JBUON 2016; 21(6): 1403-1409
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

ORIGINAL ARTICLE

Extended hepatectomy using the bipolar tissue sealer: an experimental model of small-for-size syndrome in pigs

Antonios Athanasiou [1], Michael Kontos[1], Emmanouil Pikoulis[1], John Griniatsos[1], Apostolos Papalois[2], Eleftherios Spartalis[1], Demetrios Moris[1], Evangelos Felekouras[1], Theodoros Liakakos[1]

[1]1st Department of Surgery, University of Athens, School of Medicine, Greece; [2]Experimental Research Center, ELPEN Pharmaceuticals, Pikermi, 19009 Attica, Greece.

Summary

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 a in 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*

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

Correspondence to: Antonios Athanasiou MD. 1st Department of Surgery, Laikon General Hospital, National and Kapodistrian University of Athens, School of Medicine, 2nd floor, AgiouThoma 17 str., Goudi, 15771, Athens, Greece, Tel: +30 6946122170, E-mail: antwnis_athanasiou@hotmail.com
Received: 28/12/2015; Accepted: 14/01/2016

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

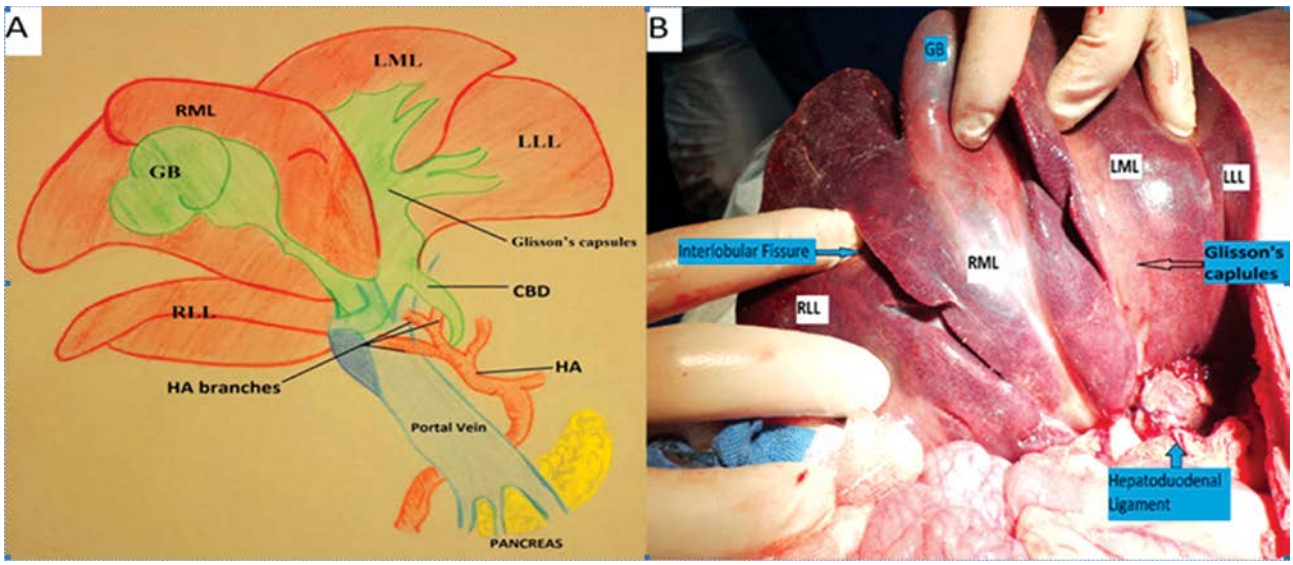

Figure 1. Diagrammatic illustration of the porcine liver anatomy. **A:** Diagrammatic illustration of the porcine liver anatomy. The interlobular fissures divide the liver into 5 distinctly independent lobes. Each lobe has its own venous, arterial and biliary draining (Glisson's pedicle). **B:** Diagrammatic 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 13 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

