An Alternative Hemostatic Dressing: Comparison of CELOX, HemCon, and QuikClot

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Abstract

Objectives: Uncontrolled hemorrhage remains a leading cause of traumatic death. Several topical adjunct agents have been shown to be effective in controlling hemorrhage, and two, chitosan wafer dressing (HemCon [HC]) and zeolite powder dressing (QuikClot [QC]), are being utilized regularly on the battlefield. However, recent literature reviews have concluded that no ideal topical agent exists. The authors compared a new chitosan granule dressing (CELOX [CX]) to HC, QC and standard dressing in a lethal hemorrhagic groin injury.

Methods: A complex groin injury with transection of the femoral vessels and 3 minutes of uncontrolled hemorrhage was created in 48 swine. The animals were then randomized to four treatment groups (12 animals each). Group 1 included standard gauze dressing (SD); Group 2, CX; Group 3, HC; and Group 4, QC. Each agent was applied with 5 minutes of manual pressure followed by a standard field compression dressing. Hetastarch (500 mL) was infused over 30 minutes. Hemodynamic parameters were recorded over 180 minutes. Primary endpoints included rebleed and death.

Results: CX reduced rebleeding to 0% (p < 0.001), HC to 33% (95% CI = 19.7% to 46.3%, p = 0.038), and QC to 8% (95% CI = 3.3% to 15.7%, p = 0.001), compared to 83% (95% CI = 72.4% to 93.6%) for SD. CX improved survival to 100% compared to SD at 50% (95% CI = 35.9% to 64.2%, p = 0.018). Survival for HC (67%) (95% CI = 53.7% to 80.3%) and QC (92%; 95% CI = 84.3% to 99.7%) did not differ from SD.

Conclusions: In this porcine model of uncontrolled hemorrhage, CX improved hemorrhage control and survival. CELOX is a viable alternative for the treatment of severe hemorrhage.

Keywords: hemostatic dressing, uncontrolled hemorrhage, CELOX, chitosan, zeolite
Despite advances in medical intervention and protective equipment, fatal traumatic hemorrhage remains one of the most challenging problems for both military and civilian medicine. Uncontrolled hemorrhage currently accounts for almost 50% of battlefield deaths before evacuation in Iraq and Afghanistan. Additionally, civilian trauma death from exsanguination approaches 80% in the United States, accounting for the second leading cause of trauma death overall. The continued military emphasis on remote operations in austere environments and increasing threat to civilian tactical law enforcement will require advances that improve the field treatment of hemorrhage in both settings.

As a result, much attention has been focused on the development of alternative methods of controlling hemorrhage, including topical hemostatic dressings. While several agents have been developed, the two most commonly utilized on the battlefield include the chitosan standard dressing (HemCon [HC], HemCon Inc., Portland OR) and zeolite powder dressing (QuikClot [QC], Z-Medica, Wallingford, CT).

The chitosan dressing is a fairly rigid wafer that forms a mucoadhesive physical barrier at the site of injury. Zeolite is a hard granule that quickly adsorbs water from blood to concentrate native elements of coagulation at the site of bleeding. However, mixed results with regard to the success of each agent have been reported in individual studies utilizing a variety of preclinical models. There have also been concerns related to side effects, specifically, thermal injury from the exothermic reaction associated with use of zeolite, although documented occurrences are relatively infrequent. Field use of these agents has reported some success in treating human wounds. Overall, recent reviews of the existing literature suggest that there is no single perfect hemostatic dressing; each has its drawbacks and benefits.

A new chitosan granular dressing (CELOX [CX], SAM Medical Products, Portland, OR) reports success in controlling hemorrhage while continuing to possess many of the ancillary characteristics of an ideal hemostatic dressing. This agent is a fine granular product that works by interacting directly with red blood cells and platelets to form a cross-linked barrier clot, independent of native factors. According to the manufacturers, it is reportedly nonallergenic, nonathermic, able to function in a hypothermic environment, and low in cost.

The purpose of this study is to compare the CX, HC, and QC dressings to standard gauze dressing (SD) in an accepted swine groin injury model. We hypothesize that CX, HC, and QC will improve hemorrhage control and survival compared with SD, HC, and QC.

