

# Topical thrombin preparations and their use in cardiac surgery

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**Abstract:** Coagulopathic bleeding may lead to increased morbidity and mortality after cardiac surgery. Topical bovine thrombin has been used to promote hemostasis after surgical procedures for over 60 years and is used frequently as a topical hemostatic agent in cardiac surgery. Recently, use of bovine thrombin has been reported to be associated with increased risk for anaphylaxis, thrombosis, and immune-mediated coagulopathy thought secondary to the production of antifactor V and antithrombin antibodies. In patients who develop bovine thrombin-induced immune-mediated coagulopathy, clinical manifestations may range from asymptomatic alterations in coagulation tests to severe hemorrhage and death. Patients undergoing cardiac surgical procedures may be at increased risk for development of antibodies to bovine thrombin products and associated complications. This adverse immunologic profile has led to the development of alternative preparations including a human and a recombinant thrombin which have been shown to be equally efficacious to bovine thrombin and have reduced antigenicity. However, the potential benefit associated with reduced antigenicity is not truly known secondary to the lack of long-term experience with these products. Given the potentially higher margin of safety and less stringent storage concerns compared to human thrombin, recombinant thrombin may be the most reasonable approach in cardiac surgery.

**Keywords:** bovine thrombin, human thrombin, recombinant thrombin, immune-mediated coagulopathy, topical hemostatic agents, thrombin

## Introduction

Coagulopathy resulting in excessive blood loss following cardiovascular surgery is a significant problem.<sup>1-4</sup> The occurrence of abnormal or excessive blood loss after cardiovascular procedures has been reported in 3% to 14% of cases.<sup>1,5,6</sup> Surgical re-exploration to identify the cause of bleeding following these procedures may be required in as many as 5% of patients.<sup>1-4</sup> Measures to control postoperative hemorrhage in cardiovascular surgery patients include transfusion of blood products and administration of various pharmacologic agents.<sup>7</sup> Both re-exploration and excessive blood product replacement can lead to serious complications including an increased infection risk, multiorgan failure, increased hospital and intensive care unit length of stay, and increased short and long-term mortality.<sup>8-13</sup> Additionally, blood transfusions may increase circulating inflammatory mediators, leading to capillary leak and tissue edema both of which may hinder or prevent immediate sternotomy closure.<sup>14,15</sup>

Topical hemostatic agents are one modality that can be employed for the control of surgical bleeding.<sup>16-22</sup> Bovine thrombin has been used as a topical hemostat for more than 60 years.<sup>17</sup> In the United States (US), it has been used in more than 1 million patient

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cases per year contributing to a significant cost estimated at US\$250 million annually.<sup>17</sup> Despite its clinical success and usefulness in surgical procedures, bovine thrombin has been associated with an increased risk of anaphylaxis, thrombosis and immune-mediated coagulopathy (IMC).<sup>23–63</sup> Such adverse effects are thought to be secondary to the production of anti-factor V and antithrombin antibodies.<sup>23–63</sup> In part because of these concerns, the US Food and Drug Administration (FDA) issued a boxed warning about these potential complications. Product labeling for bovine thrombin cautions health care professionals about the potential for severe coagulopathy and warns against re-exposure in patients with antibodies to bovine thrombin preparations.<sup>64</sup>

The adverse immunologic effects associated with bovine thrombin have led to the development of alternative preparations. Such products include human thrombin derived from human plasma and recombinant thrombin.<sup>65,66</sup> In recent trials, these products have been shown to be equally efficacious and are associated with a reduced antigenicity compared to bovine thrombin.<sup>67–70</sup> This review will attempt to put in perspective the role of topical thrombin preparations as they relate to cardiovascular surgery.

## Mechanism of action

Achieving hemostasis after vascular injury requires coordination of multiple complex pathways ultimately resulting in local vasoconstriction, platelet activation and adherence, and platelet plug formation. Additionally, the activation of the clotting cascade results in fibrin formation and cross-linking to stabilize the platelet plug.<sup>16,71–73</sup> Tissue factor expressed at the site of injury binds to factor VIIa, forming a complex which subsequently activates factor IX and factor X on the intrinsic coagulation pathway.<sup>16,71</sup> Activated factor X, catalyzed by activated factor V, subsequently converts prothrombin to thrombin.<sup>16,71</sup> Thrombin generation is also amplified by platelet mediated activation and activation of factors V, VIII and XI.<sup>16,71</sup>

Activation of thrombin is central to the final step of the coagulation cascade. Thrombin catalyzes the conversion of fibrinogen to fibrin, the induction of cross-linking factor XIII activation and the enhancement of platelet aggregation.<sup>16,17,73</sup> In addition to its direct effects on the coagulation system, thrombin causes vasoconstriction (which promotes hemostasis) and possesses other inflammatory actions important in the repair of damaged tissue.<sup>17</sup> Thrombin is rapidly neutralized by naturally circulating plasma inhibitors which limit its duration of action and maintains a local effect, thus tightly regulating the coagulation response.<sup>69</sup> Its ability to bypass initial steps of the coagulation pathway and potential to achieve a local

hemostatic effect has made thrombin attractive as a topical hemostatic agent.<sup>69</sup> Over the years, bovine thrombin has been used in multiple types of surgical procedures including cardiac, thoracic, vascular, neurologic, orthopedic, general, gynecologic, head, neck and dental surgeries.<sup>17</sup>

## Bovine thrombin

Bovine thrombin was first used in surgery in the early 1940s as a topical hemostatic agent.<sup>16,17</sup> Since that time, several formulations of bovine thrombin have been marketed. Thrombostat® (Parke-Davis, Morris Plains, New Jersey, USA) was approved in 1943 as the first topical thrombin preparation for use as a surgical hemostatic agent.<sup>16</sup> Interest in and usage of bovine thrombin for surgical hemostasis intensified following FDA approval of topical thrombin in the 1970s.<sup>17</sup> The manufacturing process of bovine thrombin continued to be revised and subsequently Thrombinar® (Armour Pharmaceutical Co, Kankakee, Illinois, USA) and Thrombogen® (Ethicon, Inc., a Johnson & Johnson company, Somerville, New Jersey, USA) were approved for use by the FDA in 1982 and 1986, respectively.<sup>16</sup> Approved for use in 1995, Thrombin-JMI® (King Pharmaceuticals, Inc., Bristol, Tennessee, USA) is the only nonhuman thrombin in use today.<sup>64</sup>

The original thrombin products, due to a less than perfect purification process, contained other bovine proteins possibly responsible for the development of cross-reacting antibodies to human coagulation factors such as factor V.<sup>16,32</sup> For example, Thrombostat® and Thrombogen® contain only 20% to 30% thrombin, the remainder being comprised of other protein contaminants.<sup>16,74</sup> Thrombin-JMI® contained 81% to 96% thrombin, reflecting significantly fewer impurities.<sup>16,47,74,75</sup> In 2008, Thrombin-JMI® underwent a change in manufacturing and was the first approved bovine thrombin to be chromatographically purified, with a factor V concentration less than 92 ng/mL, differentiating it from other topical bovine products.<sup>64,76</sup> Although the extra purification step increases the overall specific activity of Thrombin-JMI® and dramatically reduces its factor V content, the clinical significance of this purification step is unknown as the product may still contain protein contaminants that may stimulate the immune system.<sup>74,75</sup>

Over the last 20 years, published reports have begun to highlight significant side effects associated with the use of bovine thrombin. These include the development of IMC, anaphylaxis, and thrombosis which are primarily associated with the production of antibodies to the bovine product. Immune-mediated coagulopathies are caused by autoimmune reaction or cross-reacting antibodies against

elements of the coagulation cascade or thrombotic system (eg, heparin-induced thrombocytopenia, von Willebrand disease, hemophilia A) resulting in either a hypercoagulable state and potential for thrombosis or coagulopathy with hemorrhage.<sup>77</sup>

Recently, the role of bovine thrombin in the development of IMC has been extensively reviewed.<sup>16–20,50,53,77–80</sup> The use of bovine thrombin preparations may result in the development of cross-reacting antibodies to both endogenous human coagulation factors, and as mentioned above, other bovine proteins in the preparation including factor V.<sup>16,17,53,75</sup> Bovine products contain a naturally occurring carbohydrate structure (galactose  $\alpha$ -1,3 galactose,  $\alpha$ -Gal) unique to lower mammalian species.<sup>17,69,81</sup> Humans possess naturally occurring antibodies to  $\alpha$ -Gal which can mount a significant innate immune response to bovine thrombin and bovine factor V as well as to other impurities in these products.<sup>17,69,81</sup> Bovine coagulation proteins are 75% homologous to human coagulation proteins.<sup>81</sup> These structural similarities may result in antibody development that may cross-react with human coagulation factors primarily human factor V and thrombin.<sup>16,17,33,46,53,77,81</sup> In addition, patients may also paradoxically develop human coagulation protein antibodies.<sup>17,32,35,39,41,50,74</sup>

