The Use of Large Animal Models in Trauma and Bleeding Studies

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Hamostaseologie 2023;43:360–373.

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Abstract

Background Major trauma often results in significant bleeding and coagulopathy, posing a substantial clinical burden. To understand the underlying pathophysiology and to refine clinical strategies to overcome coagulopathy, preclinical large animal models are often used. This review scrutinizes the clinical relevance of large animal models in hemostasis research, emphasizing challenges in translating findings into clinical therapies.

Methods We conducted a thorough search of PubMed and EMBASE databases from January 1, 2010, to December 31, 2022. We used specific keywords and inclusion/exclusion criteria centered on large animal models.

Results Our review analyzed 84 pertinent articles, including four animal species: pigs, sheep, dogs, and nonhuman primates (NHPs). Eighty-five percent of the studies predominantly utilized porcine models. Meanwhile, sheep and dogs were less represented, making up only 2.5% of the total studies. Models with NHP were 10%. The most frequently used trauma models involved a combination of liver injury and femur fractures (eight studies), arterial hemorrhage (seven studies), and a combination of hemodilution and liver injury (seven studies). A wide array of coagulation parameters were employed to assess the efficacy of interventions in hemostasis and bleeding control.

Conclusions Recognizing the diverse strengths and weaknesses of large animal models is critical for trauma and hemorrhage research. Each model is unique and should be chosen based on how well it aligns with the specific scientific objectives of the study. By strategically considering each model's advantages and limitations, we can enhance our understanding of trauma and hemorrhage pathophysiology and further advance the development of effective treatments.

Keywords
- trauma
- hemorrhage
- coagulation
- hemostasis
- animal models

Introduction

Traumatic injuries are a leading cause of mortality worldwide, with hemorrhage being the primary cause of preventable deaths during pre-hospital and early resuscitation stages.[1] Hemorrhage accounts for up to 40% of preventable deaths within 24 hours of injury.[2] Coagulopathy, which affects at least one in four seriously injured trauma patients, has a direct relationship with injury severity, massive resuscitation, transfusion, and hemorrhagic shock. Bleeding control and hemostatic resuscitation strategies have proven effective in reducing mortality associated with hemorrhagic injuries. Early hemostatic interventions, including novel dressings, agents, and techniques, are crucial for mitigating hemorrhage and improving survival.

received
May 31, 2023
accepted after revision
July 4, 2023

Georg Thieme Verlag KG,
Rüdigerstraße 14,
70469 Stuttgart, Germany

DOI https://doi.org/10.1055/a-2118-1431.
ISSN 0720-9355.

Large animal models are used to better understand the complex processes of coagulation in trauma and to develop therapeutic interventions. Large animal models, especially pigs, have become essential tools for studying these processes because of their close anatomical and physiological similarities to humans. These models offer a more realistic representation of bleeding and clotting compared with small animal models, making them highly relevant for advancing our understanding of trauma and hemorrhage management.

Over the past several decades, considerable research has been dedicated to studying hemorrhagic shock models to better comprehend trauma pathophysiology and explore various treatment options. These studies often employ isolated models, such as controlled or uncontrolled hemorrhage, or combined injury models involving hemorrhage, bone fractures, and/or abdominal (liver or spleen). However, large animal models face challenges, such as ethical considerations, experimental design variability, species differences, and lack of suitable specific laboratory tests for different species. These difficulties hamper translating findings and therapeutic interventions from animal studies to clinical applications. Thus, there is a need for systematic evaluation of the clinical relevance of existing large animal models in studying hemostasis and bleeding control.

This review article explores isolated or combined trauma models in large animals, focusing on identifying more suitable models for investigating therapeutic approaches to prevent and treat late posttraumatic complications. We discuss the advantages, disadvantages, and challenges of various hemorrhagic shock models across different animal species and purposes while highlighting their potential to improve mechanistic understanding of pathophysiology, facilitate the development of novel therapeutic strategies, and evaluate the effectiveness of existing and emerging interventions.

Method of Literature Review

The methodology employed in this systematic review used two major databases, PubMed and EMBASE, from January 1, 2010, to December 31, 2022, with specific keywords used to narrow down the search to relevant publications. The search used the following keywords: (hemorrhage*[MeSH Terms] OR hemorrhage*[Text Words]) AND (hemorrhage*[MeSH Terms] OR hemorrhage*[Text Words]) OR bleeding*[MeSH Terms] OR bleeding*[Text Words] AND (trauma*[MeSH Terms] OR trauma*[Text Words]) AND (coagulation*[MeSH Terms] OR coagulation*[Text Words]). Small animal models were excluded from the search; NOT (rat[MeSH Terms] OR mice[MeSH Terms] OR mouse[MeSH Terms] OR rodent[MeSH Terms] OR rabbit[MeSH Terms]), with the focus being on large animal models. The inclusion and exclusion criteria were established based on the types of animals, interventions, and study designs. The search was limited to English-language publications with full-text availability. Reference lists of relevant review articles, citations, and Web sites were also screened to ensure completeness. Quality assessment was performed on all relevant studies. Only those that provided original data on hemostasis and coagulation in large animal models or tested new interventions for bleeding and thrombotic disorders in these models were included in this review.

Results

After applying specific inclusion and exclusion criteria, 75 relevant articles were identified and included in this systematic review involving four different animal species. Additionally, nine studies were identified from the reference lists of other research sources initially missed in the search process (►Fig. 1). The porcine model was the most commonly used, with 72 studies, followed by nonhuman primates (NHPs) with 8 studies, and sheep and dogs with two studies each.

