Peer Review File

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Reviewer A:

Comment 1: In both recipient and donor, it may be necessary to distinguish whether the bile leakage is from the graft (or remnant liver) cross-section or from the bile duct anastomosis. In other words, the authors should make a distinction between a hepatic parenchymal dissection problem and a bile duct cut-off or bile duct anastomosis problem.

Reply 1: We appreciate the comments very much. This is a very good question. As a retrospective cohort study, we can't identify whether the bile leakage in the recipients was from the hepatic parenchymal dissection problem or the bile duct anastomosis problem. However, the four recipients with biliary complications received grafts with multiple bile duct openings, which increased the difficulty of cholangiointestinal anastomosis (line 241-243). We speculate that unreliable biliary intestinal anastomosis resulted in bile leakage and anastomotic stenosis.

In donors, the bile leakage could be found by ICG fluorescence cholangiography. ICG fluorescence cholangiography has certain advantages in detecting bile leakage.

Changes in the text: No changes.

Comment 2: Could the author provide a new Figure with ICG showing that prevent from cutting at the bifurcation point of the two thin bile ducts as shown in Fig. 1C?

Reply 2: Thank you for your suggestion. Before applying ICG fluorescence cholangiography, we were unable to accurately determine the bifurcation of the left and right hepatic ducts. In order to avoid damaging the right hepatic duct and ensure the safety of the donor, we can only cut the left hepatic duct on the left side far from the bifurcation, resulting in the situation shown in Figure 1C. Since the application of ICG fluorescence cholangiography, we can dissect the common hepatic duct upwards, clearly display the left and right hepatic ducts, and then cut at the beginning of the left

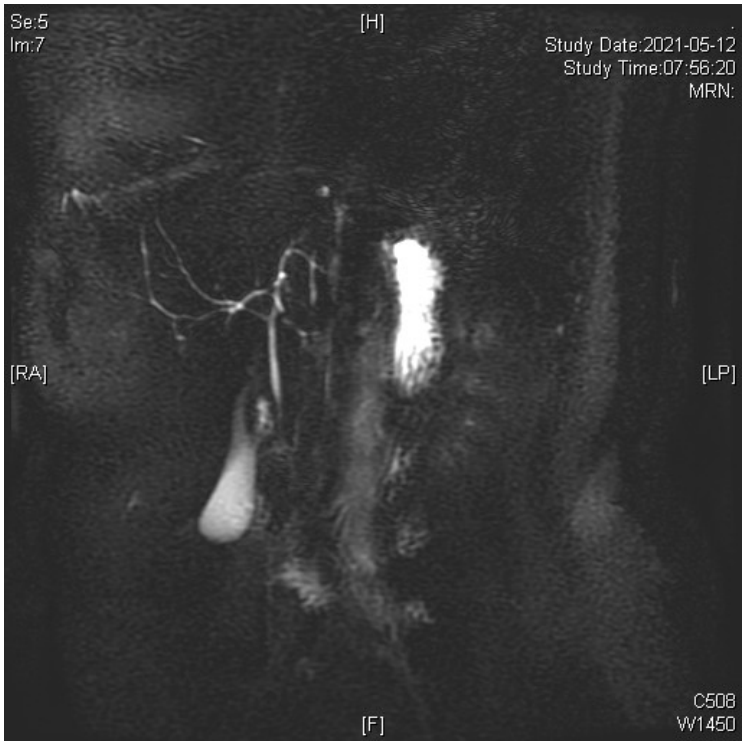
hepatic duct. While ensuring no damage to the right hepatic duct, we also ensure cutting at the primary branch of the left hepatic duct. There is no need to specifically display the secondary branch of the left hepatic duct, so we are sorry that we do not have this image.

Changes in the text: No changes.

Comment 3: Figure 1D-F shows the bile duct very well, however, had the authors identified the bile duct of the left medial segment? In other words, was there any guarantee that the bile duct of the left medial segment is preserved? The authors should explain that in detail.

Reply 3: It's also a very good question. As mentioned above, the left hepatic bile duct was cut at the beginning of it close to the bifurcation of the left and right hepatic ducts. When dividing the left hepatic bile duct, if we can clearly see the common hepatic bile duct and the right hepatic duct, we can determine the beginning of the left hepatic duct. There is no need to specifically display the bile duct of the left medial segment. Of course, if there was a serious biliary variant estimated by pri-operative MRCP, such as the left medial branch of the bile duct originating from the right anterior branch (there is no occurrence in our cases) or the right posterior branch of the bile duct originating from the left hepatic duct (see MRCP below), the graft obtain operation would be transfer to open or even be given up.

