

CASE REPORT

Severe Postoperative Immune-Mediated Coagulopathy Associated with Bovine Thrombin Exposure

George M. Rodgers, M.D., Ph.D., and Larry W. Kraiss, M.D.

Immune-mediated coagulopathy is a known risk of bovine-derived topical thrombin exposure and is the subject of a black-box warning in the package inserts of bovine-derived topical thrombin products. The manifestation of coagulopathy due to bovine thrombin exposure may range from abnormal laboratory values without clinical sequelae to severe bleeding, which can be fatal in rare instances and result in significant consumption of blood and blood products, and significantly higher treatment costs. We describe a 76-year-old woman who developed severe hemorrhagic complications after surgical exposure to bovine-derived topical thrombin. The patient was initially diagnosed with disseminated intravascular coagulation with an accompanying coagulopathy. Despite resolution of the disseminated intravascular coagulation, the patient continued to experience episodic hemorrhages accompanied by positive coagulation mixing studies and clotting factor assays indicating a significantly reduced factor V activity (< 5% of normal) that confirmed an ongoing immune-mediated coagulopathy. The presence of a factor V inhibitor was confirmed by inhibitor titer assay. After a critical care hospitalization of 64 days that required the use of 282 units of blood products and two reoperations for hemorrhage, the patient was discharged home. Immune-mediated coagulopathy resulting from bovine thrombin exposure can result in clinically severe bleeding in some patients. As illustrated by the consumption of health care resources in this case report, should such a coagulopathy develop, the costs may be considerable.

Key Words: bovine, thrombin, coagulopathy, hemostasis, antibody, topical thrombin.

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In the postoperative period, immune-mediated coagulopathy (IMC) represents one of a number of coagulation abnormalities that may affect a

patient. This coagulopathy is an acquired bleeding disorder that results when antibodies develop in response to exposure to an immunogenic antigen. These antibodies can then interfere with the normal function of the coagulation cascade. The condition is difficult to diagnose and manage partly because of the delayed appearance of clinical signs and the need to eliminate other common causes of postoperative bleeding such as phytonadione (vitamin K) deficiency or disseminated intravascular coagulation (DIC).¹ Furthermore, the

From the Departments of Medicine and Pathology, Divisions of Hematology and Oncology (Dr. Rodgers), and the Department of Surgery (Dr. Kraiss), University of Utah Health Sciences Center, Salt Lake City, Utah.

For reprints, visit <http://www.atypon-link.com/PPI/loi/phco>. For questions or comments, contact George M. Rodgers, M.D., Ph.D., Departments of Medicine and Pathology, Divisions of Hematology and Oncology, University of Utah Health Sciences Center, 50 North Medical Drive, Salt Lake City, UT 84132; e-mail: george.rodgers@hsc.utah.edu.

manifestation of IMC may range from asymptomatic laboratory abnormalities to severe postoperative hemorrhage. A relatively uncommon way to develop IMC is from surgical exposure to topical bovine thrombin. As many as one million patients are exposed to bovine thrombin each year in a variety of clinical settings.²

The primary amino-acid structure of human and bovine prothrombin differs by 10–20%, and these differences, combined with xenogenic carbohydrate side chains, form important immunogenic epitopes.^{2,3} In addition, extraneous bovine plasma proteins in these preparations, such as factor V, may generate antibodies. Immune system activation after bovine thrombin exposure may result in the formation of cross-reactive antibodies against native coagulation factors, with as many as 30% of patients with antibovine protein antibodies also manifesting antibodies against human coagulation factors.^{2,4} These autoantibodies may interfere with the normal function of the coagulation cascade, leading to excessive bleeding or thrombosis.^{2,5–9} The long-term effects of the sensitization of the immune system to nonhuman coagulation proteins remain unclear.

