MethodsX 11 (2023) 102362

Contents lists available at ScienceDirect

MethodsX

journal homepage: www.elsevier.com/locate/methodsx

Construction and validation of a novel and severe hepatic injury model in swine focuses on research and training. Observational study

Francisco José Sánchez del Valle[a,c,*], Pedro Fernández Dominguez[b], Pablo Hernández Sanz[a]

[a] Central Hospital of Defense, General and Digestive Unit, Spanish Ministry of Defense, Spain
[b] Central Hospital of Defense, Unit of Surgical Research, Spanish Ministry of Defense, Spain
[c] University of Alcalá de Henares, Madrid, Spain

ARTICLE INFO

Method name:
Incising-Penetrating, Vascular and Severe Liver Injury Model in Swine.

Keywords:
Liver [mesh term]
Surgical procedures
Operative [mesh term]
Wounds and injuries [mesh term]
models
Animal [MeSH Term]

ABSTRACT

Some hepatic wound models have been developed in pigs with the aim of reproducing liver injury; however, the wound shape, severity, and outcome differ among them. The novel injury profile employed in this study differed from that used elsewhere for standardized, repeatable, reproducible, incising-penetrating, vascular, and severe injury in swine. It is made with a cutting object that penetrates deep into the hepatic parenchyma, always affecting the two suprahepatic veins at the point where they merge into the common trunk.

The primary outcome was reproducibility and replicability of the surgical method.

The secondary outcome was the analysis of some variables (blood loss, survival, and flow) to validate the model.

- This novel method of liver injury provides a liver injury with the following characteristics: standardized, incise-penetrated, deep, bloody, and severe.
- This model can be used for research (trauma, hepato-bilio-pancreatic, pharmaceutical) and training (damage control surgery).
- Method name: Incising-Penetrating, Vascular and Severe Liver Injury Model in Swine.

Specifications table

Subject area:	Medicine and Dentistry
More specific subject area:	Establishment of animal models of specific diseases
Method name:	Incising-Penetrating, Vascular and Severe Liver Injury Model in Swine
Name and reference of original method:	N/A
Resource availability:	N/A

Background

There is not only one wound in the injured liver, and it does not have the same shape because it depends on many factors, such as the cause (knife, gunshot, blast, traffic accident), energy of the wound cause, extension of the wound, affected vascular structures

* Corresponding author at: Central Hospital of Defense, General and Digestive Unit, Spanish Ministry of Defense, Spain.
E-mail address: franciscosanchez@healthgood.es (F.J. Sánchez del Valle).

https://doi.org/10.1016/j.mex.2023.102362
Received 7 August 2023; Accepted 3 September 2023
Available online 3 September 2023

(hepatic vein, portal artery, and caval vein), and caliber of the vessels (main or branches). The outcome and treatment can differ depending on which is present; therefore, severity, outcome, and treatment can differ [1,2].

Generally, three principal types of wounds can be distinguished: penetrated or punctured, contused, and incised or cut. Punctured or penetrated wounds are deeper and less wide, incised wounds are more extended than deep wounds, and contuse wounds have irregular shapes [3].

Liver injury can be severe; therefore, it is difficult to manage posttraumatic hemorrhage. Damage-controlled surgery and abbreviated laparotomy have yielded better outcomes. In this way, there are some techniques like Pringle maneuver, perihepatic packing, angioembolization, ligation of vessels in the wound, or resuscitative endovascular balloon occlusion of the aorta (REBOA) application which are currently in the protocol of severe liver hemorrhage [4,5].

Our goal was to develop a novel liver injury method that provides specific wound characteristics (standardized, incise-penetrated, deep, bloody, and severe) and can be used for research and training.

Method details

Study design

This study (register number: ES280790000187) was performed at the Unit of Surgical Research of the Hospital Central de la Defensa Gómez Ulla (Madrid, Spain). The Committee of Ethics and the Committee of Education of the Hospital Central de la Defensa, as well as the Council of the Environment of the Community of Madrid, approved this study according to Spanish Law (RD 53/2013) and European Directive (2010/63/EU) for animal experiments. Upon completion of the experiments, the animals were euthanized using an overdose of anesthesia.

Healthy animals were used in this study. Failure to cut either vein completely was considered as a reason for excluding the data.

The study was conducted on 10 female swine (Sus Scorfa, Large White) weighing between 30.5 and 38 kg; median and Inter Quartile Range (IQR) 34.00 kg (32.75–36.12).

Pigs were checked following quarantine and their health was certified by the veterinarian in charge of the unit before inclusion in the study. The veterinarians in charge, animal handlers, and those involved in data analysis were blinded to the treatment group.

We did not compare the packing group with the non-operative group because mortality was very high and pigs died exsanguinated. Owing to ethical reasons, the non-operative group was rejected. However, other groups with different hemostatic techniques have been considered in other studies.

Anesthetic procedure and monitoring

The study was performed in an operating room for animal research using all necessary equipment for anesthesia.

The anesthetic team consisted of two veterinarians. One was responsible for anesthesia and monitoring, while the other was in charge of data collection, both clinical and analytical.

Two peripheral intravenous accesses were established using 24 G catheters placed in the marginal ear veins. One femoral artery (20 G) was cannulated to monitor the blood pressure and heart rate, and blood samples were collected. Additional monitoring included electrocardiography, pulse oximetry, vaginal temperature (vaginal) and capnography.

