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Dear editors

We appreciate the opportunity to present our microsurgical technique for establishment of a novel surgical technique as a foundation for further in-vivo liver engineering in rat to the Journal of Visualized Experiments.

Recently one research group (Pan and colleagues) was found publishing a paper regarding in-vivo liver engineering, which gives an opportunity to study liver engineering in-vivo rather than ex-vivo. However, postoperative survival was absent in his experiment. Thus, we established a novel surgical technique for in-vivo surgical single liver lobe perfusion with a long survival period of more than 1 week, which serves as a foundation for in-vivo partial liver engineering in rat. We would like to publish this procedure in JoVE, which provides an opportunity letting the audiences learn the procedure in a visualized way. This film we made will demonstrate this whole procedure step by step. We hope this technique can be used in many experimental studies where a surgical in-vivo partial liver perfusion model are needed, e.g. to study partial organ treatment by infusion with drugs, in-vivo “cell culture system” in comparison with ex-vivo, in-vivo partial organ decellularization as chemical resection, and in-vivo liver engineering.

Thanks again for reviewing our manuscript.
Best regards

Uta Dahmen

Authors' contribution

An Wang: Responsibility of designing and establishing surgical procedure, cutting and editing the film, analyzing the results and writing of the manuscript.

Jank Isabel: Assistance in editing the film and analyzing the results.

Wei weiwei: Instruction regarding micro-surgical skills

Claudia Schindler: Instruction regarding the hemodynamics of liver in rat

Uta Dahmen: Supervision of the project

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TITLE:

A Novel Surgical Technique as a Foundation for *In Vivo* Partial Liver Engineering in Rat

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KEYWORDS:

in vivo, liver perfusion, left lateral lobe, cell culture system, decellularization, recellularization, liver engineering

SHORT ABSTRACT:

We establish a novel surgical technique for an *in vivo* single liver lobe perfusion model in rat as a prerequisite for further studying *in vivo* partial liver engineering in the future.

LONG ABSTRACT:

Organ engineering is a novel strategy to generate liver organ substitutes that can potentially be used in transplantation. Recently, *in vivo* liver engineering, including *in vivo* organ decellularization followed by repopulation, has emerged as a promising approach over *ex vivo* liver engineering. However, postoperative survival was not achieved. The aim of this study is to develop a novel surgical technique of *in vivo* selective liver lobe perfusion in rats as a prerequisite for *in vivo* liver engineering. We generate a circuit bypass only through the left lateral lobe. Then, the left lateral lobe is perfused with heparinized saline. The experiment is performed with 4 groups ($n = 3$ rats per group) based on different perfusion times of 20 min, 2 h, 3 h, and 4 h. Survival, as well as the macroscopically visible change of color and the histologically determined absence of blood cells in the portal triad and the sinusoids, is taken as an indicator for a successful model establishment. After selective perfusion of the left lateral lobe, we observe that the left lateral lobe, indeed, turned from red to faint yellow. In a histological assessment, no blood cells are visible in the branch of the portal vein, the central vein, and the sinusoids. The left lateral lobe turns red after reopening the blocked vessels. 12/12 rats survived the procedure for more than one week. We are the first to report a surgical model for *in vivo* single liver lobe perfusion

with a long survival period of more than one week. In contrast to the previously published report, the most important advantage of the technique presented here is that perfusion of 70% of the liver is maintained throughout the whole procedure. The establishment of this technique provides a foundation for *in vivo* partial liver engineering in rats, including decellularization and recellularization.

INTRODUCTION:

The indications for organ transplantation are constantly expanding. In contrast, organ donation rates and overall quality of organs are declining, leading to an increasing demand for grafts. The number of candidates added to the liver transplant waiting list continued to increase (*e.g.*, in the United States, 11,340 patients were added in 2016, compared with 10,636 in 2015)¹. Despite substantial efforts, the number of available organs does not meet clinical needs. Due to the increased incidence of liver disease, many patients with end-stage liver diseases die on the transplant waiting list before a donor organ becomes available. To meet the huge demand for donor liver grafts, alternative approaches using liver tissue engineering principles are being actively pursued². Nowadays, a newly developed biological technique of liver engineering could potentially overcome this shortage.

Liver engineering consists of two steps: the generation of an acellular scaffold, followed by a repopulation of the scaffold. To obtain a biological acellular liver scaffold, the explanted liver is perfused *via* the vascular system with ionic or nonionic detergents, which can remove the cellular material from the liver. In most previous studies, a biological acellular liver scaffold was achieved by perfusion of the liver with a combination of sodium dodecyl sulfate and TritonX100. As a result, all cells were removed, whereas the structure of the extracellular matrix was maintained. The organ scaffolds were reseeded with mature cells, hepatocellular, as well as endothelial cell lines, and primary hepatocytes with or without the simultaneous application of endothelial cells or mesenchymal stem cells (MSC). Most researchers focus on *ex vivo* liver engineering³⁻¹⁴. However, in most previous studies, only small pieces of repopulated scaffold cubes were transplanted into different heterotopic implantation sites. In a few studies, partial repopulated scaffolds were transplanted as an auxiliary graft. However, the maximal reported survival time was only 72 h^{8,14}. As far as we know, orthotopic transplantation of a repopulated full liver graft has not yet been performed or published about. The long-term function and transplantation of engineered organs are still in their infancy. Therefore, an alternative approach to *ex vivo* liver engineering is needed.

In vivo liver engineering may represent an alternative to study hepatic repopulation under physiological conditions. The advantages of *in vivo* liver engineering compared to *ex vivo* liver engineering are manifold. The *in vivo* repopulated partial liver scaffold is subjected to physiological blood perfusion with proper temperature, sufficient oxygen, nutrients, and growth factors in contrast to *ex vivo* perfusion with artificial culture medium. Furthermore, the remaining partial normal liver maintains the hepatic function, principally allowing long-term survival. Since an implanted *ex vivo* engineered liver graft is still incapable of sustaining the long-term survival of experimental animals by its liver function⁸, we envision that *in vivo* partial liver engineering would ultimately become a promising model to further study the evolution of engineered livers with longer survival observations than *ex vivo*.