The studies under review utilized a range of parameters to assess the effectiveness of interventions on hemostasis and bleeding control. Physiological responses to interventions were evaluated by consistently measuring blood cell count, blood gas analysis, and hemodynamic and laboratory parameters, such as mean arterial pressure (MAP) and heart rate. Plasma-based coagulation assays, namely prothrombin time (PT), were measured in 54 studies and activated partial thromboplastin time (aPTT) in 48 studies. Viscoelastic assays, including rotational thromboelastometry (ROTEM) and thromboelastography (TEG), were extensively used in 37 and 20 studies to assess coagulation in whole blood. Coagulation activation markers, including thrombin–antithrombin (TAT) complex and D-dimer, were assessed in 18 studies, while prothrombin fragment 1 + 2 and fibrinopeptide A were evaluated in 3 studies each. Fibrinogen levels and thrombin generation (TG) were also evaluated in 27 and 14 studies, respectively, providing crucial information on TG during massive bleeding and fibrinogen availability plus consumption for clot formation. The calibrated automated thrombogram (CT), according to Hemker,[107] was the most frequently used method for TG measurement.[3–16] Platelet function was measured in 11 studies by Multiplate,[17–23] flow cytometry,[3,24] and platelet aggregometry.[25,26]

The majority of studies evaluated the effectiveness of different interventions in the management of bleeding. Thereby a reduction in blood loss served usually as the primary endpoint, with secondary endpoints encompassing assessments of coagulation parameters, hemodynamics, and pathological evaluations. Researchers used various types of hemorrhage models, controlled and uncontrolled hemorrhage models,[27] such as liver injury, femur fractures with or without hemorrhage, arterial hemorrhage, blunt chest injury with liver laceration, traumatic brain injury, and soft tissue injury. To study the impact of contribution factors causing coagulopathy, such as hemodilution (10 studies),[14,18,28–32] hypothermia (10 studies),[5,6,33–39] and acidosis, some of the trauma models incorporated those. Hemodilution models, frequently used alongside other hemorrhage models like liver injury,[5,6,8,10,40–42] provide a means of identifying potential interventions to enhance coagulation and minimize bleeding. Targeted interventions, such as the substitution of fibrinogen concentrate,[8,42,43] negative pressure wound therapy,[34] and prothrombin complex concentrate (PCC),[4,5] have been demonstrated to impact hemodilution-induced coagulopathy.

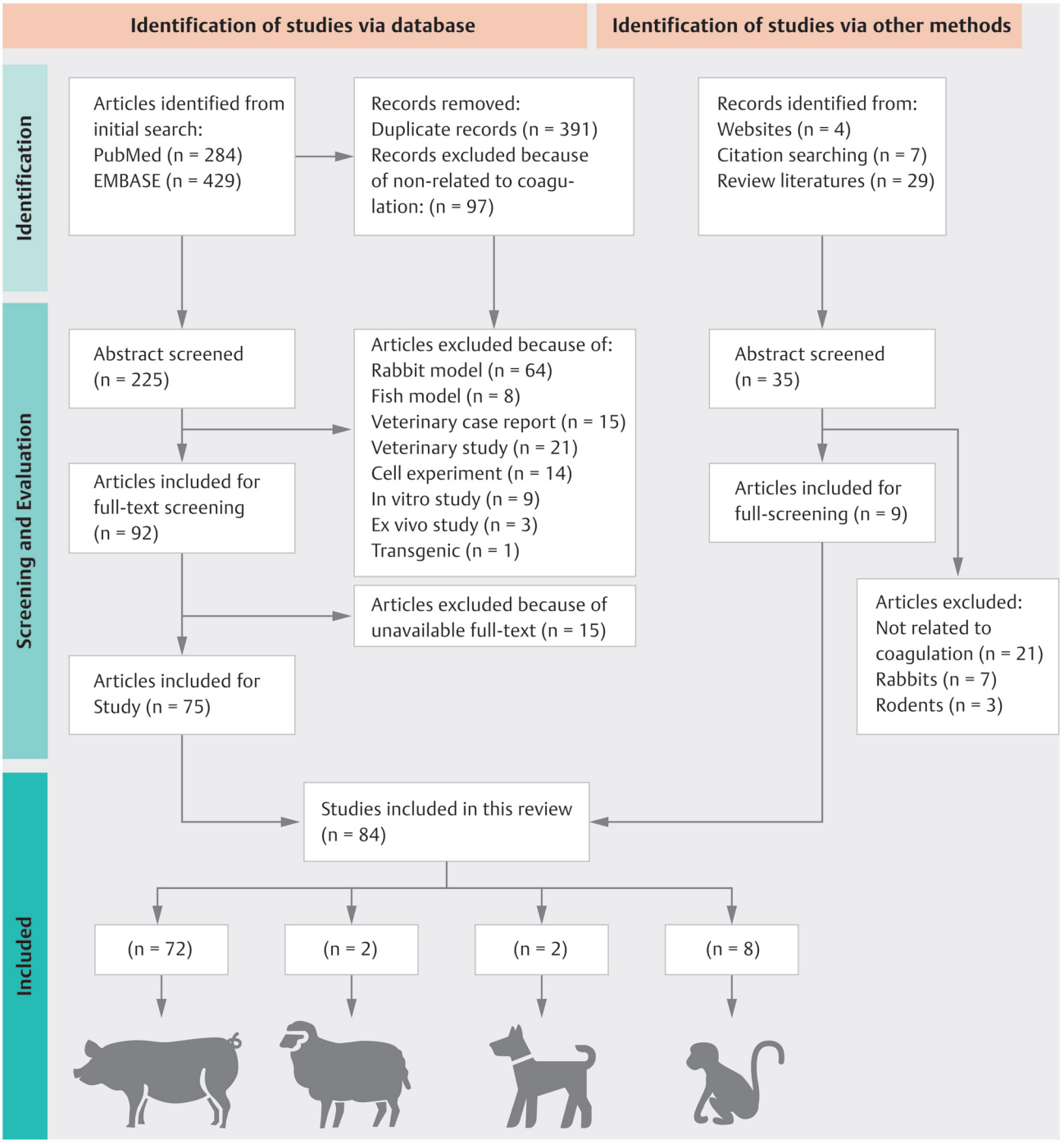

Fig. 1 Schematic diagram of the review process. This figure provides a visual representation of the systematic process used in this review article, outlining the steps taken from database search to final analysis. It showcases the rigorous methodology employed to identify, screen, and assess the relevant studies on large animal models in trauma and bleeding research.