Animals were anaesthetized (atropine sulfate, azaperone, midazolam, ketamine sevoflurane at 2%, fentanyl and were intubated with a 6.5–7.5 mm tube connected to a pump with a respiratory rate of 12 – 15 breaths/min.

The ventilator was set up in volume control mode, delivering at a rate of 12–17 breaths/min, and a tidal volume of 7–10 ml/kg

Balanced saline solution was administered prior to the surgical procedure to keep the pig in good hydration status.

Volume resuscitation

The purpose of this method is to administer the same ringer lactate solution (RLS) as the blood lost by a pig. To do this, we give from injury to min 12 Ringer lactate fluid at a rate of 1000 ml/ h; following from min 13 to min 60, at a rate of 500 ml/ h; and from min 61 to min 120, the rate was calculated in order to complete the reposition volume.

Phenylephrine was administered when the invasive main arterial pressure (iMAP) drops below 32 mm Hg, representing 40% of the basal levels.

The criteria for death and euthanization were an iMAP of 20 mm Hg and a PCO_2 of 20 mm Hg.

Surgical procedure

The surgical study team included six military surgeons. The surgery-on-surgery room team consisted of two well-trained surgeons. These two surgeons performed each operation; one of them (randomly assigned) imposed the injury and performed the surgical technique, and the other acted as an assistant. All surgeons were trained in experimental procedures prior to the study.

Surgical wound procedure

We selected two main vascular liver vessels because there was clear anatomic and echographic identification and an easy surgical approach. It was necessary to remove only the forward abdominal wall and penetrate 5 cm into the liver to reach the suprahepatic

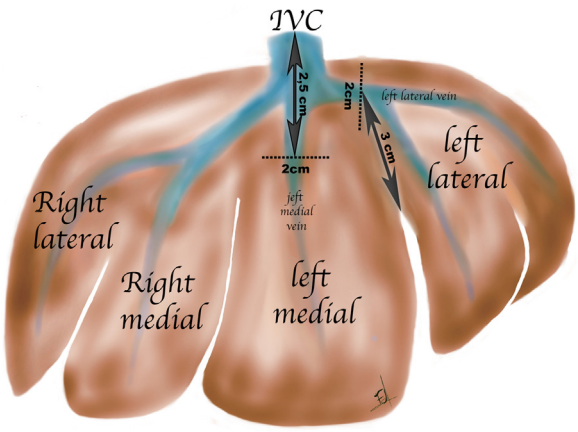

Fig. 1. Suprahepatic veins in the pig. The left medial sector (Segment IV) is transverse to a single vein that drains separately into the Inferior Vena Cava (IVC). We identified the left middle suprahepatic vein 2.5 cm below the tip of the liver. Less frequently, this branch unites with the vein from the left lateral sector to form a large common opening in the IVC. Left lateral suprahepatic vein: The left lateral sector drains through two well-defined tributaries, one from segment II and the other from segment III. Both branches unit into a common trunk before draining into the IVC. The branch was located 3 cm above the left hepatic sulcus.

vein. Finally, the great veins were very close to the heart, so they had high blood flow. Therefore, these reasons could benefit the standardization of a model of severe and bloody liver injury.

We identify anatomically the two target vascular structures as following [6].

Despite the disadvantage of being operator-dependent, an eco-Doppler scan (Fig. 2a, b) is still simple and easy to use [7], and at the same time it is a sensitive method to identify the exact location of the injury.

At the end of the experiment, once the animal was sacrificed, we removed the liver and confirmed that these two hepatic veins were cut at 100% of their diameter (Fig. 2c, d). Failure to cut either vein completely was considered as a reason for excluding the data (see above).

Therefore, liver injury was induced using a standard n 20 surgical scalpel as follows.

Left medial suprahepatic vein: On the round ligament, 2.5 cm above the tip of the liver, we made a horizontal cut, 2 cm long and 5 cm deep (Fig. 1).

Left lateral suprahepatic vein: In the left lateral lobule, 3 cm above the left hepatic sulcus, we made a vertical cut, 2 cm long and 5 cm deep.

Starting point of timing count start with the liver injury.

A major complication could be an unobserved lesion in the inferior cava vein. This complication was avoided using echography because we saw and selected only one specific vessel. In addition, the 5 cm depth of the wound did not affect the cava vein.