METHODS

Study Design

This study was a randomized, controlled, unblinded, preclinical trial using a swine model of acute hemorrhage. Four intervention groups of 12 animals each were examined using three different hemostatic agents, CX, HC, and QC, plus a standard gauze control, SD. Standard dosages and techniques, as recommended by the manufacturer, were utilized. Before this study, investigators received specialty training from the respective manufacturers. QC and HC were purchased directly from the distributor. SAM Medical Products provided CX as part of a complete, unrestricted research grant.

The protocol was approved by the Institutional Animal Care and Use Committee (IACUC). All research was conducted in compliance with the Animal Welfare Act. The animals received humane care in accordance with the Guide for the Care and Use of Laboratory Animals (National Institutes of Health Publication 86–23, revised 1996).

Animal Subjects

The study was conducted in the controlled environment of a veterinary surgical suite designed to accommodate up to four subjects at any one time. We chose farm-raised, Yorkshire swine (Sus scrofa, Blackwater Farms, Franklin, VA) as our study subjects. This choice was made due to the reliability of swine as a cardiovascular model and the ease of accessibility. We attempted to match age and weight within our groups.

Study Protocol

Forty-eight swine were fed a standard diet and observed for a minimum of 5 days. Animals were fasted the night prior to the procedure and water was provided ad libitum. Anesthesia was induced with an intramuscular injection of ketamine (20 mg/kg) and 5% inhaled isoflurane via face mask for 3 minutes. Maintenance anesthesia was set at 2% isoflurane after endotracheal intubation, and the animal remained breathing spontaneously on 21% oxygen and air administered from an MDS Matrx VMC small animal anesthesia machine (Matrx Medical, Orchard Park, NY) for the duration of the procedure. The animal was placed supine on the operating table with the front legs secured to allow adequate access to the neck. The right...
carotid artery and external jugular vein were then exposed via cutdown technique. A 22-gauge catheter was used to cannulate the carotid artery for continuous arterial blood pressure monitoring. The external jugular vein was cannulated with a 20-gauge catheter for infusion of resuscitative fluid. Continuous temperature monitoring was achieved via placement of an indwelling rectal probe, and an electric table warmer and blankets maintained a core body temperature of 36–38°C. A suprapubic bladder tap was performed under sterile procedures to remove excess urine.

After preparation of the animal, baseline vital signs were recorded every 5 minutes for 15 minutes preceding the creation of the groin injury. During this period, anesthesia was decreased to 0.5 or 1% gradually, titrated to adequate surgical pain threshold, as determined by a motor response to hoof and corneal stimulation. A complex injury was then created in the subject’s right groin to produce uncontrolled hemorrhage, as previously described by Alam et al. A No. 10 blade scalpel was used to create an oblique superficial skin incision along the right inguinal crease approximately 8 cm long. Subsequently, gentle, blunt dissection exposed the quadriceps and adductor fascial layers. This plane was then followed cranially toward the inguinal canal, until faint direct visualization of the femoral vascular bundle was achieved. Direct manipulation and exposure of the femoral vessels was avoided to prevent any spasm that could interfere with blood loss. A type K thermometer temperature probe (Extech Instruments Co., Waltham, MA) was inserted into the exposed area between the quadriceps and abdominal wall and gently secured to the muscle body with staples to facilitate the measurement of peak wound temperatures. The hemorrhagic injury was created by direct incision of the quadriceps and adductor muscles and complete transection of the femoral artery and vein. Once the wound was inflicted, the subject was allowed to bleed, unimpeded, for 3 minutes, before treatment was applied.

After creation of the injury and 3 minutes of hemorrhage, the wound was wiped with gauze. Care was taken not to disturb any preformed clot at any time during the study. The agent was then applied to the wound in accordance with the manufacturer’s instructions. CX and QC were each applied by pouring the contents of one package into the wound, followed by application of four–by-four gauze bandages (Curity, Tyco Healthcare Group LP, Mansfield, MA) and a rolled gauze bandage (Kerlix, Tyco Healthcare Group LP). Constant, one-fisted pressure from a nondominant hand was then applied for a total of 5 minutes. After direct pressure, a compression dressing (Cinchite, H and H Associates, Bena, VA) was wrapped around the pelvis in a standardized fashion and secured for the remainder of the study. HC was applied in a similar fashion. However, the product was first hand molded and preformed with a rolled bandage and applied directly over the incised vessels. This hand molding process was accomplished during the 3-minute period of uncontrolled hemorrhage. Overall bleeding time was not increased by this technique. Four–by-four gauze was then placed on top. The remaining steps of pressure and compression bandage placement were the same as with CX and QC. For the control group, four–by four gauze was inserted into the wound, followed by a rolled bandage, direct pressure, and a compression dressing in the same manner mentioned above.