►**Tables 1** and **2** summarize hemorrhage models employed in pigs, sheep, dogs, and NHPs, with detailed information on primary and secondary endpoints and the number of animals utilized in each study.

Models of Controlled Hemorrhage

Well-defined and reproducible trauma models are crucial in preclinical research to allow for a better understanding of the pathophysiology of hemorrhagic shock and the identification of potential treatments. Controlled hemorrhage models like fixed-volume and fixed-pressure ones offer high reproducibility, leading to consistent results and facilitating inter-study comparisons. Researchers often employ a hybrid strategy, combining pressure and volume control, for instance starting with a volume-controlled setup (e.g., withdrawing 35% of the total blood volume) and ceasing it when the MAP drops below a predetermined threshold.[44] Some studies, however, adopt the reverse approach.[45]

Table 1 Trauma model used in pigs

Model classification	Model of trauma	Principal outcome[a]	Peripheral outcome[a]	Coagulation tests and markers measurements	(Animals in each study)
Fixed-volume hemorrhage	Hemorrhage (30–40%)	Impact on coagulation	–	PT,[48,103,104] aPTT,[48,103,104] fibrinogen,[48,103,104] TEG,[48] ROTEM,[103,104] TAT,[48,104] (ACT, TG, coagulation factors)[48]	(60),[103] (48),[104] (16),[47] (14)[48]
	Hemorrhage (>40%)	Blood loss/survival	Impact on coagulation	PT,[30] PTI,[49] aPTT,[30,49] fibrinogen,[30,49] TEG,[30] ROTEM[49]	(20),[30] (24),[49] (15)[50]
Fixed-pressure hemorrhage	Arterial hemorrhage	Blood loss/survival rate	Impact on coagulation	PT,[31,53,58] aPTT,[31,53,58] ACT,[52] fibrinogen,[31,53,56,58] D-dimer,[31,53] ROTEM,[31] (ATIII, TAT),[53] TEG,[54] ROTEM[56]	(20),[52] (40),[31] (30),[53] (8),[54] (16),[55] (8),[56] (36)[58]
Fixed-volume and fixed-pressure hemorrhage	Hemorrhage (35%)+ MAP < 50 mm Hg	Metabolomic changes	–	NA	(12)[44]
Uncontrolled hemorrhage	Liver injury (grade III)	Blood loss/survival time	Impact on coagulation	PT,[3,60] aPTT,[3,60] ROTEM,[3,60] fibrinogen,[3,60] (TAT, D-dimer, FPA, TG, FC),[3] TEG[60]	(36),[3] (14)[60]
	Liver injury (grade V)	Blood loss/survival time	Impact on coagulation	TEG[61]	(27),[61] (19)[62]
	Blunt liver injury (not specified grade)	Blood loss/survival/ hemostatic effect	Impact on coagulation	PT, aPTT, ACT, ROTEM, TG, fibrinogen[9]	(18)[9]
	Hemodilution + liver injury	Blood loss/survival	Impact on coagulation	PT,[5,6,8,10,40–42] aPTT,[5,6,8,40–42] fibrinogen,[6,8,40–42] TAT,[5,6,8,10,42] ROTEM,[5,6,8,40–42] TG,[5,6,8,10] FC,[8] (D-dimer, FII, TG),[10] ATIII[5]	(20),[8] (18),[40] (28),[6] (32),[10] (14),[5] (9),[41] (18)[42]
	Spleen injury	Blood loss/survival	Impact on coagulation	ROTEM, TEG[67]	(44)[67]
Combined uncontrolled hemorrhage and soft tissue injury	Blunt liver injury + femur fractures	Blood loss/impact on coagulation	Hemodynamics/ survival rate/thrombin generation	PT,[7,11–16,25] aPTT,[7,11–14,16,25] ROTEM,[7,11–14,16,25] fibrinogen,[7,11–16] D-dimer,[7,11,13–16,25] TAT,[7,11,12,14–16,25] TG,[11–16,25] TT,[25] ACT,[7] FPA,[7,11] ATIII,[12–15] TEG[14]	(48),[16] (21),[25] (32),[7] (45),[11] (28),[13] (21),[12] (63),[15] (28)[14]
Combined soft-tissue injury and fixed-volume hemorrhage	Hemorrhage (30–50%) + femur fractures	Blood loss	Impact on coagulation	aPTT,[81,82] fibrinogen,[81,82] ROTEM,[81] (PT, TEG)[82]	(18),[81] (33)[82]
		Impact on coagulation	–	PT,[83–85] aPTT,[83–85] fibrinogen,[83–85] TEG,[83,84] ROTEM[85]	(23),[83] (24),[84] (30)[85]
	Hemorrhage (>50%) + femur fractures	Blood loss/survival time	Impact on coagulation	PT,[86,89] aPTT,[86,89] fibrinogen,[86,87,89] ROTEM,[86] TEG,[87,89] coagulation factors[87]	(17),[86] (21),[87] (57)[89]
		Fibrinogen synthesis	–	PT, aPTT, fibrinogen, TEG[88]	(14)[88]
		Impact on coagulation	Survival	PT, aPTT, fibrinogen, D-dimer, coagulation factors, ROTEM[32]	(20)[32]
	Hemorrhage (30–50%) + liver injury	Impact on coagulation	Systemic inflammation	PT[91]	(24)[91]
		Blood loss		NA	(22)[65]

(*Continued*)

Table 1 (*Continued*)