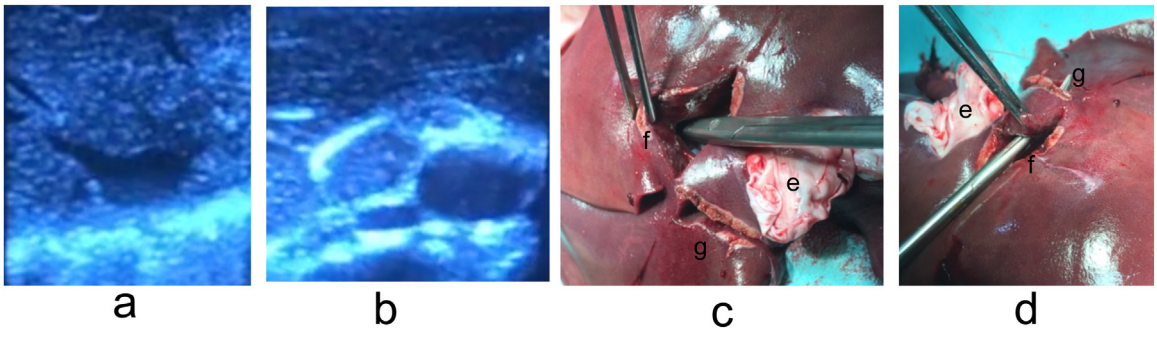

Fig. 2. Ecographic and post mortem identification of suprahepatic vessels. (a) Ecography: Left medial suprahepatic vein. Oblique transection scanning plane in the tip of the liver with a cranial inclination and an angle towards the thorax, will show the IVC entering into the liver from the thorax, and running approximately 1 cm into the parenchyma. The suprahepatic veins are visualized as tubular anechoic structures which drain in the ICV, and left middle suprahepatic vein continues running downwards. The round ligament also constitutes a useful reference to locate this vein. (b) Ecography: Left lateral suprahepatic vein. Axial scanning plane is combined with sagittal views between the left lateral lobe and the left hepatic sulcus to locate the left lateral suprahepatic vein. (c) Post mortem: Left medial suprahepatic vein. The scissor entered downwards, into the left medial vein. (d) Post mortem: Left lateral suprahepatic vein. The scissor connects the common trunk of the inferior cava vein and the left lateral vein injury (exit by the proximal vein injury). (e) Inferior Vein Cava. (f) Medial wound. (g) Lateral wound.

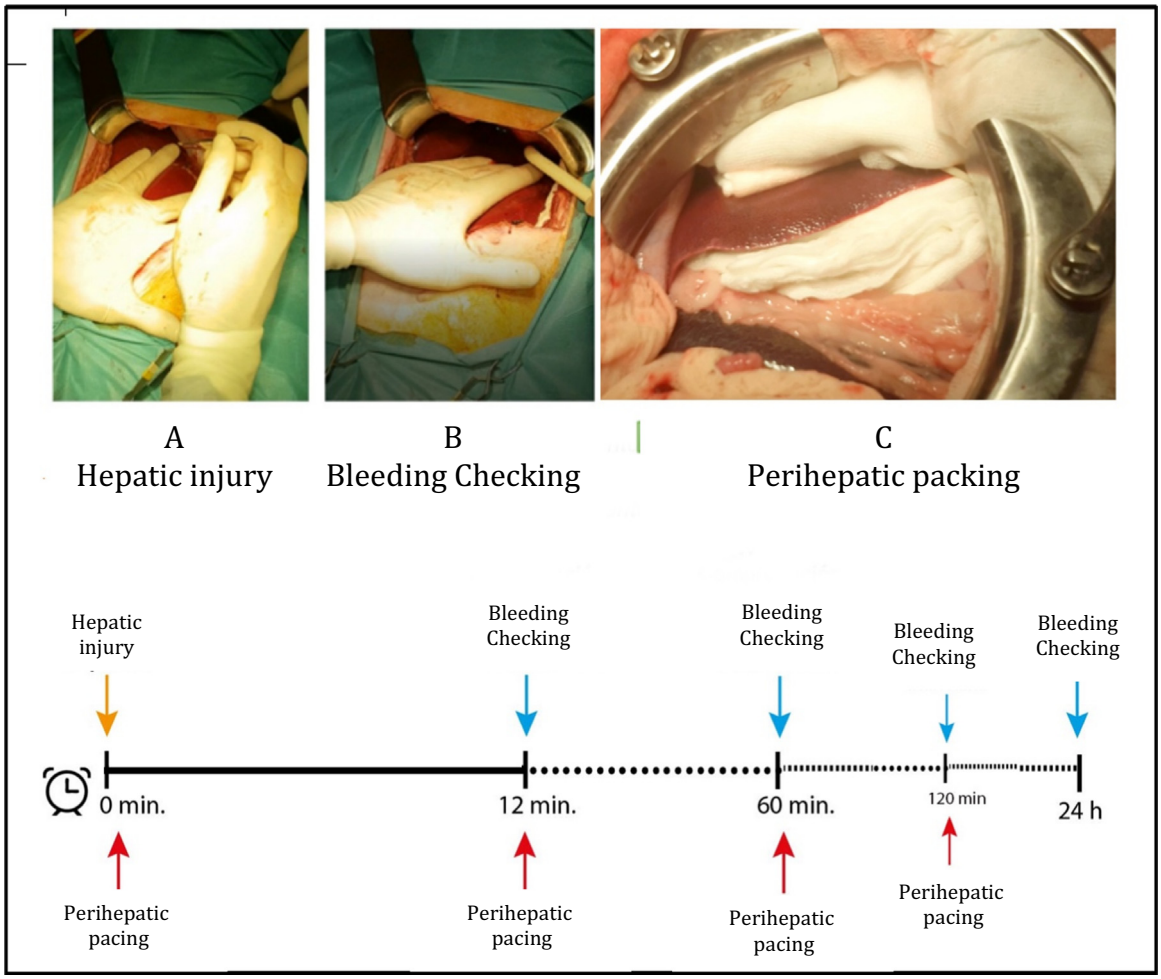

Fig. 3. Line time of the procedure. (A) Hepatic injury. (B) Bleeding checking. (C) Perihepatic packing.