89
90 Recently, one research group (Pan and colleagues) presented, for the first time, a technique of *in*
91 *vivo* liver engineering¹⁵. They achieved the isolated perfusion of the right inferior liver lobe in
92 living rats despite anatomic and technical challenges. They reported the first intraoperative
93 results of *in vivo* repopulation using a human hepatocyte cell line. However, the *in vivo* surgical
94 perfusion model of Pan *et al.* has disadvantages. They achieved single liver lobe perfusion in rats
95 at the expense of completely blocking the portal vein and inferior vena cava, which may cause
96 severe harm to the animal. The experimental rats were sacrificed after only 6 hours of
97 intraoperative observation time. Therefore, the *in vivo* liver lobe perfusion technique needs
98 further improvement to achieve postoperative survival.

99
100 We developed a novel survival model for *in vivo* liver lobe perfusion, based on previous studies
101 of the hepatic anatomy of rat¹⁶, the portal vein cannulation technique for hemodynamic
102 monitoring in mice¹⁷, and liver bioengineering^{18,19}. The key steps for the procedure are illustrated
103 in **Figures 1A - 1E**.

104
105 This technique is suitable for those who want to use this experimental *in vivo* perfusion model
106 for basic research on partial organ treatment by infusion with drugs, *in vivo* decellularization as
107 a chemical resection for organ diseases (*e.g.*, liver cancer), *in vivo* cell culture in a decellularized
108 matrix comparing *ex vivo* two-dimensional and three-dimensional cell culture systems²⁰⁻²⁶, and
109 *in vivo* liver engineering by decellularization and repopulation.

110 111 **PROTOCOL:**

112 The housing and all procedures carried out were in accordance with German animal welfare
113 legislation. All gauze, covering clothes, and surgical instruments are autoclaved and prepared
114 before the operation. All procedures are carried out under sterile conditions.

115 116 **1. Preparation of the Rat for the Surgical Procedure**

117
118 1.1. Place the rat in an induction chamber and anesthetize the rat with 4% vaporized isoflurane
119 and 100% oxygen at 0.5 L/min for about 3 min, until the rat is completely anesthetized.

120
121 1.2. Take the rat out of the induction chamber and measure its body weight.

122
123 1.3. Shave the fur of the surgical region on the abdomen.

124
125 1.4. Place the animal back into the isoflurane chamber for an additional 2 min to deepen
126 anesthesia.

127
128 1.5. Place the rat on the operation table in supine position.

129
130 1.6. Fix the anesthesia mask to the mouth region of the rat and keep the animal anesthetized
131 with a continuous gas flow of 2% vaporized isoflurane and 100% oxygen at a flow rate of 0.5
132 L/min.

1.7. Fix the limbs with pieces of tape.

1.8. Apply vet ointment on both eyes to prevent dryness.

1.9. Administer buprenorphine 0.05 mg/kg subcutaneously, to relieve pain during the operation period.

1.10. Disinfect the surgical field of the abdomen with 3 rounds of iodine tincture followed by 2 rounds of 70% alcohol.

1.11. Place sterilized gauze around the area where the incision will be made to only leave the operation field of the abdomen exposed.

1.12. Proceed to perform the operation when the toe-pinch withdrawal reflex of the rat is absent.

2. Laparotomy of the Rat

2.1. Make a transverse abdominal skin and muscle incision using scissors and an electrical coagulator.

2.2. Fix and pull the xiphoid process toward the head using a 4-0 polypropylene suture.

Note: Pay attention to lift up the xiphoid process vertically to better expose the liver, but proceed with caution to avoid severe respiratory restriction and suffocation.

2.3. Open the peritoneal cavity by pulling both sides of the abdominal walls towards the head with two subcostal hooks to expose the liver.

2.4. Cover the duodenum and small intestine in the abdominal cavity with a moistened gauze to avoid drying.

2.5. Lift left and right median lobes up by using a moistened gauze and hold them against the thorax to better expose the hilum of the liver.

2.6. Place the rat under a stereomicroscope (8X magnification).

2.7. Drop some warm saline into the abdomen and onto the surface of the liver and intestines every several minutes, to prevent drying during the whole procedure.

3. Establishment of a Bypass Passage Within the Left Lateral Lobe

3.1. Dissect the left portal vein and ligate it with a 6-0 silk suture at the base (**Figure 2A**).

3.2. Block the left hepatic artery, the left bile duct along with the left median portal vein, the left median hepatic artery, and the left median bile duct with micro clamps to prevent a flow of the perfusate to the left median lobe (**Figure 2B**).

3.3. Separate the left lateral lobe by cutting off the surrounding ligaments of the lobe with micro scissors.

3.4. Block the left lateral hepatic vein by clamping at the base of the left lateral lobe with micro clamps (**Figure 2C**).

Note: Make sure not to clamp the left portal vein as well by mistake.

3.5. Use mosquito clamps to hold the ligature of the left portal vein and keep the vein with proper tension for later cannulation.

3.6. Carefully make an incision in the front wall of the left portal vein by puncturing it with a 24-G needle-dwelling catheter (**Figure 3A**).

Note: To create a bypass, vascular access points on the left portal vein and the left hepatic vein are needed. For this step, it is preferred to create the vascular access by puncturing the vessels with a needle rather than making a larger incision using scissors. This reduces the risk of bleeding and later stenosis.

3.7. Withdraw the catheter and take the needle out of the catheter to obtain a needle-free 24-G catheter.

3.8. Connect the catheter to a perfusion tube, of which the other endpoint connects to a 20-mL syringe with 15 mL of 40 U/mL heparinized saline on a perfusion pump.

3.9. Turn on the pump for perfusing the tube to expel air out from the tube and the needle-free catheter.

3.10. Turn off the perfusion pump.

3.11. Again, insert the needle-free catheter into the left portal vein *via* the punctured incision on the vein (**Figure 3B**).