Although the true incidence of IMC associated with bovine thrombin exposure is unknown, a number of review articles and published case reports have related antibody-mediated coagulopathy to previous administration of topical bovine thrombin preparations.^{2,4–8} Given the number of more commonly occurring etiologies that can obscure a diagnosis of IMC, and low clinical awareness of the phenomenon, it is likely that the incidence is underestimated. Reported rates of antibody formation against bovine factor V and bovine thrombin after bovine thrombin exposure have ranged from as low as 12.7%¹⁰ to 21.5%¹¹ and to a high of 95%,⁴ depending on the clinical and laboratory specifics of the report and the purity of the thrombin.

We describe a patient who developed severe postoperative coagulopathy secondary to topical bovine thrombin exposure and provide a discussion of IMC, including factors that may confound rapid recognition of its presence and its associated costs.

Case Report

A 76-year-old woman with pancreatitis underwent a laparotomy, pancreatic pseudocyst excision, and a colectomy with diverting ileostomy. Her medical history was also remarkable for

glaucoma, hypertension, diabetes mellitus, and osteoporosis. Her surgical history included cholecystectomy, bladder suspension, pancreatic debridement on two occasions, and craniotomy for meningioma. The patient had documented allergies to vancomycin, fluconazole, β -lactam antibiotics, tramadol, meropenem, and lorazepam. Her coagulation studies and liver function tests were all within normal limits, and her pancreatitis was not active. The surgical procedure was uneventful, and she was discharged home.

In April 2008, 7 months after the procedure, a reversal of the ileostomy was performed, during which 5000 units of topical bovine thrombin, the only topical bovine thrombin on formulary, was administered to aid hemostasis. The patient was brought by ambulance to the hospital on postoperative day 8 and was readmitted in hemorrhagic shock. Supporting laboratory tests were significant for severe anemia (hemoglobin 8.3 g/dl [normal range 13.9–16.3 g/dl]), hematocrit 25.2% [normal range 41.0–53.0%]), blood pressure 80 mm Hg/palpable, and thrombocytopenia (platelet count $133 \times 10^3/\text{mm}^3$ [normal range $150\text{--}450 \times 10^3/\text{mm}^3$]). Table 1 shows the chronology of the patient's pertinent laboratory values after this admission. Further testing also revealed a marked coagulopathy, with prolonged prothrombin time (PT) and activated partial thromboplastin time (aPTT) values. Her fibrinogen concentration was reduced and her D-dimer level was elevated, leading to an initial diagnosis of DIC. A computed tomography scan demonstrated a large intraabdominal hematoma. The hematoma was surgically drained, and 5000 units of topical bovine thrombin were again used to aid hemostasis. The patient was then treated with antibiotics (specific agent[s] and dosage[s] could not be determined) and multiple units of blood products (packed red blood cells [PRBC], fresh frozen plasma [FFP], cryoprecipitate, and platelets).

By postoperative days 11–13, the patient's DIC had resolved, as evidenced by a 50% reduction in D-dimer level and correction of hypofibrinogenemia. However, the patient had persistent bleeding with prolonged PT and aPTT values that could no longer be explained by DIC alone. From postoperative days 13–39, the patient remained hospitalized with recurrent bleeding episodes; during this time the patient received transfusion therapy with PRBC, platelets, cryoprecipitate, FFP, and intravenous immunoglobulin (IVIG) 1 g/kg/day for 2 days (total of 132 g). The IVIG therapy was given based on

Table 1. Chronology of the Patient's Laboratory Values

Postoperative Day ^a	PT (sec)	aPTT (sec)	Other Laboratory Values
8 (readmission for hemorrhagic shock)	41.5	> 150	D-dimer 58 µg/ml, fibrinogen 102 mg/dl, platelet count 133 x 10 ³ /mm ³
11	19.3	63	Thrombin time > 100 sec, D-dimer 25.6 µg/ml, platelet count 115 x 10 ³ /mm ³
13	19.4	54	D-dimer 16 µg/ml, fibrinogen 295 mg/dl, platelet count 113 x 10 ³ /mm ³ ,
16			factor V < 5%, factor VII 107%, factor II 81%, factor IX 60%
17	17.8	64	Thrombin time > 100 sec
23	17.7	55	Platelet count 379 x 10 ³ /mm ³ (after preoperative transfusion)
28	22.6		
39 ^b	22.1	66	Fibrinogen 417 mg/dl
71	15	35	

PT = prothrombin time; aPTT = activated partial thromboplastin time.