Procedure of packing

The animals underwent extended right subcostal laparotomy. Once injured, packing was performed by placing pad dressings between the liver and diaphragm followed by additional pads under the liver. Similar compression of the left lobe was achieved by placing suprahepatic pads just anterior to the esophagus and infrahepatic pads under the left lobe [8]. The animals were treated with 10 sterilized cotton dressings (20 × 20 cm, 30 g).

Furthers looks and re-packs. (Fig. 3)

After hepatic injury, packing was maintained for 12 min, after which it was removed to verify hemostasis. Subsequently, we performed another packing, which was maintained until minute 60, when we again removed the gauze, verified hemostasis, and re-packed it. The packing was maintained for 120 min when the gauze was removed and another packing was performed. Three liver packs were used in this study.

After 120 min, if several stability parameters were met, the animals were allowed to recover from anesthesia, awakened, and kept under observation for 24 h. If not, the animals were sacrificed with an overdose of anesthesia.

Following the damage control surgery, a second surgery (second look) was performed at 24 h. The animals were anesthetized as described above and abdominal exploration was performed to quantify the study variables.

Subsequently, the experiment was completed, and the animals were euthanized with an overdose of anesthesia.

Variables

This experiment involved some variables:

Survival, defined as iMAP> 20 mmHg and TCO2 >20 mmHg

Bleeding volume. The blood was removed using a surgical aspirator (Flexivac®) and gauze packing pads. The volume of the blood loss was calculated with this formula: "v = ((b_1-a_1)+ (b_2-a_2))/1.04", where "b_1" is the weight of the tank of the surgical aspirator loaded with blood, "a_1" the dry weight of the tank (without blood), "b_2" the weight of the surgical pads soaked in blood, and "a_2" the dry weight of surgical pads (without blood) and 1.04 is the constant of pig´s blood density (Fig. 6) [9].

The total blood volume depended on weight; there was a marked physiological difference between pigs weighing 30 kg (1980 mL) and 40 kg (2640 mL). Therefore, the volume of blood loss was reported using ml/kg units and the percentage of blood loss as two methods to compare it without the influence of weight.

The percentage of total blood volume, was calculated according with the formula "v% = ((v x 100) / (w x 66))", where "v%" was the percentage of blood lost, "v" was the volume of blood lost (mL), and "w" was the weight of the pig, and 66 a constant to calculate the total volume of pig´s blood [10].

This value was calculated at four time points: 12, 60, 120, and 24 h.

Flow was calculated according the formula "f=v / t" where "v" is the volume of blood loss (ml) and "t" the time lapse (min) between we measured.

Blood sample variables were assessed for prothrombin time, partial thromboplastin time, pH, pCO_2, pO_2, base excess bicarbonate (BEB), sodium, potassium, calcium, glucose, hematocrit, and hemoglobin. Data points were extracted at baseline and 12, 60, 120, and 24 h post-surgery.

The monitored variables were the temperature, average blood pressure, and heart rate. The data points were measured at baseline and at 12, 60, 120, and 24 h post-surgery.

Data analysis

Excel and SPSS® v25 software were used for data collection and statistical analysis.

Means and standard deviations (SD) were used for continuous quantitative variables with normal distribution. For non-parametric distributions, median and interquartile range (IQR) were used.

The pig decease count as a lost value in the statistical data never counts as zero for any value.

Method validation

Baseline values are resumed in Fig. 4

Survival analysis

At 10 min, all the pigs survived. At 60 min, of 6/10 pigs survived. None of the pigs survived for 120 min. The mean (SD) survival time was 97.20 min (35.73).

Hemodynamic parameters analysis

Median (IQR) of total blood loss was 1469.00 ml (1274.00–1687.00); 44.54 ml/kg (37.16–52.04), and 67.48% (56.31–78.85). Median (IQR) of blood loss was at min 12; 798.20 ml (657.80–891.80); 22.14 ml/kg (18.62–26.66) and 33.55% (28.21–40.40). At 60 min the median (IQR) was 461.24 ml (334.10–548.60), 13.74 ml/kg (9.53–16.04) and 20.83% (14.44–24.31). At 120 min, the median (IQR) values were 366.60 ml (276.90–518.70), 10.94 ml/kg (7.60–15.27), and 16.58% (11.52–23.14), respectively Figs. 5 and 6.

From injury to min 12 the flow median (IQR) was 61.49 ml/min (50.68–68.70), from min 12 to min 60 was 8.88 ml/min (6.43–10.56) and from min 60 to 120 the flow was 5.74 ml/min (4.33–8.12).

The iMAP decreased from the baseline median (IQR) 70.00 mmHg (53.50–81.25) to 33.00 mmHg (28.50–40.75) at min 12, 23.00 mmHg (16.00–39.50) at min 60, and 21 mmHg (16.75–30.75) at 120 min.

The heart rate increased from baseline median (IQR) 81.50 bpm (72.50–90.00) until 82.00 bpm (63.50–92.25) at min 12, 75 bpm (63.50–91.50) at min 60, and 97 bpm (81.00–137.75) at 120 min.

Blood sample parameters analysis

Hemoglobin decreased until baseline median (IQR) of 8.50 g/dL (8.20–9.37) to 6.80 g/dL (6.10–9.20) at 12 min, 5.40 g/dL (4.55–6.95) at 60 min and 5.45 g/dL (4.00–6.65) at 120 min after injury.

The hematocrit decreased from the baseline median (IQR) of 25.00% (23.25–27.50) to 20% (18.00–27.00) at 12 min, 17% (15.25–20.75) at min 60 and 16% (13.75–19.50) at 120 min after injury.