Model classification	Model of trauma	Principal outcome[a]	Peripheral outcome[a]	Coagulation tests and markers measurements	(Animals in each study)
			Hemodynamic response + rebleeding		
	Femur fracture + liver injury + hemorrhage to MAP (40 mm Hg)	Physiologic parameters	Impact on coagulation	PT, aPTT, fibrinogen, D-dimer, ATIII, TEG[105]	(22)[105]
	Pulmonary contusion + liver injury + hemorrhage (30–50%)	Blood loss	Impact on coagulation	(PT, fibrinogen, ROTEM, Multiplate),[17] TEG[92,93]	(40),[17] (36),[92] (14)[93]
		Blood loss/survival	Liver, renal functions, and impact on coagulation	PT[45]	(30)[45]
	Pulmonary contusion + liver injury + blast groin injury + hemorrhage (60%)	Impact on coagulation	Viscoelastic parameters	PT, aPTT, fibrinogen, D-dimer, FC, ROTEM, tPA, PAI-1, aPC, prothrombin[24]	(26)[24]
	Femur fracture + hemorrhage (60%) + liver injury + hypothermia	Blood loss/survival	Impact on coagulation	TEG,[33,36] fibrinogen,[33,35] INR,[36] coagulation factors,[35] (PT, aPTT, ACT)[33]	(20),[36] (52),[35] (32)[33]
		Inflammation + impact on coagulation	Organ tissue damage	ROTEM, ATIII, aPC, procoagulant/anticoagulant gene markers[37]	(24)[37]
		Impact on coagulation	Plasma proteome	NA	(9)[38]
Combined TBI and hemorrhagic shock/soft tissue injury	Trauma brain injury + hemorrhage (40%)	Impact on coagulation	–	(sTM, ATIII, D-dimer, aPC, vWF, PAI-1, TF, PF 1 + 2),[74] (aPC, tPA, PF 1 + 2, E-selectin, ICAM),[75] (PT, TEG)[76]	(33),[74] (15),[75] (13)[76]
		Survival rate	–	NA	(12)[77], (22)[78]
		Platelet function	–	P-selectin,[18,79] TGF-β1,[18,79] CD40L,[18,79] (PECAM-1, CD61, CD62P)[79] (Multiplate, TEG)[18]	(10),[79] (33)[18]
	Trauma brain injury + liver injury	Blood loss/survival rate	CNS pathophysiology	PT, PFA, TEG[80]	(26)[80]
	Uncontrolled hemorrhage (250 mL) + trauma brain injury + hemorrhage (40%) + rib fracture	Platelet function	–	Multiplate, TEG, P-selectin, TGF-β1, CD40L, VCAM-1, fibrinogen[19]	(12)[19]

Table 1 (*Continued*)

Model classification	Model of trauma	Principal outcome[a]	Peripheral outcome[a]	Coagulation tests and markers measurements	(Animals in each study)
Hemodilution with or without hypothermia	Hemodilution	Blood loss/survival rate	Impact on coagulation	PT, aPTT, FVII/VIIa, TG[4]	(26)[4]
		Plasma fibrinogen level	Impact on coagulation	PTI, aPTT, ROTEM, fibrinogen[43]	(12)[43]
	Hemodilution + hypothermia	Blood loss	Impact on coagulation	PT,[34,39] aPTT,[34,39] TEG,[39] fibrinogen[34]	(26),[39] (38)[34]

Methodology abbreviations: ACT, activated clotting time; aPTT, activated partial thromboplastin time; FC, flow cytometry; INR, international normalized ratio; Multiplate, Multiplate analyzer; NA, not applicable for this study; PT, prothrombin time; PTI, prothrombin time index; ROTEM, rotational thromboelastometry; TBI, traumatic brain injury; TEG, thromboelastography; TG, thrombin generation; TT, thrombin time.
Markers abbreviations: aPC, activated protein C; ATIII, anti-thrombin III; CD40L, CD40 ligand; FII, coagulation factor II; FVII/VIIa, coagulation factor VII/activated factor VII; ICAM, intercellular adhesion molecule 1; PAI-1, plasminogen activator inhibitor-1; PECAM-1, platelet endothelial cell adhesion molecule; PF 1 + 2, prothrombin fragment 1 + 2; PFA, fibrinopeptide A; sTM, soluble thrombomodulin; TAT, thrombin-antithrombin; TF, tissue factor; TGF-β1, transforming growth factor-β; tPA, tissue plasminogen activator; VCAM-1, vascular cell adhesion molecule 1; vWF, von Willebrand factor.
[a]The principal and peripheral outcomes were not explicitly stated in all the included studies. Therefore, the outcomes reported in this table were inferred from the results and conclusions of the studies.
Ref = References.

Fixed-Volume Models

The fixed-volume model is widely utilized in hemodynamic research to investigate blood loss responses. This model involves the controlled removal of a predetermined blood volume, with model variations reflecting differences in blood volume removal, severity, and duration of hemorrhage. Despite its use and contribution to our understanding of shock, metabolic changes, hemodynamic imbalances, and their effects on coagulation and fluid resuscitation, this model has limitations. It does not completely replicate trauma-related tissue factor release and coagulation activation and considerations such as species-specific hypoxia tolerance. Also, inconsistent relationships between body weight and blood volume add further complexity.

