Purpose: After liver transplantation with a small-for-size liver graft or after extensive hepatectomy for liver malignancies or other non malignant conditions with an insufficient liver volume, the survival of patients depends on liver regeneration. This study was carried out in order to create a new porcine model for the study of small-for-size syndrome (SFSS) after extensive hepatectomy.

Methods: In the present study we used 23 domestic Landrace pigs weighing 28.3±3 kg and aged 19-21 weeks. We describe our detailed surgical procedure for 75% partial hepatectomy in a porcine model, using the saline-coupled bipolar sealing device (Aquamantys®) for hepatectomy.

Results: The Aquamantis 2.3 bipolar sealer was connected to the Aquamantis generator and was adjusted to produce 150 watts at a medium flow rate of 20 ml/min. The device temperature was programmed to remain at approximately 100° C and, as a consequence, it produced a tissue ablation without charring. The mean operating time was 153.8 min and the mean blood loss 81.9 ml. The estimated residual liver weight (ERL) was 177 g, whereas the mean proportion of ERL was 24.5%. There was no perioperative mortality.

Conclusions: A large animal model, such as pig, is extremely useful in order to reproduce and understand the SFSS. Our simple technique for successful resection of 75% of the liver in pigs, using the Aquamantys system, achieves effective and safe liver parenchymal transection with significant decrease of intraoperative blood loss and can provide useful information for researchers.

Key words: animal model, hepatectomy, saline-coupled bipolar sealing device

Summary

Introduction

Liver cancer is the 6th most common cancer globally, and the second leading cause of cancer death worldwide, mainly due to its late diagnosis and high incidence of metastasis [1]. The latest study by the American Association for Cancer Research has predicted that liver cancer will surpass prostate, breast and colon cancer in the USA by 2030 [2]. It is well known that hepatic resection is the gold standard for the treatment of liver tumors. The purpose of hepatectomy is the resection of all macroscopic disease without positive resection margins and simultaneously to retain adequate functioning liver mass [3,4]. When the residual liver volume is not adequate to maintain the metabolic demands, the patients are at high risk of developing SFSS.

SFSS is a well-recognized clinical syndrome that occurs after liver transplantation with a
small-for-size (SFS) liver graft or after extended hepatectomy for liver cancer. SFSS has emerged as a distinct clinical and pathologic entity which is characterized by delayed synthetic function, prolonged cholestasis, coagulopathy, intractable ascites and poor bile production, followed by septic complications and liver failure [5]. A large animal model, such as pig, is extremely useful in the experimental reproduction and better understanding of SFSS. The advantages of a porcine model are associated with its similarities with the human in terms of gastrointestinal anatomy, metabolism and physiology [6,7]. Literature suggests that it is not possible to establish a porcine model for SFSS after partial liver transplantation because of the porcine distinct hepatic anatomy and physiology. Liver transplantation in pigs needs a veno-venous bypass which affects the hemodynamic and pathophysiological features of SFSS [8,9].

This study was carried out in order to create a new porcine model for SFSS with extensive hepatectomy. We describe our detailed surgical procedure for 75% partial hepatectomy (PH) with the use of a saline-coupled bipolar sealing device (Aquamantys®, bipolar sealer, Medtronic, Portsmouth, USA) for parenchymal resection. Several previous reports describe porcine models of up to 70% hepatectomy with variable types of liver resection [10]. Our study constitutes a safe and easily reproducible technique for more extended hepatectomy in a porcine model in order to investigate the process of hepatic regeneration after major hepatectomy. Moreover, we discuss some critical anatomical features, the key points and the disadvantages of porcine models, based on our experience.

Methods

Animals

This protocol was approved by the General Directorate of Veterinary Services (licence No. 27/03-01-2012), according to Greek legislation regarding ethical and experimental procedures (Presidential Decree 160/1991, in compliance with the EEC Directive 86/609 and Law 2015/1992 and in conformance with the European Convention ‘for the protection of vertebrate animals used for experimental or other scientific purposes, 123/1986’). Animal handling and care was in accordance with National and European legislation about experiments in animals. This study was performed at the Experimental Research Center of ELPEN, which is located in the region of Attica. (European Ref Number EL 09 BIO 03). Twenty-three domestic Landrace pigs aged 19-21 weeks weighing 28.3±3 kg were used.