Note: Owing to the fact there is very limited space for fixing the catheter, it is not fixed at this point. Therefore, the surgeon should use care to avoid the displacement of the cannulated catheter.

3.12. Carefully make another incision at the margin of the exposed region of the left lateral hepatic vein by puncturing it with a 22- or 24-G needle-dwelling catheter (**Figure 3C**).

Note: It is recommended that the catheter be slightly smaller than the vessel.

3.13. Withdraw the catheter and take the needle out of the catheter to obtain a needle-free 22-G catheter.

3.14. Turn on the perfusion pump to perfuse heparinized saline into the left lateral lobe *via* the 24-G cannulated needle-free catheter of the left portal vein at a flow rate of 0.5 mL/min.

3.15. Use dry gauze to absorb out-flowing waste fluid around the incision area of the left lateral hepatic vein.

3.16. Cannulate the left lateral hepatic vein *via* the punctured incision of the vein with the 22-G needle-free catheter, to generate a fluid outlet to minimize intra-abdominal contamination (**Figure 3D**).

Note: It is technically difficult to fix the cannulated catheter to the liver lobe. Therefore, the surgeon should pay attention to avoid displacement of the catheter. Alternatively, without cannulation of the left lateral hepatic vein with a catheter, waste fluid can also be absorbed at the incision region of the vein only with a dry gauze.

3.17. Keep perfusing the left lateral lobe with heparinized saline for around 20 min (group 1) and then only with saline for 2 h, 3 h, or 4 h (group 2, group 3, and group 4, respectively).

3.18. Turn off the pump to stop the perfusion.

4. Physiological Reperfusion of the Left Lateral Lobe

4.1. Take off both catheters from the left portal vein and the left lateral hepatic vein.

4.2. Close the incision of the left portal vein with an 11-0 polyamide suture.

4.3. Close the incision of the left lateral hepatic vein with an 11-0 polyamide suture as well.

4.4. Unclamp the left lateral hepatic vein.

4.5. Unclamp the left median portal vein, left bile duct, and left hepatic artery.

4.6. Cut off the ligature on the left portal vein to reopen the vein.

5. Closure of the Abdominal Wall

5.1. Close the muscle layer of the abdominal wall by interrupted suturing with a 4-0 absorbable polyglactin 910 suture.

5.2. Close the skin layer of the abdominal wall by interrupted suturing with a 4-0 absorbable polydioxanone suture.

5.3. After closing the abdomen, disinfect the skin incision with 70% alcohol.

6. Postoperative Treatment of the Rat

6.1. Place the animal on a warming pad for resuscitation for about 10 min and then put it into a new cage.

6.2. Administer buprenorphine 0.05 mg/kg subcutaneously 2x a day for a consecutive 3 d postoperatively to release pain.

REPRESENTATIVE RESULTS:

Twelve male (aged 12 - 13 weeks) Lewis rats were used to assess the effect of selective liver lobe perfusion. The experiment was performed in four groups ($n = 3$ rats per group). Using different perfusion periods of 20 minutes, 2 hours, 3 hours, and 4 hours, following the steps described above, we successfully achieved *in vivo* single lobe perfusion.

***In Vivo* Perfusion of the Left Lateral Lobe:**

The accurate anatomical identification of the left portal vein and the left lateral hepatic vein and the successful left portal vein and left lateral hepatic vein cannulation can be confirmed after perfusion with heparinized saline. The first indicator of a successful identification and cannulation was the observation that blood mixed with perfusate was flowing out of the left lateral lobe *via* the fluid outlet (**Figure 4A**). The successful surgical perfusion model was further confirmed by observing the change of the left lateral lobe's color after the perfusion with heparinized saline. The color of the left lateral lobe changed from bright red to faint yellow, indicating the removal of blood from the left lateral lobe. To confirm the maintenance of the physiological perfusion of the remaining lobes, the color of the remaining liver lobes was constantly observed during the perfusion of the left lateral lobe. The correct perfusion of the left lateral lobe resulted in the lobe turning faint yellow while the remaining liver lobes maintained their bright red color throughout the whole process (**Figure 4B**).

Physiological Reperfusion of the Left Lateral Lobe:

To assess the patency of the cannulated vessels after closing the incisions of the vessels and reopening the vessels, the color change of the left lateral lobe was observed. A few red spots appeared on the surface of the perfused faint yellow left lateral lobe after reopening the left lateral hepatic vein, indicating initial retrograde perfusion in the left lateral lobe (**Figure 5A**). The surface of the targeted lobe later showed even more red spots after releasing the micro clamps of the left hepatic artery, left bile duct, and left median portal vein, implying further physiological perfusion of the left lateral lobe *via* the reopened vessels (**Figure 5B**). The surface of the targeted liver lobe turned dark red after reopening the left portal vein, confirming that the targeted liver lobe regained its full physiological perfusion *via* the left portal vein (**Figure 5C**).

Histology of the Perfused Left Lateral Lobe:

After finishing heparinized saline or saline perfusion, the selectively perfused left lateral lobe (as experimental) and the normal inferior caudate lobe (as control) were resected and fixed with formaldehyde and then subjected to a histological examination (H&E staining). In the left lateral lobe, there are no obvious blood cells visible in the branch of the portal vein, sinusoids, and central vein. As expected, red cells were visible in the branch of the hepatic artery (**Figures 6A and 6C**). In the normal inferior caudate lobe (as control), blood cells were significantly observed in the branch of the portal vein, the sinusoids, and the central vein (**Figures 6B and 6D**).

Survival Rate:

Twelve out of twelve experimental rats resulted in a 1-week survival rate of 100%. However, 3 experimental rats which underwent 4 hours of perfusion suffered temporarily from diarrhea and bloody discharge from the eyes on the second or third day postoperatively.

FIGURE AND TABLE LEGENDS:

Figure 1: Scheme of the surgical *in vivo* single liver lobe perfusion model. (A) This is a schematic drawing of the rat liver anatomy. (B) This panel shows the blockage of the left portal vein, the left hepatic artery, the left bile duct, the left median portal vein, and the left lateral hepatic vein. (C) This panel shows the cannulation of the left portal vein and the left lateral hepatic vein with catheters for fluid inlet and outlet. (D) This panel shows the perfusion of the left lateral lobe with heparinized saline by a perfusion pump. (E) This panel shows the physiological reperfusion of the left lateral lobe after reopening the blocked vessels to the lobe.