The Advanced Trauma Life Support (ATLS) defines class IV hemorrhagic shock as a situation in which the bleeding surpasses 40% of the estimated circulating blood volume. This severe blood loss is linked to a mortality rate exceeding 30% in humans.[46] In response to the need for effective study methods on coagulopathy and shock physiology, researchers have extensively used pig hemorrhage models. These models, which emulate blood loss of 30 to 40%[47,48] and more than 40%,[30,49,50] have been proven successful in consistently inducing hypotension and organ dysfunction, simulating the conditions in human patients. Various studies have examined the influence of differing fluid resuscitation strategies on coagulation in these hemorrhage models. One significant finding was that applying lactated ringers (LR) for resuscitation led to a substantial decrease in coagulation factors and subsequent TG, even though hemodynamics stabilized within 2 hours. Additionally, fibrinogen concentrations and platelet counts experienced a sharp decline after hemorrhage.[30,48] Interestingly, fibrinogen levels rebounded more quickly than platelet counts, highlighting the vital role of fibrinogen in restoring hemostasis and its functionality as an acute-phase protein.[48]

In contrast to the studies conducted using pig hemorrhage models, researchers have also been investigating therapeutic interventions in NHP model of 45% hemorrhagic shock.[51] Specifically, employing compstatin Cp40, a potential therapeutic agent, was able to improve immune response, coagulation, and organ function, while also preserving organ-barrier integrity after traumatic hemorrhagic shock.[51] This suggests that Compstatin Cp40 may have promising therapeutic potential.[51]

Fixed-Pressure Models

In the fixed-pressure model, the procedure involves constant blood extraction until a target average arterial blood pressure is reached. This model is extensively used in arterial hemorrhage research to evaluate the effectiveness of various hemostatic dressings and gauze in controlling bleeding following severe trauma.[31,52–58] Numerous studies have evidenced that certain hemostatic dressings (e.g., amylopectin, chitosan, and micronized purified polysaccharide),[52] or the direct local infusions of recombinant human activated factor VII (rFVII)[54] into a major

Table 2 Most common trauma models in sheep, dogs, and nonhuman primates

Model of trauma	Species	Principal outcome[a]	Peripheral outcome[a]	Coagulation tests and markers measurements	(Animals in each study)
Lung lobe contusions + bilateral tibial fractures + soft tissue injury in hamstring region + 20–30% hemorrhage	Sheep	Developing an ovine model of trauma and hemorrhage	–	PT, aPTT, D-dimer, fibrinogen, Multiplate, ROTEM, FV, FVIII, aPC, PAI-1, sTM[23]	(12)[23]
Paranasal sinus injury + open surgical carotid injury		Hemostasis achievement	–	PT, aPTT, fibrinogen, D-dimer[57]	(14)[57]
Liver + heart injury	Dog	Hemostasis effect on liver and heart bleeding	Adhesive effect of study material	CT, coagulation factors activity[106]	NA[106]
Spleen injury		Blood loss	Impact on coagulation	Hemostatic time[68]	(56)[68]
Uncontrolled liver hemorrhage	Nonhuman primate	Blood loss	Impact on coagulation	PT, aPTT, ATIII, fibrinogen, D-dimer, vWF, ROTEM, Multiplate[26]	(16)[26]
Midline laparotomy incision + an open mid-femur fracture		Blood loss/survival	Impact on coagulation	PT,[20,21] aPTT,[20,21] ATIII,[20,21] fibrinogen,[20,21] D-dimer,[20] vWF,[20] ROTEM,[20,21,70,71] Multiplate[20,21,70]	(24),[20] (12),[21] (40),[70] (12)[71]
		Fibrinolysis phenotypes	–	PT, aPTT, ATIII, fibrinogen, D-dimer, vWF, ROTEM[72]	(24)[72]
		Platelet function	Metabolic changes	Multiplate[22]	(27)[22]
Hemorrhage (45%)		Inflammation C3 blockade by Compstatin Cp40	Impact on coagulation + pathological changes	PT, aPTT, ROTEM[51]	(8)[51]

Methodology abbreviations: aPTT, activated partial thromboplastin time; CT, clotting time; Multiplate, Multiplate analyzer; NA, not applicable for this study; PT, prothrombin time; ROTEM, rotational thromboelastometry.
Markers abbreviations: aPC, activated protein C; ATIII, antithrombin III; FV, coagulation factor V; FVIII, coagulation factor VIII; PAI-1, plasminogen activator inhibitor-1; sTM, soluble thrombomodulin; vWF, von Willebrand factor.
[a]The principal and peripheral outcomes were not explicitly stated in all the included studies. Therefore, the outcomes reported in this table were inferred from the results and conclusions of the studies. Ref = References.

artery, can strengthen clots. This could widen the safety margin, even in high blood pressure cases. Innovative approaches such as modified setons infused with procoagulant[53] and cellulose sponges[55] have proven successful in preserving the balance between fibrinogenesis and fibrinolysis[53] and managing severe noncompressible hemorrhage.[55] Hemostatic dressings that utilize self-propelling tissue technology have successfully stopped bleeding in sheep models with minimal side effects and negligible risks of thrombosis.[57] Another development includes the FeiChuang hemostatic gauze, which was more effective at reducing blood loss in a gunshot wound model than Combat or standard medical gauze.[58] In contrast, certain novel hemostatic gauzes proved to be just as effective as the current standard for controlling bleeding at the point of injury.[31]

Models of Uncontrolled Hemorrhage

Compared with controlled hemorrhagic shock models, models of uncontrolled hemorrhage allow a more realistic clinical scenario with major trauma and uncontrolled bleeding. These models permit unrestricted bleeding independent of hemodynamics and are often used to investigate various fluid resuscitation strategies, assessing their effects on animal survival, blood loss, and hemodynamic parameters.[59] However, a significant drawback of these models is the inability to control the extent of blood loss that occurs, which could potentially limit their usefulness in specific experimental conditions.