Porcine liver anatomy

According to Couinaud, the hepatobiliary anatomy and physiology of the porcine liver are comparable to the human [11]. The porcine liver can be divided into 8 segments or 7 lobes: left lateral lobe (LLL), left medial lobe (LML), right medial lobe (RML), right lateral lobe (RLL) and caudate lobe (segment I) (Figure 1a,b). The liver lobes are separated by interlobular fissures which divide the liver into distinctly independent lobes. Each lobe has its own venous, arterial and biliary draining (Glisson’s pedicle). The RLL and caudate lobes have been reported to constitute approximately the 30% of the liver volume in the 30 kg weight range [12,13]. The gall bladder (GB) lies on the undersurface of RML. The

![Figure 1](image.png)

**Figure 1.** Diagrammatic illustration of the porcine liver anatomy. A: Diagrammatic illustration of the porcine liver anatomy. The interlobular fissures divide the liver into 5 distinctly independent lobes. Each lobe has its own venous, arterial and biliary draining (Glisson’s pedicle). B: Diagrammatic illustration of the undersurface of the liver - hepatic lobes and deep interlobular fissures. The gall bladder (GB) resides only in the RML.
lobular hepatic veins (HVs) of the porcine liver are entirely intrahepatic in contrast to humans and the hepatic parenchyma also covers part of the supra-hepatic inferior vena cava.

Results

**Anesthesia and peri-operative monitoring**

Anesthesia was achieved by intramuscular administration of 0.6 mg/kg midazolam (Roche, Athens, Greece), 0.05 mg/kg atropine sulfate (Demo, Athens, Greece) and 10 mg/kg ketamine hydrochloride (Merial, Lyon, France). Intravascular access was obtained via the auricular vein and endotracheal intubation followed (size 6.0 mm cuffed endotracheal tube, Portex, Mallinckrodt Medical, Ireland). Positive pressure was used during mechanically ventilation (Alpha Delta lung ventilator, Siare, Bologna, Italy), FiO2 was set to 21% and isoflurane and nitrous oxide were administered. The tidal volume was set at 10 ml/kg, with a respiratory frequency of 13/min to achieve a normal arterial PCO2 within the range of 35–45 mmHg. During the operation, anesthesia was maintained with propofol (0.15 mg/kg/min), fentanyl (0.6 μg/kg/min) and pancuronium (0.06 mg/kg every 20 min).

After animals were anaesthetized, more interventions took place preoperatively, with all procedures performed under aseptic conditions. Via the right common carotid artery, an intra-arterial line (7 Fr. X 16 cm, Double-Lumen, Arrow Int., Teleflex Medical, PA, USA) was placed into the aorta for the precise monitoring of arterial pressure. An intra-venous catheter (4 Fr. X 15 cm, Two-Lumen, Arrow Int., Teleflex Medical, PA, USA) was also inserted into the external jugular vein for infusion of crystalloid fluids and measurement of central venous pressure (CVP). Arterial blood gases were measured on a blood-gas analyzer, preoperatively, intra-operatively and post-operatively (IRMA SL Blood Analysis System, Diametrics Medical Inc., USA). Mean arterial pressure (MAP), electrocardiographic (ECG) monitoring, end-tidal CO2 and pulse oxymetry were monitored throughout the procedure. Blood temperature was continuously measured and was maintained at normothermia (38–39 °C) with the use of a warming pad, as described in the literature [14]. Subsequently, a suprapubic urinary catheter was inserted into the urinary bladder for drainage and monitoring of urine output. First measurements were taken 30 min after instruments were placed to allow stabilization of the animals.

**Resection technique**

The operation started with a long midline laparotomy, extending 3 cm lower to the umbilicus and 3 cm cephalad to the xiphisternum. The mobilization of the liver was achieved by dissecting all external ligamentous attachments. Subsequently, the intrahepatic portion of the inferior vena cava was dissected close to the hiatus and all hepatic veins were recognized. The CVP was kept below 3 cm H2O to minimize venous bleeding during the dissection. In the hepatoduodenal ligament (Figure 2a), the common bile duct (CBD) (Figure 2b), the hepatic artery (HA) and the portal vein (PV) were dissected and skeletonized close to the bifurcation to the LLL, LML, RML and RLL (Figure 2c,d). The lymph nodes of the hepatoduodenal ligament were removed and the PV was isolated up to the head of the pancreas. Then, the cystic duct and cystic artery were ligated and divided at the level of hilar plate. The hepatic ducts of LLL, LML and RML were dissected individually, ligated and divided.
divided using 2/0 silk, near the liver parenchyma, while any injuries to the hepatic duct of the RLL were avoided.