Antibodies to thrombin or factor V may act as inhibitors that bind to their respective targets and either neutralize thrombin or factor V, eliciting a procoagulant effect and/or promoting their clearance from the circulation.<sup>39,53</sup> Antibodies that inactivate thrombin may decrease generation of fibrin and decrease platelet activation.<sup>72,73</sup> Factor V is particularly important in that it is situated in the clotting cascade at the convergence of the intrinsic and extrinsic pathway, and as such, any inhibitory effect would have profound effect on the generation of thrombin.<sup>35,56–62,72,73</sup>

Antibody development in response to bovine thrombin exposure is reported to occur in 10% to 95% of patients.<sup>17,26,46,50,53,54,67,68,74,77</sup> Ortel and colleagues reported on 151 patients undergoing coronary artery bypass surgery or valve replacement who were exposed to bovine thrombin (Thrombogen<sup>®</sup>) and found that over 90% of patients developed antibodies to topical thrombin.<sup>50</sup> In addition, in the 105 patients who had normal antibody levels at baseline, 95% had developed antibodies after surgery.<sup>50</sup> Of interest is the fact that most patients developed antibodies to bovine factor V and factor Va (80.7% and 90.7%, respectively) most likely due to the high levels of contaminating proteins.<sup>50</sup> Antibody production to bovine thrombin occurred in 20.5% of patients.<sup>50</sup> Also of concern was that 51% of patients had elevated antibody levels to human coagulation proteins, predominately against

factor V, thrombin, or both.<sup>50</sup> Sands and colleagues reported on 88 hemodialysis patients who had been exposed to bovine thrombin and found that antibody production occurred in 30.7% of patients.<sup>26</sup> Dorion and colleagues reported an overall prevalence of bovine thrombin antibodies in patients exposed to bovine thrombin of 10%.<sup>46</sup> More recent studies comparing purer forms of bovine thrombin (Thrombin-JMI<sup>®</sup>) to human thrombin or recombinant thrombin found an incidence of antibody production to bovine thrombin to be 12.7% to 27% suggesting that the incidence is on the lower end of the range.<sup>67–69</sup> Factors affecting variability seen in antibody development may include thrombin purity, type of surgery, assay method and history of previous exposure.<sup>17</sup>

Antibodies to topical thrombin may be detected as early as 1 to 2 days and peak at 4 to 8 weeks following exposure.<sup>50,61</sup> Streiff and colleagues reported that bovine thrombin-associated inhibitors to factor V emerge a mean of 8.3 days after exposure and persist for 2.3 months.<sup>53</sup> Patients who are re-exposed to bovine thrombin may be at risk for development of antibodies to bovine thrombin and other coagulation factors.<sup>16,46</sup> Dorion and colleagues demonstrated an 8-fold increased risk in the prevalence of bovine thrombin antibodies in patients with multiple exposures compared with patients with single exposures (39% versus 5%).<sup>46</sup> In addition, Ortel and colleagues demonstrated that in patients who had elevated antibody levels to 2 or more bovine proteins prior to surgery, re-exposure posed a 5-fold greater risk for adverse events postoperatively (adjusted odds ratio: 5.40; 95% confidence interval: 1.54 to 18.8).<sup>50</sup> Also of note is that, in contrast to other immune-mediated coagulopathic responses (eg, heparin-induced thrombocytopenia), these antibodies may persist years following initial exposure and may result in significant coagulopathy with re-exposure.<sup>16,35,41,50</sup>

Over 100 case reports have been published on adverse reactions or complications associated with exposure to bovine thrombin.<sup>18,23–63</sup> In these cases, the spectrum of disease ranges from asymptomatic alterations in laboratory coagulation tests (eg, prothrombin time [PT], activated partial thromboplastin time [aPTT], international normalized ratio [INR] and thrombin time [TT]) to severe bleeding events and death.<sup>16–18,29–63,77,78,82,83</sup> Bleeding events associated with IMC may even occur several months after exposure to bovine thrombin.<sup>35,56</sup>

Cases of IMC and bleeding events have been reported in a wide range of surgical cases including neurosurgery, obstetrics, and vascular surgery.<sup>33,36,47,49,51,52,55,56,63</sup> A significant number of adverse events have been reported in cardiac surgery patients following exposure to bovine thrombin (Table 1).<sup>29–35,37–39,41–48,50,53,54,58,59,61,62</sup> Patients undergoing

Table 1 Summary of published reports addressing immunogenicity and adverse events related to the use of bovine thrombin in cardiac surgery

References	Year	Study design	Patient population	Bovine thrombin preparation	Prior thrombin exposure	Coagulation parameters	Treatment	Outcomes
Flaherty <sup>29</sup>	1989	Case report: 91 yo F (CABG, AVR)	Adult	Not reported	Not reported	<b>Nonhemorrhagic</b> Elevated PT, aPTT (POD 7), TT (POD 16)	Vitamin K	Death from respiratory failure (POD 27); TT 300 sec
Zehnder <sup>30</sup>	1990	Case report: 17 yo M (tetralogy of Fallot) Case report: 65 yo M (2nd MVR)	Pediatric Adult	Not reported Thrombostat <sup>®</sup>	Bovine (source unknown) Not reported	<b>Nonhemorrhagic</b> Prolonged TT; Antibovine thrombin antibodies <b>Hemorrhagic</b> Elevated PT, aPTT, TT (POD 7); Reduced factor V activity (1%); Antibovine thrombin antibodies; Bovine and human factor V inhibitor	None FFP, vitamin K, IVIG, prednisone, cyclophosphamide, vincristine, epsilon-aminocaproic acid, plasmapheresis	Not reported Recurrent, severe bleeding episodes; Hemothorax and soft tissue hemorrhage; Resolved
Rapaport <sup>32</sup>	1992	Case report: 59 yo F (2nd AVR/MVR)	Adult	Not reported	Not reported	<b>Hemorrhagic</b> Elevated PT and aPTT; Antibodies to bovine and human thrombin; Factor V inhibitor	PRBC, FFP, platelets, vitamin K	Nasopharyngeal bleeding; Resolved
Bänninger <sup>33</sup>	1993	Case report: 34 yo F (partial ascending aortic graft, implantation of composite-graft)	Adult	Tissucol <sup>®</sup> fibrin glue	Tissucol <sup>®</sup> fibrin glue	<b>Nonhemorrhagic</b> Elevated TT; Antibovine thrombin antibodies; Factor V inhibitor	None	Resolved
Berruyer <sup>34</sup>	1993	Case report: 59 yo M (reoperation for aneurysm of aortic arch and ascending aorta) Case report: 38 yo M (6th AVR, CABG) – Reoperation for tamponade (POD 2) – Heart transplant (POD 3) Case report: 44 yo F (TVR)	Adult Adult	Tissucol <sup>®</sup> fibrin glue Tissucol <sup>®</sup> fibrin glue	Tissucol <sup>®</sup> fibrin glue Tissucol <sup>®</sup> fibrin glue	<b>Nonhemorrhagic</b> Elevated aPTT, PT, TT; Reduced factor V activity 12% (POD 7); Antibovine thrombin antibodies <b>Nonhemorrhagic</b> Elevated aPTT, PT, TT; Reduced factor V activity 49% (POD 8); Antibovine thrombin antibodies	FFP, plasmapheresis Plasmapheresis	Resolved Resolved Death from right ventricular failure with bilateral pneumonia and sepsis (POD 16)
			Adult	Tissucol <sup>®</sup> fibrin glue	Not reported	<b>Hemorrhagic</b> Elevated aPTT, PT, TT; Reduced factor V activity 39% (POD 8); Antibovine thrombin antibodies	Plasmapheresis	Melena (POD 8); Thrombosis on TV (POD 12); Death from cardiogenic shock (POD 14)