Liver/Spleen Trauma Models

The induction of uncontrolled hemorrhage by liver injuries represents a commonly employed model. Typically, these

injuries involve liver injuries with different degrees of severity, ranging from grade III[60] to V[61,62] injuries, double liver injury,[3,9] or combined injuries with hemodilution[5,6,8,10,40–42] or femur fractures.[7,11–16,25,63] Grade III liver injuries are characterized by deep parenchymal laceration exceeding 50% of the surface area of ruptured subcapsular, resulting in moderate to severe disturbances in coagulation parameters.[64] Similarly, grade V liver injuries are associated with a high risk of exsanguination and increased mortality rates, necessitating timely and effective management strategies.[64]

Various methods have been developed to induce liver injuries in pigs. These methods include controlled mechanical impact with laparotomy,[3,5–9,11–16,25,40–42,60,63,65] closed-cavity injury models,[61,62] and surgical knives.[10] The closed-cavity injury model has been utilized to mimic noncompressible intra-abdominal hemorrhage,[61,62] while surgical knives have been used to simulate uncontrolled bleeding by removing a portion of the liver lobe.[10]

To make animal models more applicable to studying traumatic liver injury, a laparoscopic hepatectomy model in NHP was investigated.[66] This model allowed for observing both acute and long-term responses and compatibility with human-derived treatments, with physiologic, metabolic, coagulation, and inflammatory changes resembling those seen in trauma patients.[66] Despite alterations in coagulation parameters being detected, the model did not induce clinically significant coagulopathy.[66] Additionally, the same research group in another study found the absence of thrombocytopenia in this model, emphasizing the importance of appropriate animal model selection for hemostatic treatment studies.[26]

Grottke et al devised a model of blunt liver injury, enabling consistent injuries of different severities that lead to substantial alterations in all coagulation parameters, imitating the injury and treatment stages of severe trauma, including elements that promote coagulopathy, such as hypothermia, acidosis, and hemodilution.[41] The severity of liver injury significantly impacted coagulation parameters, with more severe injuries leading to greater disturbances.[41] This model has been used to investigate the effectiveness of various interventions, including direct oral anticoagulants (DOACs)-specific reversal.[3,9] Due to the adaption of the severity of liver injury, this model allows simulation of the continuous bleeding in anticoagulated pigs.

Spleen injury models are utilized to investigate noncompressible hemorrhage and evaluate the long-term effects of hemorrhage and resuscitation.[67,68] Noncompressible hemorrhage is a leading cause of preventable death in trauma situations; thus, this model is particularly relevant given the spleen's susceptibility to injury in abdominal trauma.[69] One study demonstrated that tranexamic acid (TXA) was ineffective in reducing blood loss in a pig model despite inhibiting fibrinolysis, suggesting that the blood loss induced by this model may be too severe to investigate only an antifibrinolytic.[67] Another study employed ultrasound visualization in dogs to develop an accurate model of splenic artery hemorrhage and reported promising results with microwave coagulation therapy as an alternative to thrombin injection for treating splenic hemorrhage.[68] However, the spleen injury model is nonlethal, which may limit its ability to simulate the severity of noncompressible hemorrhage in humans.

Combined Models of Hemorrhage

Hemorrhage rarely occurs in isolation in real-world medical scenarios. Therefore, realistic trauma models should consider factors that influence injury severity and response, replicating human injuries with significant blood loss, hemodynamic and metabolic changes, including shock, coagulopathy, and immune responses. Clinically, hemorrhagic shock following major trauma often occurs with other traumatic injuries, leading to shock-related organ dysfunction due to the release of cytokines and other mediators.[29] Combined models incorporating multiple injury types, including substantial bleeding, present a more accurate approach to studying trauma and evaluating therapeutic strategies. However, the development of these large animal models presents a challenge as it requires a careful balance between the severity of injuries and the prevention of immediate blood loss to facilitate the study of hemostatic interventions. Hence, these models may be particularly valuable in clinical scenarios involving trauma patients.

Liver Injury Combined with Femur Fractures

To investigate coagulation and hemostasis in response to combined traumatic insults, a combined liver injury model with femur fractures has been successfully used in pig[7,11–16,25] and rhesus macaques.[20–22,70–72] Anticoagulated pig polytrauma models have become prevalent in simulating life-threatening bleeding in trauma patients undergoing anticoagulation treatments. Various treatments, such as TXA,[16] fibrinogen concentrate,[16] nonspecific hemostatic agents (PCCs[7,11,15,16] and activated PCC [aPCC][25]), have been investigated in these models. Moreover, several studies have utilized this model to explore various strategies for managing massive bleeding associated with DOACs, specifically employing specific antidotes such as idarucizumab[11,12] or andexanet alfa.[14] An experimental model incorporating femur fractures and double liver trauma has been utilized to assess the efficacy of two distinct treatments in managing bleeding within this context.[13] Moreover, this model provides a useful tool for determining optimal treatment dosages and assessing the safety of combining medications.[13] Additionally, an NHP model of trauma has been developed to test potential therapeutic interventions, specifically investigating trauma-induced platelet dysfunction and acute suppression of fibrinolysis in the presence of tissue injury during hemorrhagic shock.[70]

Trauma Brain Injury Combined with Hemorrhage and/or Tissue Injury

Traumatic brain injury (TBI), accounting for 30% of all injury-related mortalities, frequently occurs together with hemorrhagic shock.[73] This cooccurrence immediately triggers the coagulation and complement systems, leading to subsequent endothelial shedding, activation of protein C, and inflammatory response.[74] To investigate the effects of TBI and hemorrhage on coagulation and complement systems, several

studies have used combined models, which produced a hypocoagulable state with reduced clot strength and thrombocytopenia.[18,19,74–80] The fluid percussion (employs a swift fluid injection to damage the dura),[77,80] controlled cortical impact (using an electromagnetic device for brain penetration),[18,19,74,75,79] rotational acceleration head injury (utilizing a pneumatic device),[78] and blast injury models[76] are fundamental models in TBI research. Each model offers unique insights into different forms of brain injury, furthering our understanding of injury impacts and potential treatment efficacy. Collectively, they replicate a wide range of brain injuries, from concussive impacts to rotational forces and blast injuries, reflecting diverse real-world scenarios. Various resuscitation strategies have demonstrated promising results in these models. FFP resuscitation enhances platelet function and clot strength.[19] Adding valproic acid to FFP results in early upregulation of platelet activation.[79] Moreover, using hemoglobin-based oxygen carriers has improved brain oxygenation without negatively impacting coagulation and hemodynamics.[80] Permissive hypotension has also been observed to enhance survival, hemodynamics, and the restoration of cerebral oxygenation in severe head injuries.[77] While previous large animal models of TBI and hemorrhagic shock have primarily used controlled blood loss models that did not fully simulate diffuse injuries following rapid cranium acceleration or deceleration, recent studies with a closed-head, dynamic acceleration model have revealed diverse survival rates associated with varying levels of controlled blood loss.[78]