At this stage of the procedure, it is extremely significant to identify and preserve the CHA branch for the RLL (Figure 2c,d). The hepatic artery branches of the LLL, LML and RML were dissected individually, ligated and divided using 2/0 silk (Figure 2c,d). Moreover, care was taken so as to avoid any damages to the right gastric artery. The left and right PV were carefully dissected and encircled with vessel loops (Figure 2e), to allow clamping in case of uncontrolled bleeding during hepatectomy.

Immediately after the selective ligation of the HA branches, a demarcation line of the de-vascularized area was observed between the ischemic and the normal liver parenchyma, with the RLL and caudate lobe remaining vascularized (Figure 3d,f). Because the lobular HVs of the pig liver are entirely intrahepatic as opposed to human, it was impossible to dissect and ligate the HVs. Furthermore, any dissection around the intrahepatic portion of the inferior vena cava is unsafe, especially close to the right lateral HV.

The liver parenchymal transection was carried out by using the saline-coupled bipolar sealer (Aquamantys, Bipolar sealer, Medtronic, Portsmouth, USA) as illustrated in Figure 3. The Aquamantys 2.3 Bipolar sealer was connected to the Aquamantys Generator and the generator was adjusted to produce 150 watts at a medium flow rate of 20ml/mm. This device features transcollation technology, a combination of radiofrequency (RF) energy and saline, which delivers controlled thermal energy to the liver parenchyma [15]. The device temperature is programmed to remain at approximately 100° C and as a consequence it produces a tissue ablation without charring. The liver parenchyma was divided close to the demarcation line, between RLL and RML (Figure 3c). The RML, LML and LLL were removed starting with the LLL, continuing with the LML and completing with the RML, without Pringle maneuver, leaving a minor cuff of parenchyma (Figure 3b-d, h). Any hepatic veins crossing the transection line were ligated with 3/0 Prolene. Constant suction was needed to clear the saline used from the bipolar sealer (Figure 3c).

According to research reports [12,13], the left trilobectomy (LLL, LML, RML) is considered as 70% hepatectomy in porcine models. In order to complete 75% hepatectomy, part of the RLL (segment VII) was resected (approximately 5% of total liver volume) with the bipolar sealer to achieve complete haemostasis at the cut parenchymal surface (Figure 4 f,g). The 5% of total liver volume was calculated from the weight of resected liver parenchyma (Table 1).

**Discussion**

According to the literature and our previous experiments, 80-85% hepatectomy in porcine model induces several liver damages and has a lethal result by causing acute liver failure [7,12]. On the other hand, many reports show that after 70%
hepatectomy or less, the postoperative course is uncomplicated with the reduced liver remnant to be sufficient for normal liver function [10,16]. Xia et al. [16] showed that after extended hepatectomy with part of the RLL (segment VII) and caudate lobe (segment I) as resection remnant, the survival rates, the pressure of PV, and blood liver function tests were similar to clinical SFSS. We performed 75% hepatectomy, a percentage which is capable to establish high portal pressure and multiple liver damages, but the effects are reversible and not lethal [7,17]. This porcine model can be reproduced easily with very few surgical complications and costs.

Porcine’s aspect of the hepatic hilus is similar to the human’s, as it is shown in Figure 2c. The Pinch-Burn-Cut (PBC) technique could be used for the dissection of the hepatoduodenal ligament [18]. The surrounding tissues between biliary ducts, portal and arterial vessels, which arise from the fusion of Glisson’s capsule, should be firstly dissected. Subsequently, the CBD, CHA and PV were skeletonized with safety and the periportal lymph nodes were removed. At this stage of the procedure, it is extremely significant to identify and conserve the CHA branch for the RLL, which is normally expected to be found just proximal to the PV bifurcation (Figure 2 c,d). Anatomical variations are common in the porcine liver and may be a factor for major complications.

After the ligation and division of the HA branches, a demarcation line of the de-vascularized area can be noticed on the liver surface. It is important to perform temporal clamping before dividing any HA branch and then check if the RLL and caudate lobe remain vascularized. However, an extremely small area of the RML usually remains vascularized owing to short branches which cross-supply from the RLL. The right PV should be dissected near the bifurcation in order to preserve a short tributary of the right PV. Any damage to this area must be avoided so as not to cause severe hemorrhage.

