombotic complications. By 1996, clinical reports of immune-mediated events prompted the FDA to impose a black box warning on all bovine thrombin products, warning specifically against re-exposure to bovine thrombin. Since that specific warning, numerous cases of antibody-related complications from diverse surgical settings continue to be reported, suggesting that patients and their doctors have little awareness regarding the potential risks associated with exposure to xenogeneic thrombin.<sup>12,18-29</sup> Further, clinicians cannot easily elucidate the potential relationship between anti-bovine thrombin antibodies and bleeding events due to the lack of a commercially available antibody assay.

The potential and documented adverse consequences associated with bovine and human plasma-derived thrombin strongly influenced the development of rThrombin.<sup>19-22,30,31</sup> Preclinical trials demonstrated topical rThrombin to be safe in animals.<sup>32</sup> A phase 2 trial demonstrated that rThrombin was well tolerated and minimally immunogenic and enabled the pivotal phase 3 trial

comparing the efficacy, safety, and antigenicity of rThrombin versus bThrombin. This study, in 411 patients in four surgical settings (PAB, AV graft formation, liver, and spine), was recently completed and published.<sup>12</sup> Recombinant thrombin was found to be safe and as efficacious as bThrombin. Antibodies developed postexposure in 43 of 200 patients (21.5%) exposed to bThrombin, whereas non-neutralizing antibodies to rThrombin product were detected in 3 of 198 patients (1.5%;  $P < .0001$ ) receiving rThrombin.

This report concerns the subgroup of vascular patients in the phase 3 trial. The use of topical thrombin in vascular patients differs from its use in spine and liver patients in that thrombin is used in vascular surgery as an ancillary for hemostasis at small, high pressure anastomotic arterial bleeding sites. Furthermore, patients undergoing reconstructive vascular procedures differ from other surgical populations due to the presence of reoperative scar, vasculopathy, and use of concomitant anticoagulants/antiplatelet agents. Presumably as a result of these factors alone and in combination, a numerical difference was observed in the incidence of hemostasis at 10 minutes for spine, liver, and AV procedures compared with PAB procedures. The potential statistical significance of this difference was not evaluated. Of the sites that failed to achieve hemostasis at 10 minutes, over two-thirds were PAB procedure sites.<sup>12</sup> It was this numerical trend that stimulated additional subgroup analyses in order to further examine the time to hemostasis in vascular patients.

In further analyses, the incidence of hemostasis at the time points of 1.5, 3, 6, and 10 minutes was numerically superior for the AV procedures compared with PAB procedures at all time points. The most likely explanation for this differential is the greater use of coagulation-impeding medications in the PAB group. The incidence of hemostasis in PAB procedures at 3 minutes was significantly higher with rThrombin than with bThrombin when analyzed by procedure and treatment group. At 6 minutes, this difference in hemostasis narrowed but still numerically favored rThrombin. At 10 minutes, the two groups were numerically equivalent. The reasons for this observation are not clear. This effect may not be clinically evident in surgical settings where the coagulation system is normal and not pharmacologically compromised, but in vascular reconstructive procedures this is rarely the case. Whether these differences in topical thrombin performance exist as chance observations or are the result of differences in biochemical properties cannot be determined by this study. Clarification awaits future studies of rThrombin application in patients undergoing reconstructive vascular procedures.

Although much has been documented regarding safety concerns associated with bThrombin, no obvious differences in safety were seen in vascular patients treated with bThrombin compared with those in the rThrombin treatment group. Incidence of graft occlusion and hemorrhagic, thromboembolic, hypersensitivity, and cardiac events was similar between groups and not dissimilar from that expected in this patient population. Laboratory abnormali-

ties, and in particular changes in coagulation parameters, were comparable between treatment groups.

Baseline antibodies to study drug were observed in both treatment groups. This finding is not uncommon among recombinant biotherapeutics. For example, evidence of preexisting antibodies has been observed for recombinant human interferons and interleukins.<sup>33-36</sup> The presence of anti-bThrombin product antibodies in this population is presumably related to prior exposure to bovine thrombin during previous surgical procedures. The nature and significance of this finding is unknown.

When patients with anti-bThrombin product antibodies postexposure were compared with those without antibodies, a numerically increased incidence of hypersensitivity reactions and elevation in aPTT was observed. Because antibodies formed in response to bThrombin exposure can be pathogenic, it was both timely and appropriate to compare the adverse clinical outcomes in patients who did and did not form antibodies to the bThrombin product. Not surprisingly, the small number of events and sample size of the current study preclude an unambiguous interpretation of this potential relationship.

One final noteworthy observation in this study is the prevalence of bThrombin antibodies in the vascular patient population compared with the nonvascular cohort of the overall study (spine and liver). Patients undergoing PAB and AV graft procedures had a baseline incidence of antibodies to bThrombin product of 7.4%, nearly twice that of measured antibodies to the rThrombin product. In contrast, the incidence of antibodies to bThrombin product in the spine and liver cohorts at baseline in the overall study was only 3.4% (unpublished data), less than half that in the vascular group. Unlike patients undergoing spine and liver surgery, patients with peripheral arterial disease and in need of AV vascular access frequently undergo multiple and redo vascular procedures where repeat potential bThrombin exposure is common. This unique aspect of the vascular surgery patient population is important, since following exposure to bThrombin, 27% of patients either seroconverted or increased their titer of antibodies to the bThrombin product. This translates to approximately one in four patients developing antibodies to bThrombin postoperatively, placing them at risk for known adverse events due to bThrombin-induced antibodies. This is consistent with previous reports of the immunogenicity of bThrombin in other surgical populations.<sup>27,37,38</sup>

There are certain caveats to the above observations. Since this study was not designed to detect specific statistical differences in the vascular subgroup of patients, statistical findings should be interpreted cautiously. It is certainly possible that the increased incidence of hemostasis at 3 minutes in the PAB group treated with rThrombin was observed by chance alone. Likewise, the potential correlation of the hypersensitivity adverse events and elevation in aPTT with anti-bThrombin product antibodies is an interesting observation, but a larger study specifically addressing this finding will be required to confirm or refute this observation.

This detailed analysis comparing bThrombin and rThrombin treatment in a subgroup of patients undergoing PAB and AV graft surgery provides a number of observations and raises interesting questions. First, rThrombin resulted in comparable hemostatic efficacy relative to bThrombin in vascular surgery patients, and demonstrated an increased incidence of hemostasis at 3 minutes in patients undergoing PAB surgery; whether this is a reproducible or clinically meaningful finding remains to be determined in additional studies. Clearly, both thrombins have significant activity in the first minutes after application in a surgical setting marked by anticoagulation and pathologic platelet dysfunction. Second, the rThrombin safety profile, as characterized by adverse events and laboratory evaluations, is similar to that of bThrombin, although certain adverse events were numerically more common in patients with anti-bThrombin product antibodies. Further, rThrombin does not evoke a significant immunogenic response, in contrast to bThrombin. Although there are numerous published case reports in the medical literature, clinicians may benefit from a well-controlled trial designed to detect and characterize the spectrum of adverse consequences to bThrombin product exposure.

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Conception and design: FW  
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 Statistical analysis: AA  
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