Figure 2: Intraoperative images showing the blockage of the vessels supplying and draining the left lateral lobe. (A) This panel shows the ligation of the left portal vein. (B) This panel shows the blockage of the left hepatic artery, the left bile duct, and the left median portal vein/hepatic artery/bile duct with micro clamps. (C) This panel shows the blockage of the left lateral hepatic vein with micro clamps (white arrow). The scale bars are 1 mm.

Figure 3: Intraoperative images showing the bypass circulation through the left lateral lobe. (A) This panel shows the incision in the left portal vein (white arrow) by puncturing it with a 24-G needle-dwelling catheter. (B) This panel shows the cannulation of the left portal vein for a fluid inlet with a 24-G needle-free catheter (white arrow). (C) This panel shows the incision in the left lateral hepatic vein (white arrow) by puncturing it with a 22-G needle-dwelling catheter. (D) This panel shows the cannulation of the left lateral hepatic vein for a fluid outlet with a 22-G needle-free catheter (white arrow). The scale bars are 1 mm.

Figure 4: Intraoperative images showing perfusion of the left lateral lobe. (A) This panel shows perfusate flowing into the left lateral lobe *via* the inlet (yellow catheter) and flowing out of the left lateral lobe *via* the outlet (blue catheter). The left lateral lobe was, indeed, selectively perfused, as shown by the color changing of the lobe (white arrow). (B) Note the color change of the left lateral lobe to faint yellow after the perfusion with heparinized saline, while the remaining liver lobes remain bright red (white arrows). The scale bars are 1 mm.

Figure 5: Physiological reperfusion of the left lateral lobe. (A) This panel shows the retrograde perfusion of the left lateral lobe after reopening the left lateral hepatic vein (white arrow). (B) This panel shows the physiological reperfusion of the left lateral lobe (white arrow) after releasing the micro clamps on the left median portal vein and the left hepatic artery. (C) This panel shows the complete reperfusion of the left lateral lobe (red arrow) after reopening the left portal vein and the ischemia of the left median lobe (blue arrow). The scale bars are 1 mm.

Figure 6: Histological assessments (H&E staining). (A and C) These panels show a histological assessment of the heparin-saline-perfused left lateral lobe (experimental lobe) demonstrating the absence of blood cells in (A) the portal vein and sinusoids, and (C) the central vein. (A) However, red cells are visible in the hepatic artery (black arrow) as expected. (B and D) These panels show a histological assessment of the normal inferior caudate lobe (control) revealing the presence of blood cells in all vascular structures: (B) portal vein, hepatic artery, and sinusoids (black arrows), and (D) central vein (black arrow). The scale bars are 250 μ m.

Table 1. Comparison between different models of *in vivo* liver engineering. This table demonstrates critical differences in the two model establishments between Pan *et al.*¹⁵ and our research group.

DISCUSSION:

By blocking and cannulating the left portal vein with a catheter as a fluid inlet and the left lateral hepatic vein with another catheter as a fluid outlet, we successfully generated an *in vivo* fluid bypass within the left lateral lobe, indicating that although the technique is highly challenging due to the small size of the vessels for cannulation and a high risk of causing bleeding, it is feasible. Even the rats undergoing a long perfusion period of 4 hours survived at least 1 week, showing that the rats could tolerate this surgical procedure.

In the following section, we describe the three technically most difficult and critical steps and how to successfully master them. Firstly, in the process of separation and ligation of the left portal vein, due to the close spatial relationship of the targeted portal vein with the surrounding liver parenchyma, isolation of the vessel has a high risk of causing bleeding. We recommend using micro forceps rather than scissors to separate the left portal vein, by carefully tearing apart the connected avascular tissue surrounding the left portal vein. Secondly, in terms of the incision on the front wall of the left portal vein, an oversize incision on the front wall of the left portal vein may increase the difficulty of incision repair and the chance of causing stenosis after vascular anastomosis. We recommend using a needle-dwelling catheter instead of scissors for making a properly sized incision. Thirdly, regarding the blockage of the left lateral hepatic vein, if the exposed region of the left lateral hepatic vein is blocked improperly, there will not be sufficiently exposed space on the left lateral hepatic vein for later cannulation. We suggest the clamping should be performed close to the left median lobe rather than in the middle of the exposed region of the left lateral hepatic vein.

We compare the two models of selective *in vivo* liver perfusion between Pan *et al.*¹⁵ and ours (Table 1). Firstly, the selection of the targeted liver lobe is considered a most critical step for the model establishment. We selected a rather isolated lobe, the left lateral lobe, as the targeted lobe for *in vivo* surgical perfusion model establishment. In contrast, the research group of Pan selected the right inferior lobe for the model¹⁴. Shortcomings of selecting the right inferior lobe are as followings: firstly, they had to compromise by completely blocking and cannulating the vena cava for a fluid outlet, which may negatively impact the blood circulation of the animal. Secondly, they had to block and cannulate the main portal vein for an inlet, which caused ischemia of the remaining liver lobes, and portal hypertension. As compared to the lobe targeted here, the left lateral lobe, we blocked and cannulated left lateral hepatic vein instead of the vena cava for a fluid outlet. We generated a fluid inlet by selecting the left portal vein, which has a rather large diameter, facilitating dissection, blockage, and cannulation. Therefore, directly blocking the blood flow to the vena cava and the main portal vein is avoided in the model presented here, which is critical to the survival of the rat.