Cmolik <sup>35</sup>	1993	N = 9 (5 male, 4 female) 2nd Cardiovascular operation	Adult	Thrombostat <sup>®</sup> or Thrombinar <sup>®</sup>	Not reported	<p><b>Nonhemorrhagic (N = 5)</b> Elevated PT; Reduced factor V and XI activity; Factor V inhibitor</p> <p><b>Hemorrhagic (N = 4)</b> Coagulopathic bleeding</p>	Vitamin K, FFP, platelets (N = 9); IVIG (N = 3)	Upper GI bleed, resolved (N = 1); Hemothorax and respiratory insufficiency, resolved (N = 1); Wound hematoma, bleeding from tracheostomy site, resolved (N = 1); Severe epistaxis, large hematoma, death (N = 1) Resolved
Israels <sup>37</sup>	1994	Case report: 3.5 yo F (corrective cardiac surgery)	Pediatric	Not reported	Tisseal <sup>®</sup> fibrin glue	<p><b>Nonhemorrhagic</b> Elevated PT, aPTT; Antibovine thrombin antibodies; Bovine and human factor V inhibitors</p>	None	Resolved
Muntean <sup>38</sup>	1994	Case report: 10 mo M (MVR)	Pediatric	Fibrin sealant	Tisseal <sup>®</sup> fibrin sealant	<p><b>Hemorrhagic</b> Elevated PT, aPTT; Bovine and human factor V inhibitors</p> <p><b>Nonhemorrhagic</b> Elevated PT, aPTT; Antibovine thrombin antibodies; Bovine and human factor V inhibitors</p>	PRBC, FFP, vitamin K	GI hemorrhage; Resolved
Ortel <sup>39</sup>	1994	Case report: 27 mo M (modified Blalock-Taussig shunt)	Pediatric	Not reported	Not reported	<p><b>Hemorrhagic</b> Elevated PT, aPTT; Reduced factor V activity (3.2%); Factor V inhibitor</p> <p><b>Hemorrhagic</b> Elevated PT, aPTT; Reduced factor V activity (2%); Antibovine and antihuman thrombin antibodies; Human factor V inhibitor</p> <p><b>Hemorrhagic</b> Elevated PT, aPTT; Reduced factor V activity (17%)</p>	FFP, vitamin K, IVIG, platelets	Resolved Bleeding duodenal ulcer; Fatal nonhemorrhagic CVA
		Case report: 13 yo M (multiple surgical procedures for tetralogy of Fallot)	Pediatric	Not reported	Not reported		Methylprednisolone	Minimal oozing from poorly healing mediastinal wound; Resolved
		Case report: 62 yo M (CABG)	Adult	Not reported	Not reported		FFP, vitamin K, steroids, aminocaproic acid, platelets, cryoprecipitate, PRBC, multiple secondary surgical procedures	Prolonged oozing from tracheostomy site; Resolved

(Continued)

Table 1 (Continued)

References	Year	Study design	Patient population	Bovine thrombin preparation	Prior thrombin exposure	Coagulation parameters	Treatment	Outcomes					
Christie <sup>42</sup>	1997	Case report: 78 yo M (CABG)	Adult	Thrombostat <sup>®</sup>	Not reported	<b>Hemorrhagic</b> Elevated PT, aPTT; Factor V deficiency	Steroids	Excessive oozing from lines; Resolved					
Israels <sup>43</sup>	1997	Case report: 4 yo F (extracardiac Fontan procedure)	Pediatric	Thrombostat <sup>®</sup>	Tisseal <sup>®</sup> fibrin glue	<b>Nonhemorrhagic</b> Elevated PT, aPTT; Bovine and human factor V; Bovine factor X inhibitors	None	Resolved					
Muntean <sup>44</sup> 2 year follow-up to Muntean <sup>38</sup>	1997	Case report: 4 yo M (partial correction of cardiac malformation)	Pediatric	Tissucol <sup>®</sup> fibrin sealant	Tissucol <sup>®</sup> fibrin sealant	<b>Hemorrhagic</b> Bovine and human factor V inhibitors	IVIG, rFVIIa	Pulmonary hemorrhage; Resolved					
Tarantino <sup>45</sup>	1997	Case report: 9 yo F (replacement of stenotic RV to PA conduit)	Pediatric	Not reported	Not reported	<b>Hemorrhagic</b> Elevated PT, aPTT; Antibo- vine thrombin antibodies; Bovine and human factor V inhibitors	FFP; cryoprecipitate, platelets, IVIG	Cutaneous, GI and pulmonary hemorrhage; Resolved					
Dorion <sup>46</sup>	1998	Case report: 61 yo M (2nd MVR)	Adult	Not reported	Bovine (source unknown)	<b>Hemorrhagic</b> Elevated PT, aPTT, TT (POD 8); Antibo- vine thrombin antibodies; Reduced factor V activity (4%)	FFP	Intracerebral bleed (POD 26); Resolved					
Fastenau <sup>27</sup>	1998	Case report: 69 yo M (2nd CABG, AVR)	Adult	Not reported	Bovine (source unknown)	<b>Hemorrhagic</b> Elevated PT; Antibo- vine thrombin antibodies	None	Hematoma (POD 24); Resolved					
									Adult	Not reported	12 had antibodies to bovine thrombin (95% CI 4.6–15.4); Patients receiving multiple exposures; 8 × more likely to develop antibodies than those with single exposure (P < 0.001)	N/A	2 serious bleeds in exposed patients LOS 2 × as long for those with bovine antibodies (23d vs 11d, P < 0.007)
Zumberg <sup>48</sup>	2000	Case report: 66 yo M (AVR)	Adult	Not reported	Fibrin sealant	<b>Nonhemorrhagic</b> Elevated PT, INR; Reduced factor V activity (1.6%); Antibo- vine and antihuman thrombin antibodies; Human factor V inhibitor	FFP, vitamin K	Resolved					

Kajitani <sup>47</sup>	2000	Case report: 55 yo F (ascending aorta repair; CABG)	Adult	Thrombin-JM <sup>®</sup> topical spray, Tisseel <sup>®</sup> fibrin sealant	None	<p><b>Hemorrhagic</b> Elevated PT, INR, aPTT (POD 33); Reduced factor V activity (2%); Factor V inhibitor</p>	<p>PRBC, FFP, platelets, factor VIII, cryoprecipitate, steroids, plasmapheresis N/A</p>	<p>Severe life-threatening hemorrhage (POD 33); Resolved</p>
Ortel <sup>60</sup>	2001	N = 151 (109 CABG, 42 valve surgery)	Adult	Thrombogen <sup>®</sup>	Not reported	<p>80.7% and 90.7% developed antibodies against b-FV and FVa, respectively 50% developed antibodies to b-prothrombin, 20.5% to b-thrombin 51% developed elevated antibodies to human coagulation factors (factor V, thrombin or both) Abnormal coagulation parameters seen in 41 patients</p>	<p>N/A</p>	<p>27 hemorrhagic complications, 12 thromboembolic events, 21 wound complications, 5 deaths No increased risk for adverse events in patients with prior surgical history, increased thrombin exposure, presence of antibodies to bovine or human coagulation factors post-op or abnormal coagulation studies Significantly increased risk for adverse events in patients with elevated pre-op antibody levels to 2<sup>+</sup> bovine proteins (OR 5.4, 95% CI 1.54–18.8, P &lt; 0.01) Resolved</p>
Streff <sup>3</sup>	2002	Case report: 71 yo M (CABG) Case report: 68 yo M (CABG, AVR) Case report: 79 yo M (carotid endarterectomy)	Adult Adult Adult	Not reported Not reported Not reported	Not reported Not reported Not reported	<p><b>Nonhemorrhagic</b> Elevated PT, aPTT (POD 23); Factor V inhibitor <b>Nonhemorrhagic</b> Elevated PT; Reduced factor V activity (1%); Antithrombin antibodies; Factor V inhibitor <b>Nonhemorrhagic</b> Elevated PT, aPTT; Reduced factor V activity (2%); Factor V inhibitor</p>	<p>Vitamin K Vitamin K FFP, PRBC, steroids</p>	<p>Resolved Atrial thrombus (POD 76); Resolved Resolved</p>

(Continued)



Table 1 (Continued)