Normal ranges are as follows: PT 12–15 sec; aPTT 23–36 sec; D-dimer 0–0.5 µg/ml; fibrinogen 150–400 mg/dl; platelet count 150–450 x 10³/mm³; thrombin time 16–24 sec; factor V 62–140%; factor VII 83–181%; factor II 86–150%; and factor IX 78–184%.

^aPostoperative day relative to the surgery performed to reverse the patient's ileostomy, during which topical bovine thrombin was used.

^bAfter the patient was transferred to our institution (postoperative day 37), platelet counts were not reported because the patient was receiving platelet transfusions.

anecdotal evidence demonstrating good results in previous cases.⁷ During this time, on postoperative day 16, factor assay results demonstrated a reduced factor V level (< 5% of normal); however, the diagnosis of IMC was not appreciated initially, and the patient continued to bleed and receive blood products. On postoperative day 37, a presumptive diagnosis of factor V inhibitor coagulopathy secondary to bovine topical thrombin exposure was recognized on the basis of positive mixing studies and a positive factor V inhibitor assay (1.4 Bethesda units). Arrangements were made to transfer the patient to our institution because the physicians thought she needed another surgical procedure and were unsure of how to manage the bleeding.

The coagulation mixing studies were conducted by combining patient plasma and normal pooled human plasma in a 1:1 ratio, and performing PT and aPTT assays immediately before and repeating them 1 hour later to determine if the combined plasma had returned the PT and aPTT assays to the normal range. An assay was considered positive if the combined plasma failed to return the PT and aPTT values to the normal range. A dilutional coagulopathy would have demonstrated PT and aPTT values that corrected with exogenous plasma administration, resulting in a negative study. Only a coagulation factor inhibitor, such as an antibody, would have resulted in a positive mixing study.

After transfer to our institution, the diagnosis

of IMC was confirmed by a second positive mixing study, in conjunction with elevated PT (22.1 sec) and aPTT (66 sec) values, which indicated the continued presence of an inhibitor to one or more coagulation factors. As a consequence of a confirmed IMC, all subsequent procedures and surgical interventions were performed without bovine-related products for the duration of the patient's stay in our institution. On postoperative day 39, the patient received platelets, FFP, and phytonadione. Four days later, a second hematoma (retroperitoneal, 10 cm) was evacuated and the wound debrided. Despite preoperative administration of platelets and FFP, the patient experienced melena and hematuria postoperatively. On postoperative day 59, the patient received platelets and FFP before surgery for partial wound closure with a cadaver allograft. The patient did not experience postoperative bleeding, and over the next several days, her PT and aPTT values slowly improved (Table 1).

Two months after the patient's ileostomy reversal, her coagulation studies normalized, and she was discharged with no clinical bleeding. A 1-month follow-up visit revealed no further bleeding or complications. The patient had been hospitalized in the intensive care units of two hospitals for 64 days and had received 282 units of blood products, incurring significant resource utilization and costs. A summary of her resource utilization is shown in Table 2.

Table 2. Summary of the Patient's Resource Utilization

Resource	Value or Description
ICU length of stay	
First hospital	31 days
Second hospital	33 days
Blood products	282 units (total)
Packed red blood cells	78 units
Fresh frozen plasma	129 units
Platelets	62 units
Cryoprecipitate	13 units
Intravenous immunoglobulin	132 g
Laboratory analysis (both hospitals)	Minimum daily CBC, PT, aPTT, chemistry panels
Consultations	
First hospital	Hematology, gastroenterology, pulmonary
Second hospital	Hematology, gastroenterology
Related procedures	
First hospital	1 surgery
Second hospital	2 surgeries, esophago- gastroduodenoscopy, colonoscopy

ICU = intensive care unit; CBC = complete blood count; PT = prothrombin time; aPTT = activated partial thromboplastin time.