Prothrombin time (PT) decreased from baseline median (IQR) of 14.35 s (12.20–15.27) to 13.40 (13.02–16.67) at min 12; 14.90 s (12.27–17.17) at min 60 and 12.90 s (12.77–13.77) at min 120.

Case number	Overall blood loss (ml)	Overall blood loss (ml/Kg)	Overall blood loss (%)
1	956.8	28.14	42.64
2	1435.2	43.49	65.9
3	1476.8	46.16	69.92
4	1913.6	53.16	80.54
5	1664	45.59	69.07
6	1619.28	53.09	80.44
7	1757.6	51.69	78.32
8	1461.2	38.45	58.26
9	1242.8	37.66	57.06
10	1284.4	35.68	54.06

	Overall pig´s blood (basal)	Blood loss			
		0 to 12 min	13 to 60 min	61 to 120 min	Total
ml	2263.80	798.20	461.24	366.60	1469.00
ml/kg	66.58	22.14	13.74	10.94	44.54
%	100	33.55	20.83	16.58	67.48
	Baseline	12 min	60 min	120 min	
iMAP (mmHg)	70	33	23	21	
HR (bmp)	81	82	75	97	
Hemoglobine (g/dL)	8.5	6.8	5.4	5.4	
Hemoatocrit (%)	25	20	17	16	
Protrombine (Sec)	14.35	13.40	14.90	12.90	
Flow (ml/min)	0	61.49	8.88	5.74	

Fig. 4. There are a significative amount of blood loss in the 12 first minutes. In this period, the pig loss near 1/3 of its basal blood´s volume. After that, the pig loss less blood due to the hemostatic technique of packing. The overall of blood loss was very high, near 67% of its initial volume of blood. Flow was very high in the first 12 min, but decreased after the perihepatic packing. All the values are the physiological consequence of this grave exsanguination: Hemoglobin, hematocrit, prothrombin and iMAP decreased along the observation period.

Author		Hemostatic treatment grpup	Surgical treatment group	Timelapse follow	Packing survival	Packing blood loss	AAST scale	Shape	Dimensions	Liver segment	How did they do?
Holcomb	1999	6 pigs: DFSD	6 pigs: Packing	1h	100%	1104 ml	Grave (V)	Penetrating and contuse trauma. Large and stellate wounds sith a small island of tissue in the center.	10 x 8 x 4 cm	Middle	Ring clamp modified on one arm with two 4.5 cm cutting blades set in a post at right angles to each other, forming an X.
Delgado	2008	9 pigs: Fibrin patch	8 pigs: Packing	2h	13%	4173 ml					
Pusateri	2003	6 pigs: DFSD	6 pigs: Packing	1h	55%	2973 ml					
Pusateri	2004	8 pigs: Quit Clot	8 pigs: packing	1h	12%	5338 ml					
Pusateri	2003	7 pigs: Chitosan	8 pigs: packing	1h	18%	2879 ml					
Jewelewicz	2003	Serie 1: 7 pigs: p-GlcNAc. Serie 2: 5 pigs: p-GlcNAc.	Serie 1: 7 Packing Serie 2: 5 Packing	3h	0%	3740 ml	Grave (IV)	Avulsion		Crush and avuls of the left lateral hepatic lobe and its major vesels.	
Clay	2009	6 pigs: Bloxx	6 pigs: packing	2h	50%	28.3 ml/Kg	Grave (IV-V)	incisie	35.9 cm^2	The left midle lobe was retracted upward and a large necropsy knife was used to transect the base of the lobe such that the lobar branches of the portal vein, hepatic vein and hepatic artery were involved as proximally as possible.	
Sena	2010	8 pigs: Kaolin	8 pigs: packing	2h	50%	58 ml/Kg	Grave (IV-V)	incise	36 cm2	The left medial lobe was identified and resected using a wire saw placed at the central posterior cleft and advanced anteriorly to sever the lobe.	
Schnuriger	2011	10 pigs. Non surgical	10 pigs: Packing	2, 15 min, 48 h (blood). 48 h (survival)	70%	234+478+214 ml	Grade V				
Coenye	2013	Pigs: Floseal Pigs Surgiflo					Medium (III-IV)	incise-penetrateing	12mm wide x 10 mm deep	Self made stab device consisting of four perpendicular number 10 surgical blades in the shape of a cross in all three lobes.	
Cohn	1998	7 Pigs: Pringle, suture, Avitene + Surgicel, packing	7Pigs: Pringle, suture, Avitene + Surgicel, packing	2h		267+875 ml	Grave (V)	Contuse		Firing a nail driver (low-velocity piston -type fastening tool, using a 22 caliber charge onto a solid aluminium disk (5 cm diameter, 1 cm thick) taped to the skin just below the costal margin in the midclavicular line.	
Sánchez del Valle	**2023**		10 pigs: Packing	2h	0%	1,469.00 ml. 44.54 ml/kg. 67.48 %	Grave (IV-V)	incise-penetrateing	2 wounds of 2 cm wide and 5 cm deep 40cm^2	Middle and lateral lefts lobes	Surgical scalpel nº20

Fig. 5. Studies of Hemorrhage in liver´s swine and perihepatic packing.