The Pringle maneuver during hepatectomy is traditionally used by many liver surgeons to minimize blood loss. Nevertheless, this technique is associated with ischemia-reperfusion (I/R) injury. It is well known that I/R injury is the major underlying cause of liver dysfunction during liver transplantation or extended hepatectomy [19,20]. In the present study, we did not use the Pringle maneuver during liver transection in order to avoid any risk of postoperative liver dysfunction. Instead, we used other techniques apart from inflow occlusion, which are very important during extended hepatectomy in the control of blood loss. Among them are the following: controlled CVP below 3 cm H₂O [21]; punctilious surgical techniques; extra-parenchymal ligation of the inflow vessels to the resected part of the liver [22] and more refined surgical instruments.

Belghiti’s liver hanging maneuver uses a sling passed between the liver parenchyma and the anterior surface of IVC [23]. The most important part of this technique is the blind dissection of the anterior surface of IVC. For the reason that the hepatic veins and the hepatic IVC are covered almost completely by liver tissue and the retro-hepatic dissection is impossible, the liver-hanging maneuver is hard and unachievable to perform in a porcine model. Other techniques, such as setting a clamp around the hepatic pedicle or hand-control hepatectomy with the hepatic pedicle being manipulated manually, are described in the literature [7,16,24]. However, as our experience showed, these techniques were difficult to perform and could not be reproduced without complications.

Hori et al. [10] recently described their de-

### Table 1. Liver resection extent and procedure characteristics

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
<th>Min</th>
<th>Max</th>
<th>SE</th>
</tr>
</thead>
<tbody>
<tr>
<td>Animal body weight (kg)</td>
<td>28.98</td>
<td>1.82</td>
<td>26.092</td>
<td>32.416</td>
<td>0.379</td>
</tr>
<tr>
<td>Trilobectomy* (g)</td>
<td>398</td>
<td>38</td>
<td>355</td>
<td>490</td>
<td>0.007</td>
</tr>
<tr>
<td>Weight of actual resected liver (g)</td>
<td>427</td>
<td>59</td>
<td>362</td>
<td>522</td>
<td>0.008</td>
</tr>
<tr>
<td>Estimated total liver weight**(g)</td>
<td>567</td>
<td>55</td>
<td>478</td>
<td>700</td>
<td>0.011</td>
</tr>
<tr>
<td>Estimated residual liver weight (ERL)***(g)</td>
<td>177</td>
<td>16</td>
<td>116</td>
<td>950</td>
<td>0.055</td>
</tr>
<tr>
<td>Proportion of ERL (%)</td>
<td>24.5</td>
<td>1.4</td>
<td>18.400</td>
<td>25.4</td>
<td>0.285</td>
</tr>
<tr>
<td>Operation time (min)</td>
<td>155.8</td>
<td>64.8</td>
<td>78.00</td>
<td>256.0</td>
<td>13.55</td>
</tr>
<tr>
<td>Blood loss (ml)</td>
<td>81.9</td>
<td>33.7</td>
<td>36</td>
<td>167</td>
<td>7.03</td>
</tr>
</tbody>
</table>

SD: standard deviation, SE: standard error.
*Corresponds to 70% resection according to existing knowledge [8,9]
**Estimated total liver = (weight of LLL, LML and RML) X 100/70
***Estimated residual liver = Estimated of total liver weight-Weight of resected liver
tailed surgical technique for 70% hepatectomy in pigs in order to create an insufficient remnant liver volume. They used the pean clamp-crushing technique for the dissection of the liver parenchyma without Pringle maneuver. According to their surgical procedure, it is necessary to skeletonize the Glisson’s capsule, to ligate the PV branch of the RML and to use specific devices for hemostasis. However, despite the necessity of the ligation of the PV branch to the RML, it can involve serious technical difficulties.

In this study, the liver parenchymal transection was carried out by using the saline-coupled bipolar sealer. The purpose of this device was to reduce blood loss along the resection margin. By using this device, we sealed structures less than 6 mm in diameter without generating excessive charring and eschar. The mechanism to decrease perioperative bleeding is explained by the shrinkage of type I and type III collagen fibers in the walls of arteries and veins after the effect of the radiofrequency energy [25,26]. We closed and divided with clips or ties the structures which were more than 6 mm in diameter [27].

After extensive hepatectomy with an insufficient liver volume or after liver transplantation with a SFS liver graft, the patient’s survival is intertwined with liver regeneration. A large animal model, such as pig, is extremely useful in order to reproduce and understand the SFSS. Our simple technique for successful resection of 75% of the liver in pigs using the Aquamantys system achieves effective and safe liver parenchymal transection and it can provide useful information for researchers.

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Conflict of interest

None of the authors has any financial conflict of interest to declare.

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