Changes in the text: We added some explanation to the manuscript. (see Page 5-6, line 131-132)



Comment 4: Regarding the method of evaluation of the bile duct, is the bile duct evaluated the common hepatic duct portion, the left hepatic duct, or other than this?

Reply 4: We evaluate the morphology of the bile ducts in real-time during surgery, including the common hepatic duct, left hepatic duct, and right hepatic duct. With the help of ICG fluorescence cholangiography, the division point of the left hepatic duct can be well determined.

Changes in the text: No changes.

Comment 5: The authors should describe that relationship fluorescence imaging system and optimal ICG dose or observation time.

Reply 5: This is also a very good question. Different fluorescence imaging systems may have a certain impact on the dose and observation time of ICG. This may depend on the emission and absorption wavelength of the fluorescence system. The emission wavelength of some fluorescence imaging systems on the market was limited to around 835nm and the absorption wavelengths were 800-810nm, and the difference may not be significant. In our study, we were limited to using the PINPOINT Endoscopic Fluoroscopy Imaging System/1588 Advanced Imaging Modalities Platform (Stryker

Co., Michigan, US) and did not attempt to use other systems. If there is an opportunity to use other systems, we will further report on their dosage and observation time.

Changes in the text: No changes.

Reviewer B:

Comment 1: Page 9, Line 271-272, citation no 9 Qureshima, authors mention that ICG reduces bile duct injury. Actually, in Qureshima study, there were no BDI, and both the groups have nil injury i,e. ICG was not better, and thus authors have made an error in the statement. A subsequent meta-analysis comparing IOC with ICG did not show any difference, in fact, it showed that IOC is better for intrahepatic ductal systems while ICG was better for extrahepatic ductal systems - PMID - 33398590.

I invite authors to edit and cite correctly.

Reply 1: We appreciate the comments very much. I have reviewed the literature on ICG fluorescence cholangiography and found that it is generally safe and reliable. It can prevent bile duct injury just like traditional cholangiography. So we modified the expression in the article.

Changes in the text: We modified the expression in the discussion as “it indeed prevent bile duct injury” (see Page 11, line 296)

Comment 2: Similar to above, i find another misquoting of literature on page 9, lines 300-302 when authors mention citations 29-31 that reduce bile leak after cholecystectomy and LLLH. I did not read citations 29 and 30 and leave it for authors to check. I have read citation 31 and find that there were zero bile leaks in that series which included only 1 patient with liver resection. Further, in that paper, many patients had intrabiliary ICG injection (and not intravenous). Thus, I request authors to remains cautions to exaggerate the benefit of ICG and cite properly, carefully, diligently only what the facts are and not unnecessarily err on the side of making ICG look better. Please remove citation 31, check 29-30 for accuracy and edit the statement to ensure it retains accuracy.

Reply 2: We appreciate the comments very much. We are sorry for citing citation 31 by mistake. We checked citation 29 and 30. Citation 29 titled "Intraoperative indocyanine green fluorescent imaging for prevention of bile leakage after hepatic resection" showed ICG fluorescence cholangiography could avoid the incidence of bile leakage. Citation 30 is a review which introduce the using of ICG fluorescence imaging in hepatobiliary surgery. We replaced it with two other more accurate literatures [1,2].

Changes in the text: We modified the expression in the discussion as "Many studies have confirmed that ICG fluorescence cholangiography could avoid the incidence of bile leakage both in laparoscopic cholecystectomy and LLLH" (see Page 12, line 324-326), and revised the cited literatures in the references (see page 18, line 512-517).

[1] Fan J, Li X, Peng Y, et al. Successful application of indocyanine green fluorescent imaging for the non-invasive detection of postoperative bile leakage. Photodiagn Photodyn 2022; 40:103132

[2] Hanaki T, Tokuyasu N, Sakamoto T, et al. Hepatectomy guided by indocyanine green fluorescent imaging for visualizing bile leakage (with video). Clin Case Rep 2022; 6:e05942.

Comment 3: You claim zero bile leak in ICG group but Figure 2 shows ICG image with a bile leak. This is discordant. Please edit.

Reply 3: Figure 2 indeed showed that bile leakage was observed by ICG fluorescence cholangiography during operation. Due to the discovery of bile leakage during the surgery in ICG group, we promptly sutured it to avoid the occurrence of postoperative bile leakage.

Changes in the text: We explained that the complication was the postoperative bile leakage. (see page 8, line 218)

Reviewer C:

Comment 1: It was not stated how the ICG group and non-ICG group were selected. Was ICG used in all cases after the ICG fluorescence method was introduced? Or were

there any indication criteria for using the ICG fluorescence imaging?