Discussion

This method has been used in recent studies to provide its utility [11] and is briefly described in the Materials and Methods section. Owing to the room limitation of that journal´s chapter, we now consider a specific publication method to describe and discuss it in more detail.

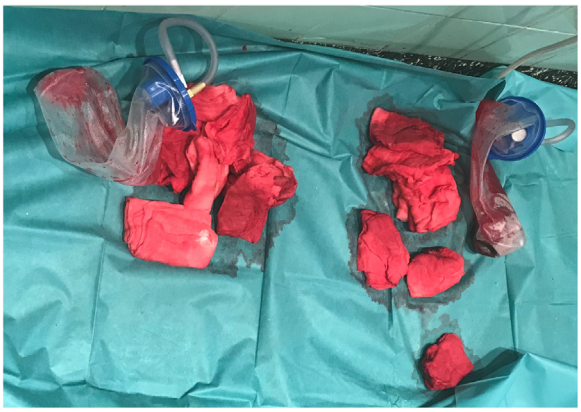

Fig. 6. Load surgical aspirator (Flexivac®) and blood-soaked gauzes pads removed after surgery at minute 12 and 60.

Injury model: material

Many studies have used pigs to induce liver injury. However, due to ethical criteria, the number of pigs used has been reduced. Many previous studies have used a sample size of five to eight pigs per group. Therefore, to the best of our knowledge, this is the largest study (10 pigs) that used standard packing as a damage control surgical technique (Fig. 4) [12–15].

Female animals were chosen because they may tolerate hemorrhage better than male animals. Androgens have been found to react in an immunosuppressive manner and may contribute to these sex-related differences [16]. In addition, laparotomy in female pigs is easier because urine leakage from the male penis, which is located within the umbilical stalk, has an extremely unpleasant odor [6].

Injury model: method

Many surgical methods have been reported, but they are not homogeneous and differ in different aspects, such as shape, extension, injury mechanism, time lapse of follow-up, and surgical technique. Consequently, they have different results in terms of blood loss, survival, flow, and analytical parameters (Fig. 4).

Standardized injury: Focusing on research and training, we attempted to standardize the liver wound because it is important to perform the same hepatic injury [17]. In our study, all pigs had identical damage, and none of them were excluded.

Wound features: We described two incisions in the left medium and left external hepatic lobes, 2 cm wide and 5 cm deep, affecting the two principal suprahepatic veins (medial left and external left) at the point where they merge into the common trunk.

From an anatomical point of view, this injury could be classified as grade V on the American Association for the Surgery of Trauma (AAST) Organ Injury Scale [18] because it penetrates deep into the hepatic parenchyma, always affecting the two suprahepatic main veins.

Physiological outcome: Some authors have described a decrease in iMAP of 22.4% in the 30 first seconds [13,19,20], a decrease of 20% during the first 5 min [21], a decrease of 41 mmHg in the first 5 min [22], or a decrease from 100 to 52 mmHg in the first 120 min [23]. Our study agrees with previous studies because the iMAP decreased by 37.00 mmHg (52.85%) (from 70.00 to 33.00 mmHg) in the first 12 min.

Therefore, from a physiological point of view, these models could be grade IV (grave lesions) on some scales, such as the World Society of Emergency Surgery (WSES), owing to hemodynamic instability when performing an injury [24].

Owing to physiological challenges, experimental liver injury can reduce survival rate. Some authors have reported the best results of up to 55% in the first hour; however, in others, there was no survival up to 2 h. In our study, the animals survived for a mean of 97.20 min and also, and there was no survival for up to 2 h.

Exsanguinate injury: Blood loss depends on the weight of the pig, which is why the volume of blood loss ranges from 669 mL to 5338 mL in a time lapse between one and three hours in different studies. In our study, from an initial volume of 2263 ml, the pig loss 1469.00 ml in two hours (representing a loss of 67.48% of the initial volume of blood). To compare our study with other studies, we calculated blood loss according to the animal weight (see above). Previous studies have described a blood loss of 28.3 58 ml/kg at two hours post- injury (Fig. 5). Our study is in the middle of both, because we observed a blood loss of 44.50 ml/kg in two hours.

Flow is used in some scales to quantify the severity of the hemorrhage. Some scales, such as the Validated Intraoperative Bleeding Scale (VIBE), consider a major severity when the flow rate is greater than 50 ml/min. [25] In our study, the flow rate was 61.49 ml/min during the first 12 min.

Owing to the large amount of blood loss, we did not let spontaneous blow after the injury and began to pack immediately after the liver insult.

Coagulopathy, hipotermia and acidosis: In our model, it was not necessary to perform an experimental coagulopathy test, because the injury was sufficient to induce physiological coagulopathy. In our study, prothrombin time (PT) decreased from the baseline median (IQR) of 14.35 s (12.20–15.27) to 13.40 s (13.02–16.67) at 12 min post-injury.

It was not necessary to induce hypothermia by placing cold packs in the abdomen or by infusing cold intravenous solutions. We achieved a baseline temperature median (IQR) of 35.55 ˚C (34.45–36.00)

Research model

A major topic in experimental research is the development of a simple model that is easily reproducible and standardized to accurately model clinical issues. Therefore, this specific group of research models (hemorrhagic shock combined with traumatic injury) provides a more accurate understanding of the life-threatening clinical conditions [26]. In addition, it is very important to have a bloody model with severe liver injury to compare the efficacy of the different hemostatic techniques because the difference can increase more in a severe than in a minor bloody wound. In the authors' opinion, this model could be used to study the efficacy and ease of use of different hemostatic agents. Therefore, further studies were conducted to replicate this model [11]. Furthermore, this model could be applied to trauma, Hepato Bilio Pancreatic, and pharmaceutical research.