Eight minutes after the initial incision, fluid resuscitation was begun with 500 mL of 6% hetastarch in lactated Ringers solution (Hextend, Hospira, Inc., Lake Forest, IL) administered via right external jugular vein over a period of 30 minutes. This method of colloid resuscitation was utilized as it is frequently used for battlefield resuscitation. Our goal was to mimic the battlefield setting as closely as possible. In the event that the animal’s respiration ceased at any point during the trial, standard manual assistance was provided for a period of up to 5 minutes. If the subject failed to respond after 5 minutes, assistance was terminated. Cardiac compressions were not administered at any point in the study. Subjects were monitored until death or for a total of 180 minutes following the time of injury. Death was defined as apnea and asystole for 5 continuous minutes. Subjects that survived through 180 minutes received euthanasia with a standard solution (Euthasol, Virbac Animal Health, Inc., Fort Worth, TX).

Following each animal’s death or euthanasia after 180 minutes, local exploration of the wound was performed to verify complete transection of the femoral vascular bundle and to evaluate placement of the agent and examine for any hematoma formation within tissue planes. Additionally, full necropsy was performed on animals that expired before completion of resuscitation to evaluate for any comorbid illnesses.

Measurements
Vital parameters (heart rate, mean arterial pressure [MAP], oxygen saturation, end-tidal carbon dioxide, respiratory rate, and rectal temperature) were measured every 5 minutes utilizing a Philips MP50 IntelliVue monitoring system (Philips Medical Systems, Böblingen, Germany). Blood was collected from the wound into preweighed suction canisters. Bandages, hemostatic agents, and table linens were weighed before and after use. Degree of hemorrhage was then determined via weight differential. Any additional bleeding after initial control of hemorrhage in the intervention group was collected in a separate suction system. Care was taken to avoid contamination from other body fluids or solids. Peak wound temperature was recorded with a type K thermometer, as mentioned in the third paragraph of Study Protocol.

Primary endpoints for this study included hemorrhage control, rebleeding, and survival. Hemorrhage control was defined as the ability of the intervention to stop bleeding after the initial application. If a wound continued to bleed without any cessation despite application of a hemostatic agent or SD, it was recorded as a failure to control hemorrhage. This was determined by direct visualization. Rebleeding was defined as any visible bleeding around the dressing after an initial period of hemorrhage control. Rebleeding could occur during any phase of the trial up until death or the 180-minute study period. This was also determined by direct visualization. Finally, survival was determined by the death criteria mentioned in the fifth paragraph of Study Protocol.
A power analysis for chi-square with three degrees of freedom, or four groups, was conducted with an assumed treatment effect of 0.50. A sample size of 48 subjects, with 12 in each treatment arm, was needed to achieve adequate statistical power (0.80).

Single-factor analysis of variance (ANOVA) was conducted to test for differences among the groups in mean weight, average peak MAP, preresuscitation blood loss, and total blood loss. Fisher’s least significant difference was performed as a post hoc test for pairwise comparisons of group means following ANOVA. Numerical values appeared to be normally distributed in the treatment groups. Subjects were cross-classified with 4 × 2 contingency tables and chi-squares with one degree of freedom. Single degree of freedom chi-squares were Yates corrected to obtain conservative tests of statistical significance. An alpha level of 0.05 was adopted for all statistical tests.

Before the start of the study, it was decided to exclude animals that did not survive through the resuscitation phase of the protocol and did not rebleed. By choosing this approach, we attempted to separate deaths from failed resuscitation and those from failed hemostasis. This second category is the goal of our study. Animals that die under the above conditions are more likely to have succumbed to massive shock and unsuccessful revival compared to inadequate hemostasis. It is also felt that to properly test the ability of a hemostatic agent, the model must reach a state where adequate hemodynamic resuscitation occurs. If a subject fails to achieve a MAP that reflects normal or above normal physiologic function, a claim of successful hemostasis for a particular agent could not be supported.