Despite the great potential for *in vivo* liver lobe perfusion, this technique has some limitations. Firstly, ischemia of the left medial lobe is inevitable, due to the blockage of the left median portal vein during the process. Secondly, based on the temporary blockage at the base of the left lateral lobe using micro clamps, this may lead to damage of the liver parenchyma surrounding the lobar base and inevitably cause a slight leakage during the perfusion with heparinized saline. Three experimental rats which were subjected to a perfusion period of 4 hours suffered temporarily from diarrhea and bloody ocular discharge, suggesting that 4 hours might be the maximum perfusion period for a rat without suffering more complications. To our knowledge, at least 3 hours is required for the decellularization of a whole rat liver *ex vivo*¹⁸. Therefore, the 4 hours of perfusion time we intended to achieve would be sufficient for further liver lobe decellularization, which is a prerequisite for liver engineering by cell repopulation of a liver scaffold.

In the future, this novel technique for an *in vivo* perfusion model may potentially be used in experimental research for partial organ treatment by infusion with drugs, in *in vivo* partial organ decellularization as chemical resection, as an “*in vivo* cell culture system”, and, possibly most importantly, for *in vivo* partial organ engineering.

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DISCLOSURES:

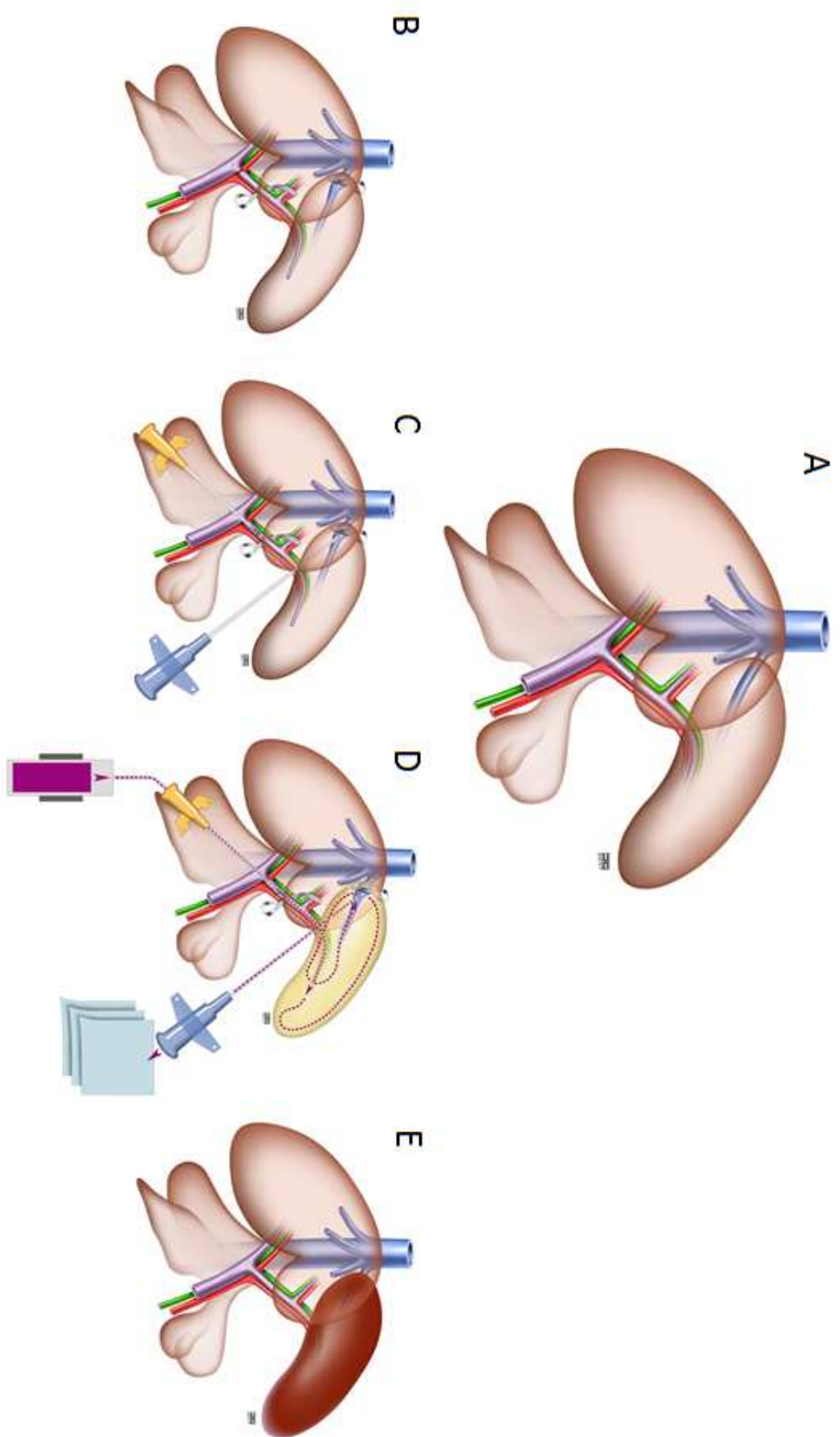
The authors have nothing to disclose.