References	Year	Study design	Patient population	Bovine thrombin preparation	Prior thrombin exposure	Coagulation parameters	Treatment	Outcomes
Winterbottom <sup>54</sup>	2002	N = 309 (cardiac, vascular or spinal surgery)	Adult	Thrombin-JMI® (156 FloSeal®, 153 Gelfoam®)	Not reported	No significant correlation between presence or absence of detectable antibodies at baseline or 6–8 wks post-op  In those with pre-op antibodies compared to those without, % of blood-related complications was 32% compared to 26% (NS); those with post-op antibodies, % of blood-related complications 20% and 22% (NS)  Trend between blood-related complications and prior surgery (P = 0.08)	N/A	384 complications in 144 patients (75 blood related)
Su <sup>28</sup>	2002	N = 162 cardiovascular surgery patients	Adult	151 exposed to bovine thrombin (source unknown); 11 unexposed	Not reported	Elevated antibody levels not statistically significant related to risk of adverse events	N/A	12 patients experienced thromboembolic complications (6 stroke, 1 aneurysm fugax, 2 graft stenosis, 1 mesenteric thrombosis, 1 PE, 1 thrombophlebitis)  Severe epistaxis; Resolved
Kirkeby <sup>58</sup>	2005	Case report: 31 yo M (VAD)	Adult	Not reported	Not reported	<b>Hemorrhagic</b> Elevated aPTT, INR (POD 40); Reduced factor V activity (22%); Factor V inhibitor	Vitamin K, FFP, cryoprecipitate, rFVIIa	Resolved
Lawson <sup>59</sup>	2005	Case report: 74 yo M (CABG)	Adult	Thrombin-JMI®	Bovine (source unknown)	<b>Nonhemorrhagic</b> Elevated PT, aPTT, INR (POD 7); Elevated antithrombin factor V IgG and antihuman V IgG (POD 12); Reduced factor V activity (9.2%)	None	Resolved
Crow <sup>61</sup>	2007	Case report: 16 mo F (pulmonary angioplasty)	Pediatric	Not reported	Not reported	<b>Hemorrhagic</b> Reduced factor V activity (POD 16); Factor V inhibitor	IVIg, exchange transfusion	Excessive intraoperative bleeding; Resolved



Savage <sup>62</sup>	2007	Case report: 3 mo M (shunt replacement, atrial septectomy)	Pediatric	Not reported	Bovine (source unknown)	<b>Nonhemorrhagic</b> Elevated PT, aPTT; Petechial rash (POD 10); Reduced factor V activity (1%); Human factor V inhibitor	IVIG, steroids, FFP, vitamin K	Resolved
		Case report: 11 yo F (revision of Fontan)	Pediatric	Not reported	Not reported	<b>Nonhemorrhagic</b> Elevated PT, aPTT; Antibovine thrombin antibodies; Human factor V inhibitor	Vitamin K	Resolved

**Notes:** Table 1 addresses the summary of reported experiences related to the use of topical bovine thrombin in cardiac surgery patients. These experiences include a variety of reports addressing the incidence of antigenicity as well as the clinical spectrum of adverse events ranging from asymptomatic elevation in coagulation studies to life-threatening hemorrhage events.

**Abbreviations:** aPTT, activated partial thromboplastin time; AVR, aortic valve replacement; b-FV, bovine thrombin factor V; b-prothrombin, b-thrombin; bovine prothrombin; b-thrombin, bovine thrombin; CABG, coronary artery bypass graft; CI, confidence interval; CVA, cerebrovascular accident; d, days; F, female; FFP, fresh frozen plasma; FVa, factor Va; GI, gastrointestinal; INR, international normalized ratio; IVIG, intravenous immune globulin; LOS, length of stay; LVAD, left ventricular assist device; M, male; mo, month; MVR, mitral valve replacement; N/A, not applicable; NS, not statistically significant; OR, odds ratio; PA, pulmonary artery; PE, pulmonary embolism; POD, postoperative day; post-op, postoperative; PRBC, packed red blood cells; pre-op, pre-operative; PT, prothrombin time; rFVIIa, recombinant activated factor VII; RV, right ventricle; sec, seconds; TT, thrombin time; TV, tricuspid valve; TVR, tricuspid valve replacement; VAD, ventricular assist device; wks, weeks; x, times; yo, year-old.

cardiac surgical procedures seem to be at increased risk for the development of antibodies to bovine products and associated complications.<sup>16,33,53</sup> This may be due, in large part, to the frequency of use in this population and the potential for re-exposure.<sup>50,53</sup> Cases of IMC and adverse events have also been reported with pediatric patients.<sup>31,37,38,43,44,61,62</sup> This may be of considerable importance in pediatric cardiac surgery due to the high likelihood of re-exposure with re-operation in patients with complex congenital heart lesions.<sup>31,37,38,43,44,61,62</sup>

Recent reviews have highlighted the point that the true incidence of IMC and bleeding events in patients following exposure to bovine thrombin may not be well appreciated and may be underestimated.<sup>16,18,77</sup> The diagnosis of IMC is often under-recognized either secondary to its asymptomatic effect on laboratory parameters with no bleeding or bleeding that is mistakenly attributed to another cause or underreported in cases where the diagnosis is made.<sup>77</sup> Bleeding may occur months after exposure and the physician may not be aware of previous exposure.<sup>35,56</sup> Use of bovine thrombin is often not well documented in patient records. Thrombins are often used as a component in combination hemostatic agents like fibrin (eg, fibrin sealants) or with other substrates like absorbable gelatin sponges and granules, gauze sponges, collagen and cellulose preparations.<sup>19,22,69,72</sup> Documentation of use of these products in the medical record is often very difficult to verify making it problematic to document a history of exposure to bovine thrombin.<sup>18,63,78</sup> In addition, there are no commercially available tests to screen for the presence of antibodies to bovine thrombin further complicating this issue.<sup>17,18,77,78</sup> Attempts at estimating the incidence of bleeding with IMC have been addressed in a few studies.<sup>46,53</sup> Dorion and colleagues reported an overall incidence of severe bleeding in 1.7% of patients exposed to bovine thrombin.<sup>46</sup> Streiff and colleagues, in a review of 126 cases of factor V inhibitors of which 58 were bovine induced, reported that bleeding developed in 33% of patients who developed bovine thrombin inhibitors which was fatal in 6%.<sup>53</sup> Clinicians need to maintain a high level of suspicion for IMC especially in higher risk populations like cardiac, vascular, and neurosurgical populations where previous exposure to bovine thrombin may be high.<sup>53,63,80</sup>

The course of events surrounding the potential risk involved with the use of bovine thrombin led the FDA in 1996 to issue a boxed warning for bovine thrombin cautioning about possibility for development of potentially serious coagulopathies related to its use and that patients with antibodies to bovine thrombin should not be re-exposed.<sup>64</sup> Since that time, Thrombin-JMI<sup>®</sup> has modified its purification

process to further reduce factor V in the bovine product in an attempt to further reduce the antigenicity of the product, however, the potential advantages of this reduction remain unknown and the most recent package labeling continues to carry the warning.<sup>16,64,76</sup> Unlike the bovine thrombin product, human and recombinant thrombin products do not carry a boxed warning in their package labeling.<sup>65,66</sup> Whether reduced antigenicity of these products will translate into lower or negligible rates of IMC is not known at this time.<sup>16</sup> Recently published guidelines for perioperative blood transfusion and blood conservation in cardiac surgery developed by a combined task force of the Society of Thoracic Surgeons and The Society of Cardiovascular Anesthesiologists have recommended against the use of topical hemostatic agents that employ bovine thrombin for blood conservation in cardiac surgery procedures because of the potentially harmful immunologic reactions that may occur with this drug.<sup>84</sup>

Often, the initial indication for IMC secondary to bovine thrombin exposure is alteration in coagulation parameters (eg, PT, aPTT, INR, TT) in the presence or absence of bleeding.<sup>16,53,63,80</sup> A comparison of these coagulation parameters against values obtained prior to bovine thrombin exposure may indicate the presence of an acquired coagulopathy and may support the diagnosis of bovine thrombin induced IMC.<sup>62,63,80</sup> However, other causes of acquired coagulopathies, all which may be present in this population including therapeutic anticoagulation (eg, warfarin, heparin), hypothermia, acidosis, hemodilution, sepsis, disseminated intravascular coagulation, liver disease, and vitamin K deficiency need to be ruled out.<sup>21,80</sup> Correction of above abnormalities and administration of fresh frozen plasma (FFP) ± vitamin K should allow for correction for acquired coagulopathy.<sup>21,80</sup> However, coagulopathy may not respond to FFP in patients with bovine thrombin induced IMC secondary to increased titers of circulating antibodies.<sup>62,80</sup>