Although it is well known that the liver must be packed from 24 to 72 h [27], we removed it four times in this study: at 12, 60, 120, and 24 h. This limitation is necessary in this research model to obtain specific time points to check for hemostasis (control group) and to compare it with another surgical technique (study group).

Surgical training model

The principles of damage control surgery have demonstrated improved survival; however, few animal models have included these rules [23]. Consequently, it is critical to develop new surgical animal models that focus on training novel surgeons.

Following these recommendations, we adapted the damage control principles to experimental surgery in animals, performing liver packing and closing the abdomen in less than one surgical hour. After one hour, the pig was intubated and monitored under intravenous resuscitation as a critical unit. Because of the impossibility of being intubated at all times, if the pig fulfills several parameters (see above), it is awakened two hours after injury. Finally, a second surgery (second look) was performed 24 h after hepatic insult.

Moreover, the features of the injury (penetrated, deep, severe, and bloody) could be similar to injuries performed with a knife, sword, or other kind of stab weapon.

When more resources or damage control techniques were applied, higher survival rates were observed. Moreover, in the Spanish Army and Navy, we applied this method to train surgical teams at different meetings, appointments, or trainings prior to military missions.

Limitations

The current method has lower "clinical value" than "experimental value." For instance, in real life, packing is not removed, laparotomy is performed after a time lapse of spontaneous internal bleeding, or there are more abdominal lesions and resuscitation maneuvers. However, these limitations are necessary to control for unexpected variables and provide more scientific accuracy.

Ethics statements

The Committee of Ethics and the Committee of Education of the Hospital Central de la Defensa, as well as the Council of the Environment of the Community of Madrid, approved this study according to Spanish and European Law (RD 53/2013) and EU Directive 2010/63/EU for animal experiments and complied with the ARRIVE guidelines.

Declaration of Competing Interest

The author is a research member of Transbiomat (research group of the "Universidad Complutense de Madrid").

The authors declare that they have no known competing financial interests or personal relationships that could have influenced the work reported in this study.

CRediT authorship contribution statement

Francisco José Sánchez del Valle: Methodology, Conceptualization, Writing – original draft, Validation, Writing – review & editing. **Pedro Fernández Dominguez:** Conceptualization, Methodology, Writing – review & editing. **Pablo Hernández Sanz:** Methodology.

Data availability

Data will be made available on request.

Acknowledgments

The commander and crew of the Frigate Santa María in the Indic Ocean supported the onboard writing of this manuscript.
To J Barroso for helping us with the literature search.
To C. Gutierrez for helping us with the statistic study.

Funding

90% Open access fees have been supported by a grant (PID 2021-123045OB-100) from the public agency (Ministry of Science and Technology of Spain).

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.mex.2023.102362.