Discussion

In 1996, a black-box warning was added to the prescribing information of stand-alone bovine-derived thrombin.² The warning states that reexposure of patients with antibodies to bovine-derived plasma proteins is contraindicated and that the immunologic basis of the condition should be taken into account when undertaking any intervention.⁹ Unfortunately, there is no routinely available assay to detect these antibodies, making presurgical screening and postsurgical diagnosis difficult. Nor do standard coagulation tests predict which patients will develop bleeding complications.

Immune-mediated coagulopathy secondary to bovine thrombin exposure is an acquired bleeding disorder that can be difficult to diagnose and manage. Suspicion of IMC is the first challenge, followed by confirmation and management. Therefore, for any patient with unexplained postsurgical bleeding or elevated PT, aPTT, or thrombin time values without clinically evident bleeding, a diagnosis of IMC should be given consideration.¹ This is especially true for any patient with known exposure or who is highly likely to have been exposed to bovine thrombin and for those patients who have had more common causes of coagulopathy resolved or ruled out.¹ The opportunities for previous

exposure to bovine thrombin are many, given its use in various specialty procedures such as vascular, cardiovascular, neurologic, and dental.² Ideally, a complete surgical history and medical record review would detect previous exposure. However, use of bovine thrombin is so common that it is often not recorded in the operative notes, and patients are rarely informed of its use.

In our patient, bovine thrombin was the only topical thrombin on formulary at the first institution during the time her surgery was performed. The method of how the topical thrombin was employed in her case was unknown; however, thrombin may be used as a reconstituted spray, an unreconstituted powder applied directly to the bleeding site, or with a gelatin sponge soaked in reconstituted liquid. An evaluation using the Naranjo adverse drug reaction probability algorithm¹² indicated a probable (score of 5) relationship between our patient's IMC and the use of bovine thrombin.

Initial exposure to bovine thrombin may prime the immune system such that subsequent exposures result in a secondary anamnestic immune response that produces antibodies that may cross-react with native coagulation factors.^{2,4} This, in turn, may increase the patient's risk for developing clinical sequelae.⁴ Enhanced purification of the only bovine formulation still available in the United States was reported and evaluated in 2008—significantly reducing the presence of highly immunogenic, contaminating bovine plasma proteins such as factor V—but the process has not succeeded in eliminating them entirely.¹³ Furthermore, since bovine and human coagulation proteins are only approximately 80–90% homologous, the bovine proteins remain a potential source of immunoreactivity.² It is uncertain whether the enhanced purification process will affect the clinical manifestations of IMC, and the clinical significance of these findings is unknown.

Generally, after bovine thrombin exposure, factor V inhibitors appear after a mean post-exposure interval of 8.3 days.⁷ These inhibitors remain detectable for a mean of 2.3 months and have been detected up to 3 years later.^{7,14} Clinical manifestations range from laboratory abnormalities not associated with hemorrhage to epistaxis, hematuria, or, as in our patient, severe, potentially life-threatening hemorrhage.^{5,15–17}

Severe cases of IMC produce increased morbidity, prolong hospitalization, and consume expensive resources.^{18,19} Successful management of our patient required a lengthy intensive care

Table 3. Costs of Resource Utilization

Resource	Total Cost/ Unit (\$)ª	Estimated Units	Cost (\$)
ICU length of stay (no ventilator)	4022/day ²¹	64 days	257,408
Blood products			
Packed red blood cells	1459/unit ²²	78 units	113,802
Fresh frozen plasma	72/unit ²³	129 units	9288
Platelets	656/unit ²¹	62 units	40,672
Intravenous immunoglobulin	180.50/g ²⁴	132 g	23,826
Total estimated cost			444,996

ICU = intensive care unit.