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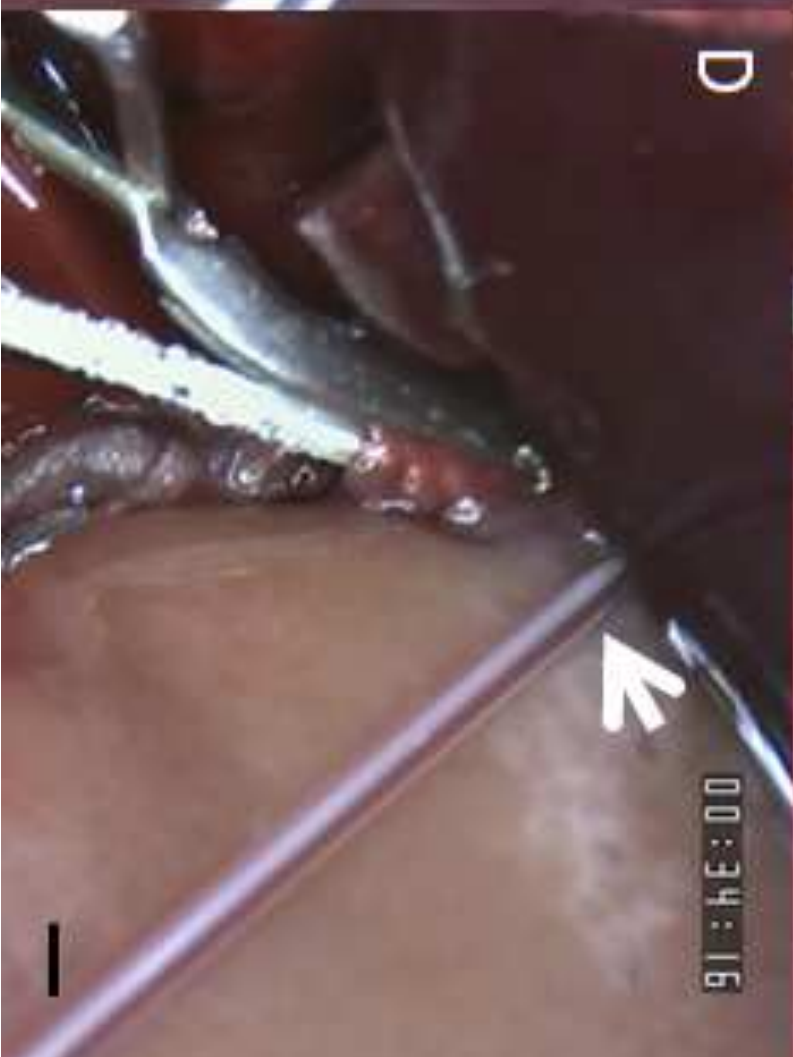
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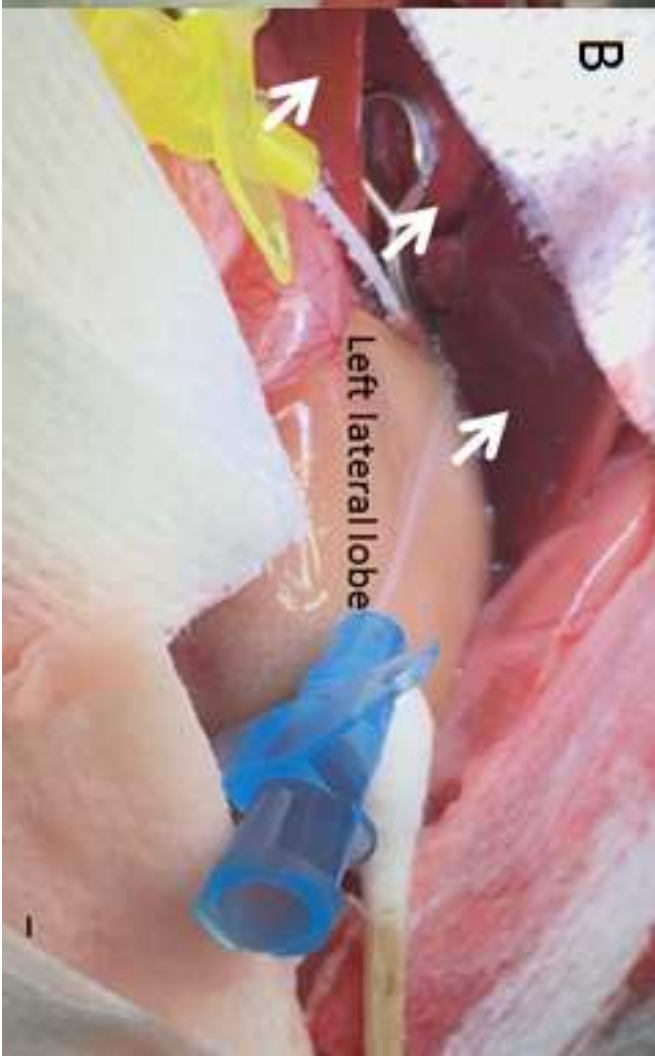
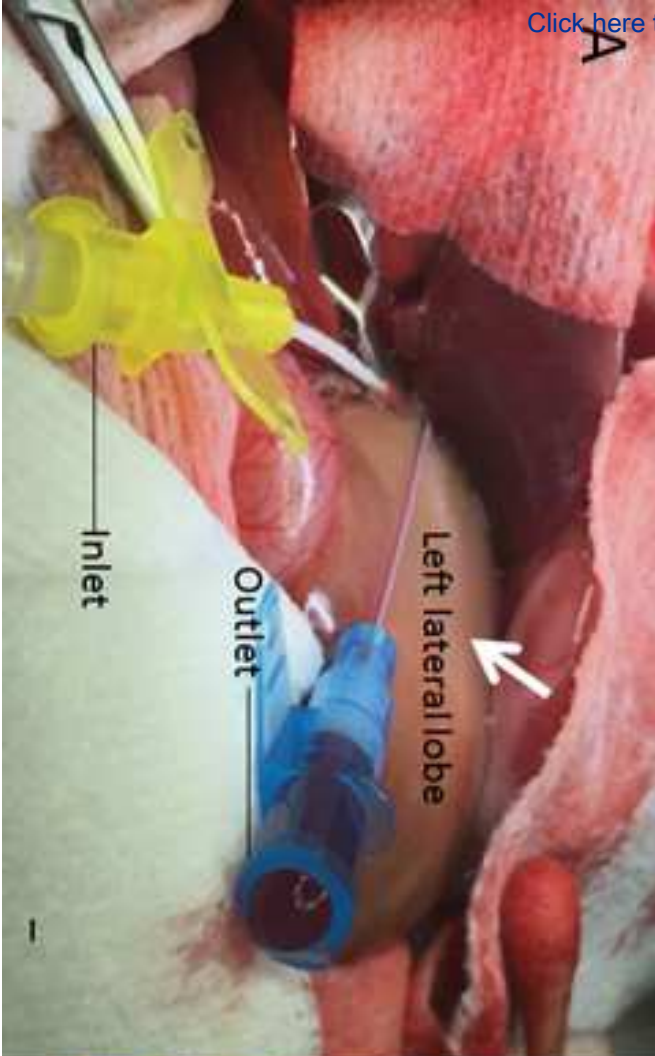