References

[1] G. Parra-Romero, G. Contreras-Cantero, D. Orozco-Guibaldo, A. Domínguez-Estrada, J.M.M. del Campo J de, L. Bravo-Cuéllar, in: Abdominal trauma: Experience of 4961 Cases in Western Mexico, 87, Cir y Cir (English Ed, 2019, pp. 183–189. Mar 1.
[2] A.B. Christmas, Management of hepatic trauma in adults. In: UpToDate 2020;1–31. Available from: https://www.uptodate-com.m-hcd.a17.csinet.es/contents/management-of-hepatic-trauma-in-adults?search=liverinjury&source=search_result&selectedTitle=3~150&usage_type=default&display_rank=.
[3] H. Durán Sacristán, Compendio de cirugía. McGraw-Hill, 2002 ; Available from: https://www.casadellibro.com/libro-compendio-de-cirugia/9788448604219/843087.
[4] C.A. Ordoñez, M.W. Parra, M. Millán, Y. Caicedo, M. Guzmán-Rodríguez, N. Padilla, et al., Damage control in penetrating liver trauma: fear of the unknown, Colomb Med. 51 (4) (2020) e4134365 (Cali, ColombDec.
[5] F.C.R.C.C. Ordonez, Liver trauma: WSES 2020 guidelines Federico, World J. Emerg. Surg. [Internet] 15 (24) (2020) Available from:, doi:10.1186/s13017-020-00302-7.
[6] M. Bekheit, P.O. Bucur, M. Wartenberg, E. Vibert, Computerized tomography-based anatomic description of the porcine liver, J. Surg. Res. 210 (2017 Apr) 223–230.
[7] D. Sebastián, D.J. Castro, Liver segmentation: ultrasound evaluation, Rev. Arg. Diag Por Imagenes. 1(2) (2012) 55–62. Available from: https://www.webcir.org/revistavirtual/articulos/junio13/argentina/faardit_ingles_a.pdf.
[8] C.E. Lucas, A.M Ledgerwood, Perihepatic Packing, in: Encyclopedia of Intensive Care Medicine [Internet], Springer Berlin Heidelberg, Berlin, Heidelberg, 2012, pp. 1717–1722, doi:10.1007/978-3-642-00418-6_493. Available from:.
[9] A. Madrid, in: Mundi-Prensa (Ed.), Aprovechamiento De Los Subproductos Carnicos, 1999, pp. 35–43.
[10] A. Fülöp, Z. Turóczi, D. Garbaisz, L. Harsányi, A. Szijártó, Experimental models of hemorrhagic shock: a review, Eur. Surg. Res. 50 (2) (2013) 57–70 Eur Chir Forschung Rech Chir Eur.
[11] S.D.V.F. José, D.N. Luis, G.M. Juan, D.P. Antonio, S.R. Lidia, Utility of microporous polysaccharide hemospheres in severe hepatic trauma: experimental study of hemostatic strength and ease of use, Injury 54 (2) (2023) 339–344 Feb.
[12] Holcomb, Dry fibrin sealant dressings reduce blood loss, resuscitation volume, and improve survival in hypothermic coagulopathic swine with grade V liver injuries, J. Trauma. 47 (2) (1999) 233–240.
[13] A.V. Delgado, B.S. Kheirabadi, T.M. Fruchterman, M. Scherer, D. Cortez, C.E. Wade, et al., A novel biologic hemostatic dressing (fibrin patch) reduces blood loss and resuscitation volume and improves survival in hypothermic, coagulopathic swine with grade V liver injury, J. Trauma Inj. Infect. Crit. Care 64 (1) (2008) 75–80.
[14] A.E. Pusateri, C.W. Goodwin, A.T. McManus, D.T. Zolock, J.L. Sondeen, R.L. Cornum, et al., Dose-dependent actions and temporal effects of angiotensin ii on microvascular permeability, J. Trauma 55 (3) (2003) 518–526.
[15] D.D. Jewelewicz, S.M. Cohn, B.A. Crookes, K.G. Proctor, Modified rapid deployment hemostat bandage reduces blood loss and mortality in coagulopathic pigs with severe liver injury, J. Trauma 55 (2) (2003) 275–281.
[16] M.W. Wichmann, R. Zellweger, C.M. DeMaso, A. Ayala, I.H. Chaudry, Enhanced immune responses in females, as opposed to decreased responses in males following haemorrhagic shock and resuscitation, Cytokine 8 (11) (1996) 853–863 [Internet]Available from: https://www.sciencedirect.com/science/article/pii/S1043466696901143 .
[17] M. Welch, J. Barratt, A. Peters, C. Wright, Systematic review of prehospital haemostatic dressings, BMJ Mil. Health 166 (3) (2020) 194–200 Jun 1.
[18] E.E. Moore, T.H. Cogbill, G.J. Jurkovich, S.R. Shackford, M.A. Malangoni, H.R. Champion, Organ injury scaling: spleen and liver (1994 revision), J. Trauma 38 (3) (1995) 323–324 Mar.
[19] A.E. Pusateri, H.E. Modrow, R.A. Harris, J.B. Holcomb, J.R. Hess, R.H. Mosebar, et al., Advanced hemostatic dressing development program: animal model selection criteria and results of a study of nine hemostatic dressings in a model of severe large venous hemorrhage and hepatic injury in Swine, J. Trauma 55 (3) (2003) 518–526 Sep.
[20] A.E. Pusateri, A.V. Delgado, E.J. Dick, R.S. Martinez, J.B. Holcomb, K.L. Ryan, Application of a granular mineral-based hemostatic agent (QuikClot) to reduce blood loss after grade V liver injury in swine, J. Trauma Inj. Infect. Crit. Care 57 (3) (2004) 555–562.
[21] B. Schnüriger, K. Inaba, G. Barmparas, P. Rhee, B. Putty, B.C. Branco, et al., A new survivable damage control model including hypothermia, hemodilution, and liver injury, J. Surg. Res. 169 (1) (2011) 99–105.
[22] M.J. Duggan, A.Y. Mejaddam, J. Beagle, M.A. Demoya, G.C. Velmahosa, H.B. Alam, et al., Development of a lethal, closed-abdomen grade v hepato-portal injury model in non-coagulopathic swine, J. Surg. Res. 182 (1) (2013) 101–107 Jun 1.
[23] S.M. Cohn, J.H. Cross, M.E. Ivy, A.J. Feinstein, M.A. Samotowka, Fibrin glue terminates massive bleeding after complex hepatic injury, J. Trauma 45 (4) (1998) 666–672 Oct.
[24] F. Coccolini, F. Catena, E.E. Moore, R. Ivatury, W. Biffl, A. Peitzman, et al., WSES classification and guidelines for liver trauma, World J. Emerg. Surg. (2016) 1–8 [Internet]Available from:, doi:10.1186/s13017-016-0105-2.
[25] K.M. Lewis, Q. Li, D.S. Jones, J.M.D. Corrales, H. Du, P.E. Spiess, et al., Development and validation of an intraoperative bleeding severity scale for use in clinical studies of hemostatic agents, Surgery 161 (3) (2017) 771–781 (United States) [Internet]Available from:, doi:10.1016/j.surg.2016.09.022.
[26] A. Fülöp, Z. Turóczi, D. Garbaisz, L. Harsányi, A. Szijártó, Experimental models of hemorrhagic shock: a review, Eur. Surg. Res. 50 (2) (2013) 57–70.
[27] A.J. Nicol, M. Hommes, R. Primrose, P.H. Navsaria, J.E.J. Krige, Packing for control of hemorrhage in major liver trauma, World J. Surg. 31 (3) (2007) 569–574.