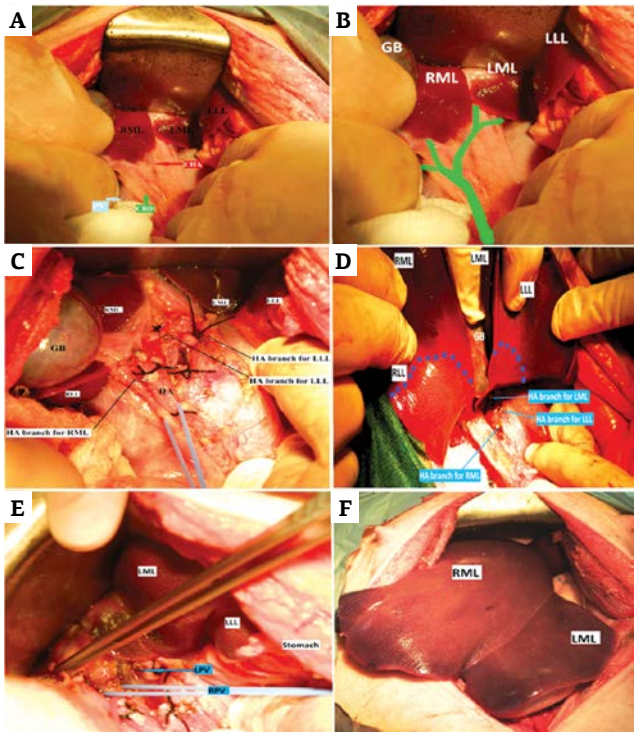

Figure 2. Dissection of the hepatoduodenal ligament. **A:** Hepatoduodenal ligament. The extrahepatic triad consists of the HA, PV and CBD. **B:** Location of CBD. The course of hepatic duct of each lobe. **C:** A surgical view after the division of the HA branches to the LLL, LML and RML. The HA is encircled with blue vessel loop and is located to the left of the CBD and anterior to the PV. **D:** 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. **E:** The left and right PV were carefully dissected and encircled with vessel loops. **F:** Ishemic RML and LML after the division of their HA branches.

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 Aquamantis 2.3 Bipolar sealer was connected to the Aquamantis 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 3 b-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).