Once other causes of acquired coagulopathy are excluded, a plasma mixing study should be performed.<sup>62,80</sup> Clotting times should normalize or decrease to near normal levels in patients who have a factor deficiency. However, if a circulating inhibitor of coagulation is present, clotting times will not correct which may be indicative of antibodies to factor V or thrombin. In instances where an abnormal mixing study is found, testing for specific factor activity (eg, factor V) should be performed.<sup>62,80</sup> If possible, studies to identify cross-reacting antibodies to establish presence of antifactor V or antithrombin antibodies may be performed.<sup>62,80</sup> However, abilities to test for antibodies are limited by laboratory capability, expense and turn-around time.<sup>63,80</sup> Therefore,

a presumptive diagnosis may be made for bovine induced IMC in patients with a new onset unexplained coagulopathy, a history or suspicion of bovine thrombin exposure, and an abnormal mixing study.<sup>63,80</sup>

There is no standard treatment protocol established at this time for the treatment of IMC secondary to bovine thrombin inhibitors.<sup>39,53,80,83</sup> What information that does exist for the treatment of this disease is derived from various case reports and case series.<sup>39,53,80,83</sup> Treatment strategies have usually been driven based on severity of clinical manifestation.<sup>53,62,80</sup> Patients who present with abnormal coagulation test without bleeding require no treatment other than careful monitoring and follow-up with serial testing.<sup>53,61,62,80</sup> In most cases, coagulation parameters will normalize spontaneously over time. However, repeat exposure to bovine thrombin products should be avoided.<sup>39,53</sup> The indication and benefit of any invasive procedure needs to be carefully considered in these patients.<sup>53</sup>

In patients with mild to moderate bleeding events, corticosteroid therapy has been advocated to assist in reduction of antibody titers and to decrease recurrence.<sup>53,61,62</sup> Supportive transfusion with red blood cells may be used, if required.<sup>53</sup> Use of plasma components like FFP and cryoprecipitate, as expected, would not be effective alone secondary to the circulating inhibitors present causing inactivation of the clotting components.<sup>53,80</sup> Transfusion of platelets, however, may be useful to treat bleeding secondary to factor V within platelet granules potentially being less accessible to circulating inhibitors and therefore protected from antibody exposure.<sup>53,56,62,85</sup> In patients with severe bleeding episodes or in patients refractory to above measures, more aggressive immunosuppressive management strategies have been required. Strategies using cyclosporine, cytotoxic chemotherapeutic agents (eg, cyclophosphamide, azathioprine), intravenous immune globulin, and plasmapheresis have reported varying success rates.<sup>30,31,44,45,53,57,60–62,80,83</sup> Even with use of the above aggressive strategies, fatalities have been reported in a significant number of patients with bovine thrombin induced IMC.<sup>53,80</sup> Reported experiences using recombinant activated factor VII for severe bleeding in patients with bovine thrombin induced IMC have been unsuccessful.<sup>57,58</sup>

In addition to bleeding, use of bovine thrombin has also been associated with thromboembolic complications.<sup>26–28</sup> Fastenau and colleagues demonstrated that exposure to Thrombogen<sup>®</sup> appeared to stimulate the development of antiphospholipid antibodies in 6 of 7 patients following left ventricular assist device placement.<sup>27</sup> Sands and colleagues demonstrated that patients with polytetrafluoroethylene grafts

and elevated antibody levels to topical bovine thrombin had significantly more vascular access thrombosis.<sup>26</sup>

It is important to realize that although patients may have markedly prolonged clotting times, they are not necessarily auto-anticoagulated and may be at risk for thrombosis.<sup>39</sup> However, routine monitoring of oral anticoagulation may be prohibitive in the presence of certain inhibitors (eg, factor V). This has led to devastating hemorrhage in patients in whom anticoagulants were used; therefore, anticoagulation should be used only when life-threatening thrombosis is present.<sup>53</sup>

Exposure to bovine thrombin may sensitize patients and cause an IgE mediated anaphylaxis with repeat exposure.<sup>23–25,77</sup> Like bovine thrombin-induced IMC, this risk is compounded by the fact that previous exposure to bovine thrombin is often poorly documented and difficult to verify and there is no readily available assay to detect the presence of IgE antibodies to bovine thrombin.<sup>80</sup>

Administration of any biologic product from an animal or exogenous human source is associated with a risk for transmissible disease.<sup>16,77</sup> Therefore, a theoretical risk for the transmission of bovine viral pathogens and prions to patients receiving bovine thrombin exists.<sup>16,77</sup> Studies in experimental models demonstrate that solvent-detergent treatment and nanofiltration steps used in manufacturing dedicated to the clearance of any viral contamination were able to reduce titers to bovine viral and prion elements.<sup>16,77,86</sup> However, whether this completely eliminates risk is unknown.<sup>16,77,87</sup>

## Human thrombin

In August 2007, human thrombin (Evithrom®; Johnson & Johnson Wound Management, Somerville, New Jersey, USA) received FDA approval as the second topical thrombin product commercially available for use as a hemostatic agent.<sup>65</sup> The product is isolated from pooled, cryo-poor, human plasma obtained from licensed plasmapheresis centers in the United States.<sup>19,65,77</sup> Individual plasma units are tested for blood-borne diseases such as HIV type 1 and 2 antibodies, hepatitis B surface antigen, hepatitis A, B and C viruses, and parvovirus B19.<sup>19,20,65,77</sup> The collected plasma is subsequently processed through a series of separation and filtration steps. Once the chromatographic purification step is complete, the solution is incubated with calcium chloride in order to isolate and activate prothrombin to thrombin.<sup>19,65</sup> The solution then undergoes purification through a series of steps which include ultrafiltration, vapor heat treatment, solvent-detergent treatment, sterile filtration and freeze-drying.<sup>19,20,65</sup> The solvent-detergent treatment and nanofiltration are targeted steps for the inactivation and removal of viruses.<sup>19,65</sup>

Because the human thrombin product is made from human plasma, it may carry a risk of transmitting infectious agents, such as viruses, and theoretically, the Creutzfeldt-Jacob disease (CJD) agent.<sup>65,77,88</sup> The risk of transmitting an infectious agent has been reduced by screening plasma donors for prior exposure to certain viruses, by testing for the presence of certain current virus infections, and by inactivating and removing certain viruses. Despite these measures, such products can still potentially transmit disease. There is also the possibility that unknown infectious agents may be present in such products, as no procedure has been shown to be completely effective in removing viral infectivity from derivatives of human plasma.<sup>19</sup>

Human thrombin has been shown to be as effective as bovine thrombin in a phase 3, prospective, randomized, controlled, double-blind study of 305 patients at 22 centers in the United States undergoing cardiovascular, neurologic or general surgical procedures (Table 2).<sup>67</sup> Patients with known antibodies to bovine thrombin were excluded from the study. Treatments were applied with an absorbable gelatin sponge (Surgifoam®; Johnson & Johnson, Somerville, New Jersey, USA). Patients with oozing or bleeding of mild intensity that could not be controlled by other surgical techniques, were randomized to either Thrombin-JMI® and Surgifoam® (n = 152) or Evithrom® and Surgifoam® (n = 153). Treatment with human thrombin was as successful as treatment with bovine thrombin in achieving hemostasis within ten minutes of product application. The secondary efficacy endpoint of hemostasis within 6 and 3 minutes of product application was also achieved. Occurrence of adverse events was not statistically different between the two groups. In the Evithrom® group, at least 1 serious adverse event was reported for 26/153 (17%) subjects compared with 17/152 (11%) subjects treated with bovine thrombin. Pruritus and procedural complications were more commonly seen with the human-derived thrombin product. None of the adverse events were considered causally related to thrombin administration.<sup>65,67</sup>

Overall, human thrombin is generally accepted as relatively safe; however, there is no assurance that the product is completely free of blood-borne pathogens. The viral reduction process used in the manufacturing of the product has significantly reduced the risk of transmission of viral pathogens, but not eliminated it.<sup>19,20</sup> The potential to transmit infections from infected plasma donors should still be considered.<sup>20</sup> A recent study estimated that plasma-derived human thrombin carries an infective risk for parvovirus of  $7 \times 10^{-8}$  to  $2 \times 10^{-10}$  per vial. The risk of hepatitis has the highest margin of safety reported at approximately  $8 \times 10^{-22}$  per