Hemorrhage Combined with Femur Fractures

Simultaneous femur fracture and hemorrhage can cause significant tissue damage, inflammation, and impaired coagulation due to the release of bone marrow debris and fat into the bloodstream, activating the coagulation system and leading to disseminated intravascular coagulation (DIC) syndrome. Preclinical studies have primarily focused on evaluating the combined effects of trauma and hemorrhage on coagulation impairments, clotting status, and blood loss.[32,81–88] Hemorrhage severity greatly impacts the metabolic markers,[86,87] physiological and coagulation responses observed in animal models, with varying percentages used to mimic different degrees of blood loss seen in trauma patients. Studies have found that hemorrhage and tissue injury significantly reduced arterial blood pressure, cardiac index, hemoglobin, hematocrit, and platelet count. Coagulation function was affected by apheresis, hemorrhage, and resuscitation with different solutions, with a reduction in fibrinogen levels resulting in a significant decrease in clot strength.[81–84] Therapy with high-dose fibrinogen concentrate and the transfusion of platelets were found to restore coagulation function.[85] Also, resuscitation with shed whole blood or FFP has demonstrated greater efficacy than PlasmaLyte in maintaining blood pressure and TEG maximum amplitude.[89]

Hemorrhage Combined with Liver Injury

These models simulate hypovolemia and hemodynamic instability due to hemorrhage, severe bleeding, and coagulopathy resulting from liver injury.[90] Studies have evaluated interventions under varying degrees of hemorrhage severity to gain insight into their potential impact. Several experimental porcine studies have compared the effectiveness of different perihepatic packing techniques[65] and coagulation factor therapies.[91] These interventions have shown promising results in reducing blood loss,[65] improving survival,[65] and attenuating the development of acute trauma coagulopathy and systemic inflammation.[91]

Blunt Chest Injury Combined with Hemorrhage and Liver Injury

This trauma/hemorrhage model is utilized to induce endotheliopathy of trauma, which leads to coagulopathy and multiple organ failure, including acute respiratory distress syndrome.[17,24,45,92,93] Previous studies utilizing this model successfully induced TIC, as evidenced by significant differences in TEG parameters[92] and principal component analysis of ROTEM.[17] PCC induced more rapid clot propagation than FFP in treating coagulopathy in pigs but resulted in lower clot strength and a higher degree of clot lysis at later time points, indicating a delayed consumptive coagulopathy.[92] Furthermore, studies have demonstrated that the ovine model of traumatic coagulopathy is essential for exploring the complex interactions between hemodynamic, metabolic, and coagulation functions in sheep.[23]

Hemorrhage Combined with Femur Fractures, Liver Injury, and Hypothermia

Animal models that simulate multiple traumatic injuries are useful for studying the complex interactions between injuries that can exacerbate tissue ischemia, organ dysfunction, and failure. Porcine models have been widely used to investigate the effects of various treatments such as intravenous vitamin C administration,[37,38] lyophilized plasma administration,[35,36] and resuscitation fluid regimens[33,36] on inflammation,[35,36] coagulation function,[33,35,36] plasma proteome,[38] and end-organ histology.[37] Studies using these models have shown that high-dose vitamin C[37] and lyophilized plasma resuscitation fluids[35,36] have beneficial effects on coagulation function and inflammatory markers. A 1:1 ratio of plasma to red blood cells[33] and rFVIIa administration[28] have also shown promise in reducing blood loss and restoring abnormal coagulation function in coagulopathic pigs.

Discussion

In this review, we evaluated various animal models used in trauma and bleeding research to identify areas for improvement and provide insights into more clinically relevant and reliable models. Essential criteria for clinically relevant trauma models include significant tissue injury, severe bleeding, shock, and a level of trauma severity that closely reflects real-life clinical situations.[94] Ideally, these models also consider a realistic time frame before resuscitation is initiated.[94] However, achieving these parameters is often challenging, particularly in models simulating severe hemorrhage. The extent of blood loss in these models can significantly affect the survivability of the animal, and without immediate

intervention, such as the administration of blood products like red blood cells, survival duration may be markedly limited.

Several models have been used to mimic clinical scenarios and to understand the pathophysiological aspect of traumatic insults. Controlled hemorrhage models that use MAP as the variable are considered more clinically relevant and reliable than fixed-volume models. Although fixed-volume models offer the advantage of assessing compensatory hemodynamic mechanisms, they produce variable outcomes due to the undefined degree of hypotension.[29] On the other hand, while fixed-pressure models offer better control over hypotension, uncontrolled hemorrhage models provide a more realistic representation of real-life situations.[29] Standardized traumatic insults have been developed to improve the reliability of uncontrolled hemorrhage models. Effective treatment for both models should address alterations in coagulation through standard-of-care resuscitation.

While investigating pathophysiological changes following trauma and shock, it is also important to consider differences between species. NHP models have been shown to share many similarities with human physiology in critical areas such as pharmacokinetics, pharmacodynamics, immunology, genetics, and hemodynamics. Also, Tarandovskiy et al found that humans, baboons, and rhesus macaques had the most similar simultaneous thrombin and plasmin generation assay parameter values compared with other species.[95] Despite this, only a small percentage (10%) of studies reviewed here used NHPs as research subjects due to ethical considerations, animal welfare concerns, costs, and availability.