RESULTS

A total of 58 animals were procured for this study. All animals were female and between 10 and 14 weeks of age. Mean weights (represented as weight ± SD) of the 48 animals included in this study were 35.5 ± 1.1 kg (range 33.6–36.8 kg) for SD, 34.9 ± 0.7 kg (range 33.6–36.4 kg) for CX, 36.0 ± 1.4 kg (range 34.1–38.2 kg) for HC, and 35.5 ± 1.2 kg (range 34.5–37.7 kg) for QC.

Two animals served as pilot subjects and eight animals were excluded from the study. Of the eight excluded subjects, one received CX application, three received HC, three received QC, and one received SD. All of the excluded animals died within 15 minutes of injury and none rebled following treatment application. Additionally, approximately half of these subjects exhibited some manifestation of cardiac infarction, arrhythmia, or respiratory illness either immediately after the injury or during necropsy.

This model reproduced a severe hemorrhage in each of the subjects. As expected, initial bleeding at time of incision appeared quite brisk and slowed toward the end of the 3-minute bleeding time, consistent with a shift from arterial to venous hemorrhage as a primary source. Mean initial blood loss (represented as volume ± SD) was 48.8 ± 6.3 mL/kg in the SD group, 46.4 ± 5.2 mL/kg in the CX group, 9.448.1 mL/kg in the HC group, and 46.5 ± 4.9 mL/kg in the QC group (p = 0.761). Using an accepted value for the total circulating volume of swine of 70 mL/kg, mean proportional blood loss in each group was 69.7, 66.3, 68.7, and 66.4%, respectively. These values exceeded the accepted 40% blood loss definition for Class IV hemorrhagic shock.

All four dressings were able to control the initial hemorrhage in 100% of cases. However, as the subjects were resuscitated, 10 of 12 (83%, 95% CI = 72.4% to 93.6%) SD animals rebled, and 6 of 12 (50%, 95% CI = 35.9 to 64.1%) of those cases did not reach a secondary hemostasis. At necropsy, surrounding hematoma was apparent in all SD animals that rebled. There were no hematomas apparent in rebleeding animals from other groups. Statistical analysis revealed that each of the three hemostatic agents proved superior to SD with respect to rebleed. Rebleeding occurred in no (0%) CX subjects (p < 0.001), 4 of 12 (33%, 95% CI = 19.7% to 46.3%) HC subjects (p < 0.038), and 1 of 12 (8%, 95% CI = 3.3% to 15.7%) QC subjects (p = 0.001) (Figure 1).

Among the three hemostatic agents, there were significant differences in rebleeding (p = 0.049). HC instances of rebleed were associated with an application of the dressing that adhered tightly to the soft tissue surrounding the vessels but did not seal the actual vascular injury. In HC applications that successfully prevented rebleeding, the dressing was also tightly adhered to the vessels on necropsy.

Survival was determined by a subject’s ability to maintain vital signs for 180 minutes after infliction of the injury. Only CX improved survival significantly compared to SD in this study. Survival was achieved in 100% of CX subjects (p = 0.018), compared to 50% (95% CI = 35.9% to 64.2%) in the SD group. Eight of 12 (67%, 95% CI = 53.7% to 80.3%) HC subjects survived, and 11 of 12 (92%, 95% CI = 84.3% to 99.7%) QC subjects survived (Figure 2). Each death in the HC group was associated with rebleed and failure of the dressing to adhere to the vasculature. It was noted upon necropsy of the sole death within the QC group that the applied agent did not

![Figure 1](image-url)
appear to directly cover the incised vessels, but rather had migrated into an adjacent soft tissue void. Most deaths occurred between 60 and 90 minutes postinjury, and all were associated with rebleeding. Death generally occurred within 20 minutes of rebleeding. Significant differences were found among the hemostatic agents in survival rates ($p = 0.049$).

While preresuscitation blood loss was similar among the four treatment groups, there was a statistically significant difference in total blood loss within the groups. Average total blood loss (represented as volume $\pm$ SD) was $54.0 \pm 7.2$ mL/kg in the SD group, $46.4 \pm 5.2$ mL/kg in the CX group, $50.1 \pm 11.0$ mL/kg in the HC group, and $46.5 \pm 4.9$ mL/kg in the QC group ($p < 0.050$) (Figure 3).