**Table 2** Summary of trials with human and recombinant thrombin

References	Year	Study design	Patient population	Thrombin regimen	Endpoints	Results/adverse events
Doria <sup>67</sup>	2008	R, DB, AC, MC Phase III study Ethicon (sponsor)	N = 305 Patients undergoing cardiovascular, neurologic and general surgeries ≥ 1 bleeding site (mild to moderate)	Human (n = 153) vs bovine (Thrombin-JMI <sup>®</sup> , n = 152) thrombin Applied topically to bleeding site in combination with absorbable gelatin sponge	Primary: cessation of bleeding at 10 min Secondary: cessation of bleeding at 3 and 6 min, safety, antibody seroconversion	For all procedures, 97.4% of patients in each group achieved hemostasis within 10 min of application (RR = 1) No difference in hemostasis seen between thrombin products for surgery type or for assessments at 3 and 6 min Similar ADEs between products; 12% experienced ≥ 1 ADE possibly related to treatment Antibody seroconversion seen in 3.3% of patients given human thrombin and 12.7% of patients given bovine thrombin (P = 0.01)
Chapman <sup>91</sup>	2006	R, DB, PC, MC Phase II study ZymoGenetics (sponsor)	N = 130 AV graft placement for hemodialysis (25%), major hepatic resection (22%), PAB surgery (21%) or spinal surgery (32%)	rThrombin (n = 88) vs placebo (n = 42) Applied topically to bleeding site in combination with absorbable gelatin sponge Open-label rThrombin permitted if hemostasis not achieved in 10 min	Primary: safety Secondary: incidence of hemostasis within 10 min Study not powered to assess efficacy	Incidence of ADEs similar between groups (<5% related to treatment); rThrombin had higher incidence of nausea (45% vs 26%), constipation (27% vs 12%), insomnia (19% vs 5%), and vomiting (13% vs 2%) compared with placebo Incidence of hemostasis within 10 min: HR 1.3 for rThrombin vs placebo (no statistical analysis) % of bleeding sites requiring rescue therapy: rThrombin 10% (9/93), placebo 20% (18/90)
Chapman <sup>68</sup>	2007	R, DB, MC Phase III study ZymoGenetics (sponsor)	N = 411 Vascular procedures [PAB surgery or AV graft placement for dialysis] (40%), spinal surgery (30%) or hepatic resection (30%)	rThrombin (n = 205) vs bovine (Thrombin-JMI <sup>®</sup> , n = 206) thrombin Applied topically to bleeding site in combination with absorbable gelatin sponge	Primary: incidence of hemostasis within 10 min Secondary: safety and presence of antibodies to thrombin	Open-label rThrombin was as effective as rescue therapy for 95% (19/20) of bleeding sites Antibodies to rThrombin developed in 1 patient in each treatment group during trial Incidence of hemostasis within 10 min: rThrombin 95.4%, bovine thrombin 95.1% (Lower limit of 95% CI for absolute difference, -3.73%, excluding the pre-specified value of -15%) Nearly all patients experienced an ADEs within 1 month of procedure; rates of possible treatment-related ADEs were similar in 2 groups Antibodies (seroconversion or ≥ 1 unit increase in titer) were present in 21.5% of patients given bovine thrombin and 1.5% of those given rThrombin (P < 0.0001)

Weaver <sup>69</sup>	2008	R, DB, MC Subgroup of phase III study (Chapman <sup>68</sup> ) ZymoGenetics (sponsor)	N = 164 Vascular cohort (PAB and AV graft procedures)	rThrombin (n = 82) vs bovine (Thrombin-JMI) <sup>®</sup> , n = 82) thrombin Applied topically to bleeding site in combination with absorbable gelatin sponge	Primary: incidence of hemostasis within 10 min Secondary: incidence of hemostasis at 1.5, 3 and 6 min, safety, presence of antibodies to thrombin	Incidence of hemostasis within 10 min: rThrombin 91%, bovine thrombin 94%; % achieving hemostasis at 3 min was higher in rThrombin vs bovine group undergoing PAB surgery (P = 0.046) No significant differences between rThrombin and bovine thrombin were observed for ADEs, perioperative transfusions, peri- and postoperative bleeding, laboratory values and thromboembolic events Antibodies (seroconversion or $\geq 1$ unit increase in titer) were present in 27% of patients given bovine thrombin and 0% of those given rThrombin (P < 0.0001) 15.6% patients seropositive and 84.4% seronegative for pre-existing anti-bovine thrombin antibodies; 2% patients had pre-existing anti-rThrombin antibodies No anti-rThrombin antibodies developed on day 29 (seroconversion or $\geq 10$ -fold increase in titer); no difference between seropositive or seronegative groups Incidence of ADEs and laboratory values similar between groups; 4 patients experienced possible treatment-related ADEs (erythema, incision-site erythema, pruritus, PE) Blood products used by 22% patients; 28% seropositive, 21% seronegative LOS: 5.5 days seropositive, 3.0 days seronegative
Singla <sup>70</sup>	2009	OL, SG, MC Phase IIIb immunogenicity and safety study ZymoGenetics (sponsor)	N = 206 Spinal (43%), arterial reconstruction (36%), AV vascular access (22%) surgical procedures	rThrombin applied topically during surgical procedure All patients confirmed (45%) or highly likely (55%) previous bovine thrombin exposure within 3 years	Primary: incidence of anti-rThrombin at day 29 between patients seropositive or seronegative for pre-existing anti-bovine thrombin antibodies Secondary: safety, blood product use and LOS	

**Note:** This table summarizes the results of published clinical trials with human or recombinant thrombin preparations.

**Abbreviations:** AC, active control; ADEs, adverse event; AV, arteriovenous; CI, confidence interval; DB, double blind; HR, hazard ratio; LOS, length of stay; MC, multicenter; min, minutes; OL, open-label; PAB, peripheral artery bypass; PC, placebo-controlled; PE, pulmonary embolism; R, randomized; RR, relative risk; rThrombin, recombinant thrombin; SG, single-group; vs, versus.



vial.<sup>89</sup> To date, there have been no reported cases in the United States of CJD or viral seroconversion. In the United Kingdom, 4 cases of probable transmission from transfusion of prion-contaminated blood products have been reported; however, there are no cases of transmission by pooled-human plasma products.<sup>77,90</sup> The true risk of transmission is unknown and therefore the product labeling of Evithrom<sup>®</sup> carries a warning directed at the risk of prion and viral disease transmission.<sup>65</sup> Patients who potentially may receive human product during surgery should be counseled on the risks and benefits prior to undergoing treatment.

There is some evidence that the use of human thrombin products result in a lower frequency of cross-reacting antibodies. The current formulation has not been associated with the risk of antiovine factor V development or potential factor V antibody formation.<sup>20</sup> In the Phase 3 study by Doria and colleagues, 3.3% of patients exposed to human thrombin developed antibodies to at least 1 of the antigens, compared with 12.7% of the patients treated with bovine thrombin.<sup>67</sup> None of the patients treated with Evithrom<sup>®</sup> developed detectable antibodies to either human thrombin or to human factor V or Va.<sup>67</sup> However, there was no difference in adverse events between the two groups.<sup>67</sup> Consequently, it is unknown whether the improved immunogenicity profile will translate to improved safety profiles in the clinical setting.<sup>16</sup>

Although reconstitution of Evithrom<sup>®</sup> is not necessary prior to administration, the storage requirements must be considered. Unlike the other formulations, human thrombin must be stored in the freezer and after thawing, is stable for no more than 30 days when refrigerated and for no more than 24 hours when stored at room temperature.<sup>65</sup>

## Recombinant thrombin

Recombinant thrombin (Recothrom<sup>®</sup>; ZymoGenetics, Inc., Seattle, Washington, USA) received FDA approval for use as a topical hemostatic agent in January 2008.<sup>66</sup> To date, it is the first thrombin product which was developed via recombinant technology and as a therapeutic preparation that is free of adventitious agents and nonthrombin immunogenic factors.<sup>88</sup> Recombinant thrombin (rThrombin) is produced from the precursor recombinant prethrombin-1 derived from Chinese hamster ovary (CHO) cell cultures.<sup>16,20,77,88,91</sup> Enzymes derived from snake venom are used to activate prethrombin-1 to alpha-thrombin.<sup>20,66</sup> The alpha-thrombin is purified to rThrombin in a chromatographic process that includes solvent-detergent treatment and nanofiltration.<sup>66</sup> A disulfide-linked dimer, rThrombin, composed of a 36-amino acid A-chain and a 259-amino acid B-chain is

identical in amino acid sequence to naturally occurring human thrombin.<sup>66</sup> Structurally and functionally, it is nearly identical to plasma-derived human thrombin.<sup>20,66,88</sup> The cell line used to manufacture rThrombin has been extensively tested and shown to be free of known infectious agents. The cell culture process used in manufacturing employs no additives of human or animal origin. The purification process involves solvent-detergent treatment and nanofiltration steps dedicated to viral clearance.<sup>66,88</sup> When compared to bovine thrombin, rThrombin has greater purity and it is essentially free of nonthrombin proteins.<sup>20</sup>