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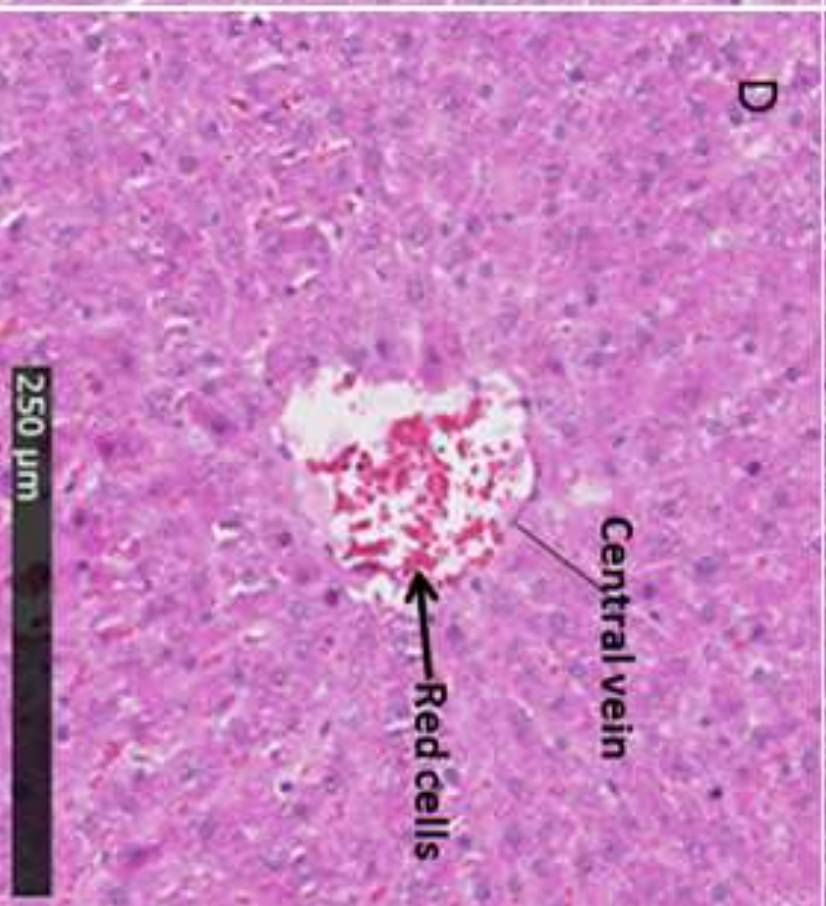
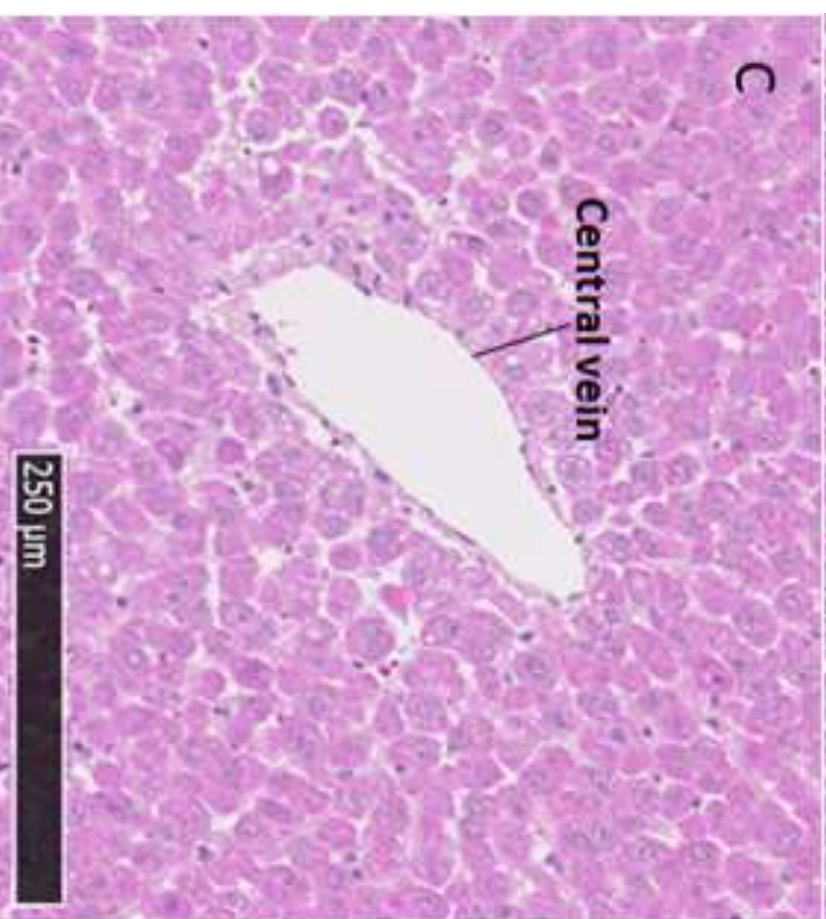
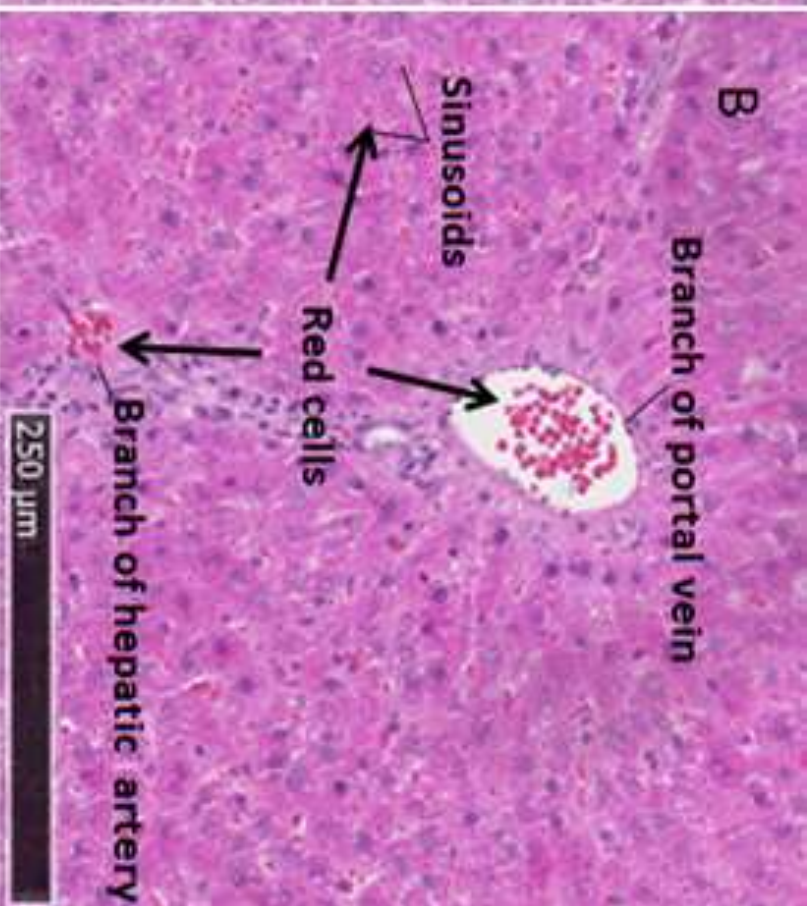
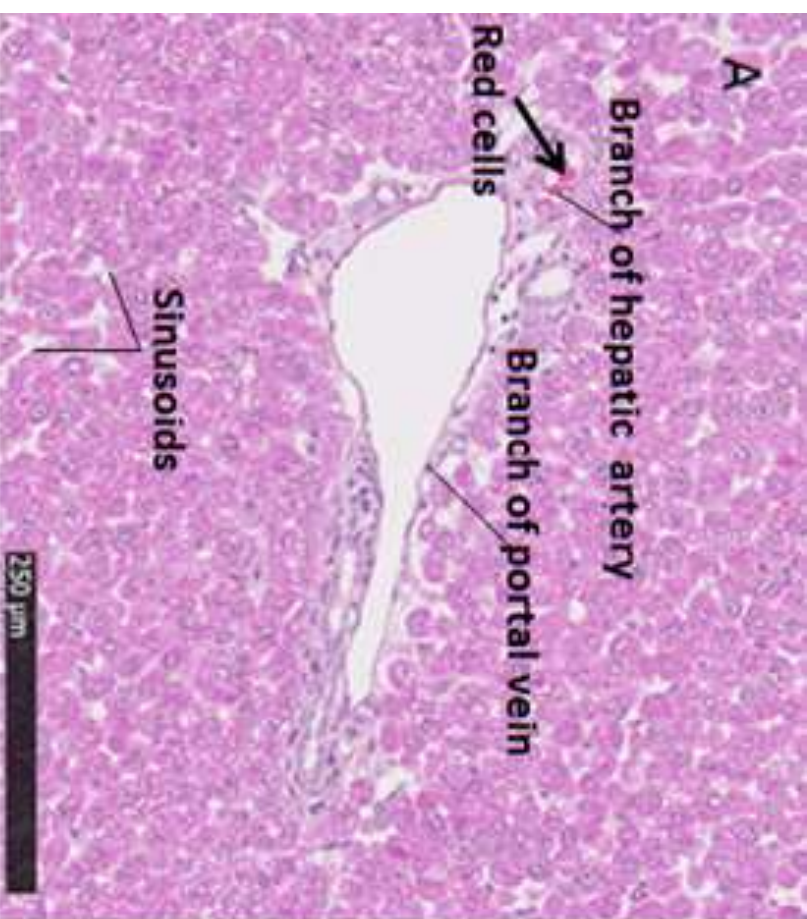