Mean arterial pressure is the best measure of hemodynamic compromise and resuscitation, and continuous monitoring ensured the animal was stable before injury. Stability was determined by assessing MAP every 5 minutes for 15 minutes before the time of injury. Post-resuscitative MAPs were measured continuously for the duration of the procedure. The average trend for each group can be seen in Figure 4. The downward trend for SD and HC within the first 90 minutes includes subjects that died in association with rebleeding. Average peak postresuscitative MAPs (represented as MAP $\pm$ SD) were $60.3 \pm 19$ mm Hg (range 29–84 mm Hg) for SD, $71.6 \pm 13$ mm Hg (range 52–106 mm Hg) for CX, $65.8 \pm 10$ mm Hg (range 52–85 mm Hg) for HC, and $67.3 \pm 13$ mm Hg (range 36–82 mm Hg) for QC. Although the SD group demonstrated the lowest average postresuscitative MAP, there were no significant differences among the four groups ($p = 0.298$). All three hemostatic agent groups achieved a minimum mean postresuscitative MAP greater than 65 mm Hg.

Upon measurement of wound temperatures, only the QC group was found to generate heat. The average maximum temperature in wounds treated with QC, $61.0^\circ C$ was statistically different compared to $37.6^\circ C$ in CX, $38.2^\circ C$ in HC, and $38.8^\circ C$ in SD ($p < 0.001$).

**DISCUSSION**

The objective of this experiment was to compare a new chitosan-based product, CX, and two commonly used hemostatic agents, HC and QC, in a head-to-head trial against standard gauze dressing in a lethal hemorrhagic groin injury. We chose to utilize a validated model for hemostatic research that most clearly represents combat-related groin wound and treatment algorithms. The rationale for duplicating a groin injury reflects current trends in battlefield trauma, where the most common types of injury seen are to the extremities, groin, and axillae.$^{13,14}$ While some wounds are superficial and can be easily abated with direct pressure, tourniquets, or other conventional techniques, we are most concerned with injuries that, through their anatomic location, cannot be controlled. A high groin injury is an example of this type of wound and is the reason for its use in this experiment.

Additionally, the model was intended to produce a lethal hemorrhagic injury and replicate medical response in a battlefield environment as closely as possible. To create a variety of high-pressure/high-flow and low-pressure/low-flow states, bleeding time has varied in previous studies.$^6-10,15-18$ We chose to replicate a 3-minute bleed, which has clearly been demonstrated to produce a fatal wound.$^7$ While we are not aware of any existing field data that document average
time to intervention in the operational environment, we believe that 3 minutes of bleeding is a gross estimate of the likely delay to care encountered on the battlefield. Both limited and delayed resuscitation most closely resemble current recommendations and realities of field combat casualty care. We believe that the incorporation of a hemostatic agent, placement of a compression dressing and limited colloid resuscitation adequately, although not perfectly, reflect these recommendations. Our model demonstrated blood loss, vital sign changes, and mortality rates of SD controls that approximated, or were more severe than, those of previous studies.

In this study, CX behaved in a similar fashion to other chitosan dressings. It did not generate any significant heat during use; average wound temperature was 37.2°C. Additionally, like other chitosan-based products, CX is easily removed. Once reacted, it forms a soft, mildly sticky, gel-like mass that can be removed with manual extraction. Residual material was easily washed from the wound with simple saline lavage. However, we believe one of the greatest benefits of this agent is the ability to employ it in a granular form. A nonexothermic dressing that can conform to wound contours is highly desirable in certain situations. In many ways, CX seems to combine the benefits and eliminate the concerning properties of both HC and QC. It was discovered to be at least as effective as HC and QC in controlling hemorrhage and the only agent to significantly improve survival over SD. However, this statistical superiority in survival over other agents is predicated on one subject. We believe further tests need to be conducted before CX can claim clinical superiority in terms of survival. However, we feel that our study shows that CX is at least as effective as HC and QC in this area.

One unexpected observation noted during the experiment is that the actual reaction layer of CX appears to be approximately 1 mm thick. This characteristic results in the formation of a sphere of unused agent surrounded by a soft “shell” of reacted product. At necropsy, these spheres were opened and the unutilized product was inspected. Anecdotally, the material was reapplied in some pilot animals and appeared to control hemorrhage. This “built-in reusability” that could potentially control unexpected rebleeding identifies a new potentially desirable characteristic in hemostatic agents, not seen in hemostatic dressings currently in use. Further investigation of this property is required.