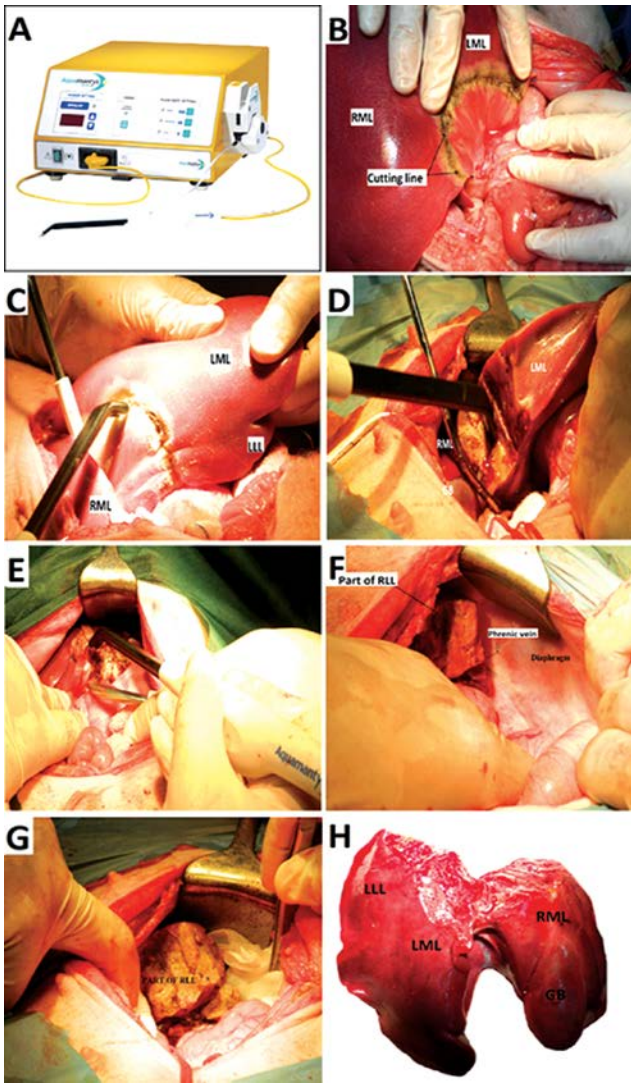

Figure 3. Liver resection using the bipolar sealer. **A:** The Aquamantis Pump Generator. **B:** A cutting line was set with the bipolar sealer according to the demarcation line. **C, D, E:** Liver resection using the bipolar sealer, structures less than 6 mm in diameter were sealed without generating excessive charring and eschar or high temperature.Constant suction was needed to clear the saline used from the bipolar sealer. **F,G:** All of the liver's lobes were resected except part of the RLL and the caudate lobe. **H:** The posterior aspect of the specimen after extensive hepatectomy.

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%

Table 1. Liver resection extent and procedure characteristics

Parameter	*Mean*	*SD*	*Min*	*Max*	*SE*
Animal body weight (kg)	28.98	1.82	26.092	32.416	0.379
Trilobectomy* (g)	398	38	335	490	0.007
Weight of actual resected liver (g)	427	39	362	522	0.008
Estimated total liver weight**(g)	567	55	478	700	0.011
Estimated residual liver weight (ERL)***(g)	177	16	116	950	0.035
Proportion of ERL (%)	24.5	1.3	18.400	25.4	0.285
Operation time (min)	153.8	64.8	78.00	256.0	13.53
Blood loss (ml)	81.9	33.7	36	167	7.03

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

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 billiary 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 caudane 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 H_2O [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-

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.

Acknowledgements

This study was funded by Scholarship – Grant by the Experimental - Research Center ELPEN Pharmaceuticals (E.R.C.E). The authors are grateful to Mrs. Maria Karamperi, Mrs Popi Tsarea and Miss Elefteria Karampela as well as to the rest of Elpen's team for their skillful technical assistance.

Conflict of interest

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

References

1. Galle PR. Management of liver cancer. Digestive Dis 2016;34:438-439.
2. Li L, Johnson M, Calabrese C et al. Identification of highly metastatic disseminating tumor cells in late-stage liver cancer lung metastasis. Cancer Research 2016;76:B38-B38.
3. Liver Resection of Secondary Liver Cancer. In: Li K, Yang J, Liu C, Chen P (Eds): Operative Techniques in Liver Resection. Springer, 2016, pp 153-157.
4. Zhang S, Yue M, Shu R, Cheng H, Hu P. Recent advances in the management of hepatocellular carcinoma. JBUON 2016;21:307-311.
5. Taniguchi M, Shimamura T, Todo S, Furukawa H. Small-for-size syndrome in living-donor liver transplantation using a left lobe graft. Surg Today 2015;45:663-671.
6. Court FG, Wemyss-Holden SA, Morrison CP et al. Segmental nature of the porcine liver and its potential as a model for experimental partial hepatectomy. Br J Surg 2003;90:440-444.
7. Court FG, Laws PE, Morrison CP, Teague BD, Metcalfe MS, Wemyss-Holden SA, Dennison AR, Maddern GJ: Subtotal hepatectomy: a porcine model for the study of liver regeneration. J Surg Res 2004;116:181-186.
8. Ito T, Kiuchi T, Yamamoto H, Oike F et al. Changes in portal venous pressure in the early phase after living donor liver transplantation: pathogenesis and clinical implications. Transplantation 2003;75:1313-1317.
9. Yoshizumi T, Taketomi A, Soejima Y et al. The beneficial role of simultaneous splenectomy in living donor liver transplantation in patients with small-for-size graft. Transpl Int 2008;21:833-842.
10. Hori T, Yagi S, Okamua Y et al. How to successfully resect 70 % of the liver in pigs to model an extended hepatectomy with an insufficient remnant or liver transplantation with a small-for-size graft. Surg Today 2014;44:2201-2207.
11. Couinaud C. [Liver lobes and segments: notes on the anatomical architecture and surgery of the liver]. Presse Med 1954;62:709-712.
12. Kahn D, Hickman R, Terblanche J, von Sommoggy S. Partial hepatectomy and liver regeneration in pigs--the response to different resection sizes. J Surg Res 1988;45:176-180.
13. Iida T, Yagi S, Taniguchi K, Hori T, Uemoto S. Improvement of morphological changes after 70% hepatectomy with portocaval shunt: preclinical study in porcine model. J Surg Res 2007;143:238-246.
14. Ingram DL, Legge KF. Variations in deep body temperature in the young unrestrained pig over the 24 hour period. J Physiol 1970;210:989-998.
15. Marulanda GA, Ulrich SD, Seyler TM, Delanois RE,