Sheep have been identified as a valuable animal model for coagulation and hemostasis, given their physiological similarities to humans in routine and certain coagulation tests.[96] These similarities include comparable cardiorespiratory and hemostatic functions, as evidenced by similar results in tests such as PT, aPTT, fibrinogen assays, as well as ROTEM parameters.[23,96,97] Sheep models have been used successfully to mimic the trauma-induced coagulopathy (TIC) and involvement of the activated protein C (aPC) pathway, characterized by hyperfibrinolysis and depletion of factor V.[23] However; TG data show differences in endogenous TG potential between sheep and humans. Sheep exhibit markedly accelerated TG kinetics compared with humans, likely due to their higher sensitivity to the human tissue factor used in the assay.[97] Sheep exhibit distinct differences in secondary hemostasis compared with humans. These include the rapid initiation of the contact activation pathway, elevated levels of factor VIII, low levels of protein C, increased clot firmness, and reduced capacity for clot lysis.[96] Limitations of using sheep as a model included cost, size, and the lack of breed-specific reference ranges in coagulation. Although dogs are one of the species most similar to humans in coagulation, especially in the acceleration of coagulation, there are still significant differences between dogs and humans, particularly in the extrinsic activation of coagulation.[98] Moreover, using dogs in experimental studies is restricted due to ethical concerns about their status as companion animals.

Pigs are the predominant animal model used in hemostasis and coagulation research, accounting for 85% of studies in this review. Pigs remain a popular choice in preclinical research due to their similarities to humans in organ size, blood volume, some functional coagulation proteins, and hemodynamic response.[29,59,94] In the context of TIC, research involving pigs has highlighted the critical role of aPC and its response following TBI and hemorrhage.[75] These findings suggest aPC operates as a compensatory mechanism in response to activated coagulation in TIC and inflammatory pathways, thereby affirming the relevance of pigs in coagulation research.[74] However, inducing coagulopathy in pigs can be challenging. These arise from differences in compensatory mechanisms, variations in vasopressor receptors, and differences in inflammatory and immunological responses between pigs and humans.[29] Some studies have introduced hemodilution prior to injury to establish a standardized coagulopathy.[41] However, this approach might not accurately represent what occurs in real clinical situations, especially when permissive hypotension is part of the treatment process.

Compared with humans, pigs have shown a lower TG potential[95,99] and maximum clot lysis,[99] pointing to the differences between coagulation pathways of humans and pigs. Results from ROTEM demonstrate variability in the coagulation state of the pig model as a hypercoagulable species.[97,100] These variations depend on the specific test (EXTEM[100] or NATEM[97]; considering clotting time, maximum clot firmness (MCF), and clot formation time), and other experimental factors such as pig breed, age, and the anesthesia protocol used.[99,100] Furthermore, FibTEM testing has shown that pigs have higher fibrinogen levels with less platelet contribution to clot strength compared with humans, leading to a higher MCF.[99] Regarding the similarities of fibrinolytic pathways between pigs and humans, thromboelastographic findings suggest a pronounced delay in clot lysis in porcine whole blood compared with human blood.[101] However, other studies have noted similar D-dimer reference intervals in both species, indicating comparable fibrinolysis.[100] When utilizing pigs as experimental models for fibrinolysis studies, researchers must be cautious due to the species propensity for thrombus formation in smaller vessels and arteries, as well as specific markers measured in the study. Moreover, variations in study outcomes underscore the necessity for meticulous methodological considerations when comparing results across studies. For instance, differing tissue factor concentrations used in TG assays may complicate comparisons.

Large animal models allow researchers to conduct controlled experimental studies, enabling them to adapt the experimental variables and assess interventions with high precision. Nevertheless, it is crucial to recognize the limitations associated with translating findings from animal models to real-world clinical scenarios. Animal models cannot fully address genetic variability, environmental effects, and other crucial aspects of human biology.[102] Improving animal models is crucial for understanding human traumatic injuries and investigating therapeutic interventions. Current models primarily focus on the initial response to injury, which is insufficient to capture the full clinical picture. As complications may emerge later, more extended observation

periods are needed to evaluate treatment impacts during intensive care. The essential use of anesthesia in experimental models can potentially obscure the natural physiological stress responses to hemorrhage and resuscitation, such as effects on sensorimotor functions, cardiovascular actions, and metabolic demands.[28] Early use of mechanical ventilation with positive pressure could alleviate the progression of lung failure in models featuring experimental chest trauma.[27] These factors should be considered when evaluating the outcomes of such studies. Further investigation, including the importance of genetic differences and data integration from various models, is needed to address the complex pathophysiology of trauma and hemorrhage.

Additionally, ethical considerations of animal research should also be taken into account. Alternative approaches, such as advanced imaging, multiomics analysis, in vitro models, computer simulations, and humanized animal models, can offer valuable insights while ensuring animal welfare. While we should continue to improve and develop these alternatives, the use of large animal models remains essential for certain areas of biomedical research. Integrating these approaches with animal models and clinical data allows for more comprehensive and robust development of effective treatments. With the advances in animal welfare and ethical standards, we should aim to refine, reduce, and replace (the 3Rs principle) the use of animal models where possible and justified.

Conclusion

This review underscores the significance of refining animal models in trauma and bleeding research to better represent human conditions. While pigs are the predominant species used, each animal model offers unique advantages and challenges. Extended observation periods, considering species-specific differences in coagulation profiles, and innovative approaches like genetics and alternative methods are essential for developing more comprehensive models. Integrating animal research with ethical considerations and alternative techniques will pave the way for more effective treatments and improved patient outcomes.

Conflict of Interest

O.G. has received research funding from Abiomed, AstraZeneca, Alveron, Bayer Healthcare, Biotest, Boehringer Ingelheim, CSL Behring, Ferring, Octapharma, Novo Nordisk, Nycomed, and Portola. He has also received honoraria for lectures and consultancy support from Abiomed, AstraZeneca, Bayer Healthcare, Boehringer Ingelheim, CSL Behring, Octapharma, Sanofi, Shire, Takeda, Pfizer, and Portola.

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