In this study, subjects treated with HC experienced incidences of rebleed and mortality more often than those treated with either CX or QC and fewer than SD. We found this one-sided wafer to be very effective when it worked as intended; but when it failed, it was fatal. The predominant reason for the wide spectrum of results in this model appears to be the physical form of the dressing. Application of HC was more difficult than that of the other agents. This seems to be due to the rigid nature of the wafer combined with the narrow wound structure and poor visibility. Drawbacks from the inflexibility of this dressing have been observed in other studies as well. Although applied in a consistent manner, the dressing did not always adhere to the incised vessels themselves, but rather to the surrounding tissue. In contrast, successful HC applications were tightly adhered to the vessels. The difficulty in placement suggests that broad and planar vice deep and narrow wounds would be more suitable for HC, when applied in its recommended manner. An inability for universal application may translate to an increased training requirement. HemCon, Inc. has recently indicated that they have modified the SD to make it significantly thinner and more flexible. Some studies have suggested that the effectiveness of this product may be related to the batch production process. However, this assertion was not evaluated in this experiment. Overall, 8 of 12 (67%) subjects treated with HC survived to the end of the experiment, but statistical significance was not achieved when compared to SD.

As mentioned, QC works via an exothermic reaction. In fact, the heat generated at the site of QC application has created concern over thermal injury to human tissue. As has been seen in previous studies, our experiment confirmed a quick, and relatively brief, increase in temperature to an average of 61.0°C. In recent months, Z-Medica has created a new formulation that reportedly does not employ a significant exothermic reaction. One study shows that this new formulation does not produce thermal injury. Recently, QC has proven effective at controlling hemorrhage in several studies. It also performed very well in our experiment. Eleven of 12 (92%) subjects survived the duration of the study, the single death related to an incident of rebleed. Compared to SD, survival of QC subjects was not found to be significant (p = 0.072). At necropsy of this single fatality, it was found that a large majority of the product had migrated into a tissue void lateral to the vascular bundle, leaving only a small amount of product to interact directly over the incised vessels. Although the dressing was applied in accordance with the manufacturer’s instructions, migration of the product likely contributed to this single fatality. While QC is relatively easy to use mechanically, a degree of extra training may be required to make appropriate utilization decisions considering the risk of thermal injury.

LIMITATIONS

While the study was designed to replicate a combat injury and subsequent field medical response, there are various additional factors that may influence the effectiveness of hemostatic dressings in a field environment. The impact of movement during transportation of the patient from the field, for example, was not examined in this study. Likewise, the type, size, and location of the trauma may limit application of some types of agents. This model replicated an injury with a fairly large exposure; however, a design that emulates smaller wound openings more consistent with penetrating injuries from a projectile may yield varying results. For example, lightweight granular products, such as CX and QC, may pose an obstacle to placement. Additionally, secondary components, such as burns and pressure injuries, associated with penetrating and blast injuries, may affect results. High-pressure bleeds may
also yield different results. Finally, due to the inherent differences in the physical and chemical properties of each of these agents, this study could not be blinded. Until a reproducible model that adequately accounts for all of these factors is developed, extrapolation from the animal lab to the battlefield may prove difficult.

A bias could have arisen in the decision to exclude animals that did not complete the resuscitation phase of the protocol. While the rationale for this approach was described above, we performed a secondary analysis that included both study animals and those subjects eliminated due to the exclusion criteria. To determine survival differences among the four groups, a chi-square with three degrees of freedom was conducted to reveal an overall significance of p = 0.052. We subsequently performed a pairwise comparison using Yates’ corrected chi-square to test for differences in survival between CX and SD. Consistent with our study data, the results indicated a significant difference (p = 0.034).

CONCLUSIONS

It is widely recognized and accepted that early control of hemorrhage can improve immediate and delayed mortality through the prevention of massive blood loss, hypotension, coagulopathy, metabolic derangements, and infection.21–23 The results of this study demonstrate that, in a porcine model of uncontrolled hemorrhage, CX improved hemorrhage control and survival. CX is a viable option for the treatment of severe hemorrhage.

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