Mont MA. Reductions in blood loss with a bipolar sealer in total hip arthroplasty. Expert Rev Med Devices 2008;5:125-131.

16. Xia Q, Lu TF, Zhou ZH et al. Extended hepatectomy with segments I and VII as resection remnant: a simple model for small-for-size injuries in pigs. Hepatobil Pancreat Dis Int 2008;7:601-607.
17. Arkadopoulos N, Defterevos G, Nastos C et al. Development of a porcine model of post-hepatectomy liver failure. J Surg Res 2011;170:e233-242.
18. Park YK, Kim BW, Wang HJ, Xu W. Usefulness of the Pinch-Burn-Cut (PBC) technique for recipient hepatectomy in liver transplantation. Korean J Hepatobil Pancreat Surg 2012;16:13-16.
19. Clavien PA, Selzner M, Rudiger HA et al. A prospective randomized study in 100 consecutive patients undergoing major liver resection with versus without ischemic preconditioning. Ann Surg 2003;238:843-850; discussion 851-842.
20. Theodoraki K, Tympa A, Karmaniolou I, Tsaroucha A, Arkadopoulos N, Smyrniotis V. Ischemia/reperfusion injury in liver resection: a review of preconditioning methods. Surg Today 2011;41:620-629.
21. Li Z, Sun YM, Wu FX, Yang LQ, Lu ZJ, Yu WF. Controlled low central venous pressure reduces blood loss and transfusion requirements in hepatectomy. World J Gastroenterol 2014;20:303-309.
22. Capussotti L, Muratore A, Ferrero A, Massucco P, Ribero D, Polastri R. Randomized clinical trial of liver resection with and without hepatic pedicle clamping. Br J Surg 2006;93:685-689.
23. Kokudo N, Imamura H, Sano K et al. Ultrasonically assisted retrohepatic dissection for a liver hanging maneuver. Ann Surg 2005;242:651-654.
24. Ladurner R, Hochleitner B, Schneeberger S et al. Extended liver resection and hepatic ischemia in pigs: a new, potentially reversible model to induce acute liver failure and study artificial liver support systems. Eur Surg Res 2005;37:365-369.
25. Kaibori M, Matsui K, Ishizaki M et al. A prospective randomized controlled trial of hemostasis with a bipolar sealer during hepatic transection for liver resection. Surgery 2013;154:1046-1052.
26. Kaparelos D, Moris D, Kontos M et al. Microwave versus saline-linked radiofrequency (Aquamantys) assisted liver resection in a porcine liver resection model. A safety and feasibility pilot study. JBUON 2016;21:412-418.
27. Curro G, Lazzara S, Barbera A et al. The Aquamantys(R) system as alternative for parenchymal division and hemostasis in liver resection for hepatocellular carcinoma: a preliminary study. Eur Rev Med Pharmacol Sci 2014;18:2-5.