ªNondrug costs were normalized from reference year to 2008 (year when this case occurred) dollars using the consumer price index for medical care services available from the U.S. Department of Labor, Bureau of Labor Statistics.²⁵

unit (ICU) stay (total of 64 days), the use of 282 units of blood products, and the administration of 132 g of IVIG—a significant expenditure of health care resources for a potentially preventable problem (Table 2). To estimate the cost associated with our patient's case, published cost data on hospital duration, blood products, and therapeutic interventions were utilized. Previous studies have estimated that when transfusion is required for any reason, the average length of stay often increases by a factor of 2.0–2.5 times²⁰ at a cost of about \$4022/day for an ICU bed without a ventilator.²¹ Based on published cost data, the approximate cost of PRBC transfusion is \$1459/unit,²² FFP approximately \$72/unit,²³ and platelets \$656/unit.²¹ The cost of IVIG was \$180.50/g.²⁴ Costs were normalized from reference year to 2008 (year when this case occurred) dollars by utilizing the consumer price index for medical care services available from the U.S. Department of Labor, Bureau of Labor Statistics.²⁵ Using the available published information, an estimated cost for hospitalization and blood products was over \$440,000 as a consequence of this patient suffering an adverse event (Table 3). Costs of IMC not factored into this estimate include those associated with consultations, reoperations, imaging studies, and laboratory tests, as well as some therapeutic interventions and the transfer of patient between hospitals. Therapies used in other published cases of IMC and representing further or alternative hidden costs have included cyclophosphamide therapy, plasmapheresis, and steroid treatments.⁷

Given the challenges in diagnosing and managing IMC, the best option is prevention. In

cases where an acquired inhibitor secondary to bovine thrombin exposure has been diagnosed, or in patients with a documented or likely history of bovine thrombin exposure (including patients with a past surgical procedure for which topical thrombin is commonly used), alternative approaches to achieve intraoperative hemostasis are recommended.

As demonstrated in this case report, IMC presents with clinical heterogeneity and is often difficult to diagnose. The clinical presentation may be masked by other conditions, causing IMC to be misdiagnosed or overlooked, and therefore underreported. If IMC is suspected, ordering the appropriate diagnostic tests, including a coagulation mixing study and factor V assay to identify the presence of a factor V inhibitor, and consulting with a hematologist and/or a transfusion specialist can lead to an early, accurate diagnosis, allowing for the focused management of the underlying immune problem. In severe cases, advanced hematology and critical care support are essential. Ultimately, bovine thrombin–induced IMC is an iatrogenic event and is therefore preventable. Alternatives to topical bovine thrombin that are approved by the FDA, such as recombinant thrombin and pooled human plasma thrombin, are commercially available and should be considered as hemostatic options—especially in patients with known or suspected previous exposure to bovine thrombin.

Conclusion

The clinical presentation of our patient in this case report is consistent with previously published reports of hemorrhagic bovine

thrombin-associated IMC and is an extreme example of the costs that may be associated with this adverse event. Laboratory analysis demonstrated significantly elevated PT and aPTT values, which did not correct with therapies directed at an initial diagnosis of DIC. Positive coagulation mixing studies consistent with the presence of an inhibitor aided in establishing the diagnosis of IMC, along with clotting factor assays that indicated significantly reduced (< 5% of normal) factor V activity. Additional confirmatory data were supplied by an inhibitor titer assay demonstrating a factor V inhibitor. The patient spent 64 days hospitalized and required 282 units of blood products and two reoperations for hemorrhage. Due to her clinically severe bleeding, consumption of health care resources and related costs were considerable. In patients with known or suspected previous exposure to bovine thrombin, other hemostatic options should be used.

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