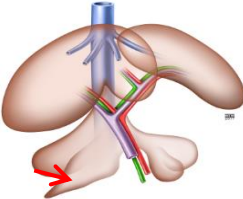
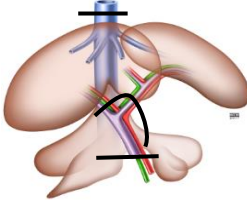


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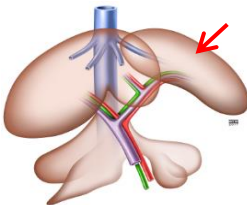
Parameters	Result
Research group	Pan et al 2016 [15]
In-vivo	Yes
Target liver lobe	Right inferior lobe 
Blockage of vena cava and main portal vein	Yes 
Ischemia of the remaining lobes	Yes
Cannulation of vena cava	Yes
Cannulation of main portal vein	Yes
Portal hypertension	Yes
1 week survival rate	Sacrificed intraoperatively

is

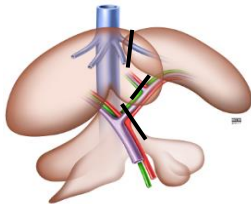
Own group

Yes

Left lateral lobe



No



Only left median lobe

No

No

No

100%

Name of Material/ Equipment	Company	Catalog Number	Comments/Description
Perfusion Pump Perfusor VI	B. Braun, Melsungen		
Catheter			
Versatus-W Catheter	Terumo	SR+DU2419PX	24G, 0.74×19mm
Versatus-W Catheter	Terumo	SR+DU2225PX	22G, 0.9×25mm
micro surgical instrument			
micro scissors	F·S·L	No. 14058-09	
micro serrefine	F·S·L	No.18055-05	
Micro clamps applicator	F·S·L	No. 18057-14	
Straight micro forceps	F·S·L	No. 00632-11	
Curved micro forceps	F·S·L	No. 00649-11	
micro needle-holder	F·S·L	No. 12061-01	
general surgical instruments			
standard sissors	F·S·L		
mosquito clamp	F·S·L		
serrated forcep	F·S·L		
teethed forcep	F·S·L		
needle-holder	F·S·L		
suture			
4-0 prolene	ethicon		
4-0 ETHICON*II	ethicon		
6-0 silk	ethicon		
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