In a phase 2, randomized, double-blind, placebo-controlled, efficacy and safety analysis of rThrombin conducted by Chapman and colleagues, 130 adult patients were randomized to receive a single application of either rThrombin or placebo (Table 2).<sup>91</sup> Patients were included if they were undergoing arteriovenous (AV) graft formation for hemodialysis access, major hepatic resection, peripheral arterial bypass (PAB) surgery or spinal surgery. Additional inclusion criteria were age  $\geq 18$  years, normal platelet and coagulation parameters, no known antibodies or hypersensitivity to thrombin or other coagulation factors, and no known bleeding or hematologic disorders. The study was not powered to detect differences in outcome measures and no formal statistical hypotheses were tested. Recombinant thrombin was added to either Gelfoam<sup>®</sup> (Pharmacia, Kalamazoo, Michigan, USA) or Surgifoam<sup>®</sup> prior to application. Time to hemostasis (TTH) was measured for a maximum of 10 minutes. If hemostasis was not achieved by that time, open-label rThrombin was permitted as rescue therapy. Therefore, some patients randomized to placebo received open-label rThrombin and some patients received more than one dose of rThrombin. Rescue therapy was needed for 18 of 90 placebo treated bleeding sites (20%) compared with 9 of 93 rThrombin treated bleeding sites (10%). When rThrombin was used as the rescue therapy, it was efficacious in 19 of 20 bleeding sites (95%).<sup>20,91</sup> One patient in each group (2.4% placebo, 1.2% rThrombin) developed specific antibodies to rThrombin. At baseline, antibodies specific to rThrombin were present in 2 patients in the placebo group and 5 in the treatment group.<sup>91</sup> Anti-rThrombin antibodies did not neutralize native human thrombin and were not associated with any bleeding adverse events.<sup>66,91</sup>

In a phase 3, double-blind, randomized, comparative study, Chapman and colleagues compared the efficacy and tolerability of rThrombin with Thrombin-JMI<sup>®</sup> in 411 adults undergoing hepatic resection (n = 125, 30%), spinal surgery (n = 122, 30%), PAB surgery (n = 88, 21%) or AV

graft formation ( $n = 76$ , 18%) (Table 2).<sup>68</sup> Patients were included if they were age  $\geq 18$  years, and had no history of heparin-induced thrombocytopenia and no known sensitivity to bovine thrombin components, bovine materials, or porcine collagen. Patients were excluded if they had ever been treated in a clinical study of rThrombin, had undergone a therapeutic surgical procedure or been treated with an experimental agent within 30 days, or had received blood products within 24 hours before operation. Participants were randomized to receive either rThrombin ( $n = 205$ ) or Thrombin-JMI<sup>®</sup> ( $n = 206$ ). Both treatments (1000 units/mL) were applied topically to bleeding sites with an absorbable gelatin sponge. The primary efficacy endpoint was the TTH, summarized as the incidence of hemostasis within 10 minutes. Secondary endpoints included the incidence and severity of adverse events, the incidence and grade of clinical laboratory abnormalities and the incidence of antiproduct antibodies. Overall, the incidence of TTH within 10 minutes after a single application of study drug was 95.1% in the bovine thrombin group and 95.4% in the recombinant thrombin group. The percentage of patients achieving hemostasis at 1.5, 3, 6 and 10 minutes did not differ between the groups.<sup>20,66,68</sup> Topical recombinant thrombin was noninferior compared to bovine thrombin at improving surgical hemostasis in this study.<sup>20,68,92</sup>

Among the 411 patients treated with study drug, all but 2 patients (1 in each group) reported adverse events. Most events were moderate in severity and had similar incidence in the rThrombin and bovine thrombin treatment groups. The most common adverse event reported was incision site complication (63% for both groups). Other frequent adverse events were procedural pain (rThrombin 29%, bovine thrombin 34%) and nausea (rThrombin 28%, bovine thrombin 35%).<sup>68</sup> Serious adverse events were reported by 18% of patients treated with rThrombin and 22% with bovine thrombin. Serious adverse events included those that necessitated hospitalization, were life-threatening or resulted in significant disability or death.<sup>68</sup> Compared with bovine thrombin, rThrombin was associated with a significantly lower incidence of post-treatment antiproduct antibody development ( $P < 0.0001$ ).<sup>68</sup> Specific antiproduct antibody development was reported in 1.5% of patients (3/198) receiving rThrombin compared with 22% of patients (43/200) receiving bovine thrombin.<sup>68</sup> Anti-product antibody development was defined as either seroconversion or a greater than one-titer unit change.<sup>68</sup> None of the antibodies in the rThrombin group neutralized native human plasma thrombin activity.<sup>66,68</sup> Antibodies against bovine thrombin were not tested for neutralization of native human thrombin.

Because of the reported association between antibodies to bovine thrombin and adverse clinical outcomes, a post-hoc evaluation of the potential association of postbaseline anti-product antibodies with certain adverse clinical outcomes was performed.<sup>68</sup> Development of antibodies did not lead to any adverse events such as excessive bleeding in either group.<sup>66</sup> At baseline, positive antiproduct antibody titers were found in 1.5% of patients (3/198) in the recombinant thrombin group compared with 5% of patients (10/200) in the bovine thrombin group.<sup>92</sup>

The immunogenicity and safety of recombinant thrombin was evaluated in a phase 3b, open-label, single-group, multi-site study of 206 vascular and spinal surgery patients at high risk for preexisting antibovine thrombin product antibodies (Table 2).<sup>70</sup> Patients aged  $\geq 18$  years who had a history of an operation with documented or highly likely bovine thrombin exposure within the previous 3 years were included in the study. Patients were excluded if they had a known hypersensitivity to rThrombin and/or if cardiopulmonary bypass or aortic arch involvement occurred during the surgical procedure. The primary objective was to compare the incidence of antibodies to rThrombin at day 29 between patients who were seropositive and patients who were seronegative for preexisting antibodies to bovine thrombin. Singla and colleagues conducted a chart review and reported 45% (94/206) had a definite previous exposure to bovine thrombin, 55% (114/206) had a likely exposure and  $< 1\%$  (1/206) had an unknown exposure history. Preoperatively, 15.6% (32/206) of patients had preexisting antibovine thrombin antibodies.<sup>70</sup> The incidence reported by Singla and colleagues in this study population was much higher than that reported by Chapman and colleagues in the phase 3 study (5%, 10/200).<sup>68,70,92</sup> Of those patients with bovine thrombin antibodies, only half had a documented history of bovine thrombin exposure (47%, 15/32). Interestingly, 2% of patients in the phase 3b study had preexisting anti-rThrombin antibodies; however, there was no known prior exposure to the recombinant thrombin product. Post exposure, no patients became antibody positive (seroconversion or  $\geq 10$ -fold increase in titer) for rThrombin antibodies by day 29.<sup>70</sup>

Adverse events and laboratory results in this surgical population were similar for patients who were seropositive or seronegative for preexisting antibovine thrombin antibodies. Four patients experienced adverse events that were considered potentially treatment related, including erythema, incision-site erythema, pruritus and pulmonary embolism. Blood product usage and median hospital stay were higher in the seropositive group (28% of patients and



5.5 days, respectively) compared to the seronegative group (21% of patients and 3.0 days, respectively); however, statistical significance was not reported by the authors. Singla and colleagues concluded that the incidence of anti-rThrombin antibodies was comparable for patients who were seropositive or seronegative for pre-existing antbovine thrombin antibodies and therefore, pre-existing antibodies did not affect the immunogenicity of rThrombin. Recombinant thrombin administration in patients with documented or highly likely previous exposure to bovine thrombin was well tolerated and the immunogenicity of rThrombin was not affected by the presence of antbovine antibodies.<sup>70</sup>

Results of clinical trials have demonstrated comparable efficacy and safety of rThrombin to bovine thrombin in addition to a significant decrease in immunogenicity.<sup>16,68</sup> A non-significant trend toward faster time to hemostasis was noted in favor of recombinant thrombin.<sup>68</sup> Additionally, rThrombin, when compared to bovine thrombin was associated with a significantly lower incidence of post-treatment antiproduct antibody development ( $P < 0.0001$ ).<sup>68</sup> Specific antiproduct antibody development was reported in 1.5% of patients (3/198) receiving rThrombin compared with 22% of patients (43/200) receiving bovine thrombin.<sup>66,68</sup> It is unclear whether any of the 3 patients exposed to recombinant thrombin had prior exposure to bovine thrombin. Development of antibodies did not lead to any adverse events such as excessive bleeding in either group.<sup>68</sup> Although associated with a lower incidence of antibody formation, it is yet to be determined if the lower frequency of cross-reacting antibodies is associated with a lower frequency of IMC.<sup>77</sup> To date there have been no case reports published in the available literature. However, in the published clinical trials, there are limited data ( $n = 6$ ) available on repeat exposure to rThrombin.<sup>66</sup> In addition to its improved immunogenicity profile, recombinant thrombin is virtually identical to native human thrombin in its amino acid sequence, structure and physiochemical characteristics.<sup>88</sup> Produced within a CHO cell line, recombinant thrombin is also free of any bovine or human derived proteins. Furthermore, there are no identifiable infectious agents within the current preparation.<sup>77</sup>

There are still potential risks associated with recombinant thrombin. No specific adverse events have been established as adverse reactions causally related to rThrombin administration.<sup>66</sup> The most common adverse events seen in phase 3 of the large, randomized controlled trial by Chapman and colleagues, included incision site complication (63%), nausea (28%), procedural pain (29%), constipation (22%) and pyrexia (20%). However, none of these are thought to

be a direct effect of thrombin administration.<sup>68</sup> Recothrom<sup>®</sup> carries similar contraindications and warnings as the other formulations. It is not to be used for direct injection into the circulatory system or for the treatment of severe or brisk arterial bleeding. It is also contraindicated in patients with known hypersensitivity to the product, any of the product ingredients or hamster protein.<sup>66,92,93</sup>

## Economic impact

When comparing the three commercially available thrombin products, there is little difference in the listed wholesale prices. The current average wholesale prices per international unit for bovine, human and recombinant thrombin are US\$0.018, US\$0.02, and US\$0.021, respectively.<sup>94</sup> These prices do not reflect hospital acquisition prices based on volume usage or group purchasing nor do they reflect direct and indirect costs associated with administration of the products.<sup>83</sup> Direct costs, such as storage and inventory issues, are higher for human thrombin as it has to be stored in the freezer and after thawing is stable for no more than 30 days when refrigerated and no more than 24 hours when stored at room temperature.<sup>65</sup> Additionally, indirect costs may vary between the different formulations. Such costs may include specialized distribution, administration, monitoring systems for high-risk drugs and/or cost of treating drug adversity.<sup>83</sup> Although the incidence of coagulopathies associated with thrombin use is unknown, the cost of treating such acquired problems may be high and more than likely is associated with additional hospitalization costs.

## Future considerations and controversies

With the recent introduction of the newer topical thrombin formulations combined with the heightened awareness of the adverse effect profile associated with bovine thrombin, a few key issues surrounding the proper selection of topical thrombin products have been raised. To date, there are no direct, controlled, randomized trials demonstrating the effectiveness of bovine thrombin on improving patient outcomes.<sup>17,20</sup> At the time of approval, bovine thrombin was shown only to reduce the intraoperative blood loss in a limited number of surgical cases with no immunologic data obtained.<sup>17</sup> Only recently has the efficacy of topical thrombin products been evaluated in a randomized, double-blind comparison fashion.<sup>67-69</sup> In such trials, the newer human or recombinant thrombin products were compared with bovine thrombin.<sup>67-69</sup> Future trials may benefit from inclusion of a placebo control group to better define efficacy of topical

thrombin administration as a hemostatic agent in surgical patients.<sup>20,78</sup>

Additionally, the true incidence of IMC with the more purified bovine thrombin product available today is not known. Many of the original reports of antibody products and IMC occurred at a time when more impure bovine thrombin products, such as Thrombogen<sup>®</sup> and Thrombostat<sup>®</sup>, were used.<sup>29–39</sup> In 2001, Thrombin-JMI<sup>®</sup> was remanufactured to reduce the levels of bovine-related contaminants, such as factor V, in the products.<sup>16</sup> However, reports of IMC and antibody production have occurred with the more purified bovine thrombin products.<sup>56–63,67–70</sup> These cases may suggest that the degree of purity of the thrombin preparations may be only part of the explanation for the pathogenesis of these events.<sup>17,59</sup> Beginning in 2008, the manufacturing of Thrombin-JMI<sup>®</sup> incorporated a further purification step to enhance purity.<sup>76</sup> The effect this will have on antibody production and incidence of IMC is unknown at this time.

Lastly, will decreased antibody production with human or recombinant thrombin decrease the risk of adverse events associated with bovine thrombin use? Comparator trials using human or rThrombin have demonstrated decreased antibody production compared to bovine thrombin but have shown no difference in side effect profiles.<sup>67–69</sup> The association of postexposure antbovine thrombin antibodies with adverse clinical outcomes was addressed in one of these studies.<sup>69</sup> Patients who developed antibodies to bovine thrombin had a numerically increased incidence of hypersensitivity and elevation of aPTT compared to antibody negative patients. However, this was not significant secondary to small numbers of events and sample size.<sup>69</sup> These studies used the original Thrombin-JMI<sup>®</sup> product. It is unknown how antibody production with the 2008 reformulation compares with human or recombinant thrombin. Due to the newness of these agents, experiences addressing the long-term outcomes in patients receiving human or rThrombin are lacking. Additionally, the true risk of re-exposure to these agents on adverse outcomes is unknown. However, in patients with a high incidence of previous exposure to bovine thrombin or seropositive for antbovine thrombin antibodies, the use of recombinant thrombin appears to be safe.<sup>70</sup> Future studies will be required to address whether the potential improved immunogenicity of these products translates into improved outcomes.

## Conclusion

Excessive blood loss following cardiac surgical procedures can be extremely problematic. Topical thrombin has been used adjunctively during cardiac surgical procedures to

control intraoperative bleeding. Use of topical bovine thrombin in surgical populations, especially in cardiac surgery patients, has been associated with adverse outcomes including anaphylaxis, thrombosis and IMC secondary to cross-reacting antibodies, all of which may increase in incidence upon re-exposure. The clinical spectrum of IMC may range from asymptomatic alteration in coagulation factors to life-threatening hemorrhage and death. The clinical diagnosis of IMC is often difficult to make secondary to poor documentation of previous exposure, lack of a commercially available laboratory assay to test for the presence of antibody and the lack of recognition of IMC in the differential diagnosis. Treatment options in patients with bovine thrombin induced IMC for severe bleeding are marginally effective. The overall incidence of IMC secondary to bovine thrombin is not truly known and may be unrecognized or underreported. These circumstances led the FDA to place a boxed warning for topical bovine thrombin products as well as a negative endorsement for use in perioperative blood transfusion and conservation in cardiac surgery by The Society of Thoracic Surgeons and Cardiovascular Anesthesiologists. Most reports concerning adverse events with topical bovine thrombin were associated with the use of older, more impure formulations. However, adverse events have been reported with the newer, more purified products, suggesting that the degree of purity may only partially explain its potential pathogenesis.

The adverse immunologic profile associated with bovine thrombin has led to the development of alternative products, including a human and a recombinant thrombin formulation. These preparations appear to be equally efficacious, are associated with reduced antigenicity and have similar acquisition costs. However, the true benefit and associated risk are unknown secondary to a lack of long-term experience and re-exposure to these products. Future placebo-controlled studies comparing the most recent formulations may be required to address the role of antigenicity and overall patient outcomes associated with the use of topical thrombin products.

The potential for increased adverse events associated with the use of bovine thrombin in cardiac surgery patients, especially when considering the increased risk of re-exposure either at the time of surgery or during future surgical procedures, may justify the use of newer nonbovine alternatives. Recent studies comparing human and recombinant thrombin have demonstrated equal efficacy with decreased antigenicity compared to bovine thrombin. However, plasma-derived human thrombin carries a potential risk of transmitting infectious agents and may have higher direct costs secondary to storage and inventory considerations. Therefore, secondary

to a potentially higher margin for safety and less stringent storage concerns, recombinant thrombin may be the most reasonable approach if topical thrombin use is required in cardiac surgery patients.

## Disclosures

The authors declare no conflicts of interest.

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