Reply 1: Thank you for your suggestion. We may not have expressed it very clearly in the method. In fact, after we had the Endoscopic fluorescence imaging system since November 2019, ICG fluorescence cholangiography was used in all donor operations. So the cases before Nov. 2019 belonged to non-ICG group and the cases after Nov. 2019 belonged to the ICG group.

Changes in the text: We added some explanation in the methods. (see Page 5, line 116, 120-121)

Comment 2: It was stated that the difference in operative time between the two groups was not affected by the learning curve, but can we assume the same for the presence of bile duct complications? If the non-ICG and ICG groups were performed at different period of time, it would be reasonable to assume there would be a difference in the incidence of complications.

Reply 2: Thank you for your question. There is indeed a difference in enrollment time between the two groups. The cases before Nov. 2019 belonged to non-ICG group and the cases after Nov. 2019 belonged to the ICG group. In the discussion, we also discussed about whether the difference of biliary complications is caused by the learning curve (page 13-14, line 364-381). We found that the learning curve really caused the difference between the operation duration and warm ischemia time, However, we believe that the learning curve of LLLS only requires about 25-30 operations. It was showed in our previous study that the operation duration of the subsequent 25 operations was significantly shorter than that of the initial 25 operations (144.8 ± 18.3 versus 170.2 ± 33.6 minutes; P=0.01), and the intraoperative blood loss was significantly reduced (134.8 ± 89.2 versus 186.0 ± 100.5 mL; P=0.05) [1]. However, no further improvement was made later. The operation duration in the ICG group which was performed in the latter period was as same as the subsequent 25 operations (138.8±24.6 versus 144.8 ± 18.3 minutes, P=0.84). In this study, four grafts (case 12, 31, 39, 44) had multiple bile duct openings in the non-ICG group, in which three ones belonged to the subsequent 25 operations. Biliary complications of corresponding

recipients who received grafts with multiple bile duct openings also occurred in the subsequent 25 ones. Three donors in the non-ICG group (case 8, 35, 41) suffered bile leakage, in which two of them belonged to the subsequent 25 operations. Therefore, we believe that the occurrence of biliary complications is related to the use of ICG fluorescence cholangiography, but not to the learning curve. Furthermore, it was reported that in pure laparoscopic living donor right hepatectomy, learning curve which increases proficiency and cooperation only has a greater impact on reducing operation duration, while it has no influence on the accurate judgment of the division point of bile duct [2]. Of course, in the last paragraph of the discussion, we acknowledged that this study is a retrospective study, not an RCT study, which is indeed a limitation.

Changes in the text: No changes.

[1] Lu L, Wang ZX, Zhu WW, et al. Left Hepatic Vein Preferential Approach Based on Anatomy Is Safe and Feasible for Laparoscopic Living Donor Left Lateral Sectionectomy. Liver Transpl 2021; 27: 88-95.

[2] Lee KW, Hong SK, Suh KS, et al. One Hundred Fifteen Cases of Pure Laparoscopic Living Donor Right Hepatectomy at a Single Center. Transplantation 2018; 102: 1878-84.

Comment 3: It should be clearly stated how the bile duct cut line was determined in the non-ICG group. It is stated that the technique is described in reference 13, but even that reference did not describe how the bile duct cut line was determined. As well, how they determine the bile duct cut line the in ICG group? Was the bile duct divided just peripherally part of B4 branch? Or, were B2 and B3 bifurcations identified and bile duct was divided just central part of B2/B3 bifurcation?

Reply 3: Thank you for your suggestion. We have added how to determine the division point of the bile duct in both non-ICG group and ICG group in the methods.

Changes in the text: We have modified the methods as advised. (see Page 6, line 138-141 and line 159-161)

Comment 4: Was good bile duct recognition possible in all patients in the ICG group?

It was mentioned that the ICG fluorescence must be confirmed by dissecting the surrounding fatty and connective tissue. Were there any cases in the ICG group in which the biliary images were poorly identified?

Reply 4: All the bile duct shape can be displayed in the patients in ICG group. Because the penetration force of fluorescence observed by the PINPOINT Endoscopic Fluoroscopy Imaging System is 8mm. For donors with an average BMI of 21-23 kg/m^2, the surface of the bile duct is rarely covered with thick fat and connective tissues. Even for donors with fat and connective tissues, simple dissection and removal of these tissues can make fluorescence imaging very obvious.

Changes in the text: No changes.

Reviewer D:

Comment 1: Authors should mention How do the authors cope with donors of LDLT who have an allergy for iodine? ICG administration should be avoided for these patients.

Reply 1: Thank you for your suggestion. ICG is manufactured by multiple companies around the world and has been in use clinically for hepatic function studies since 1960. The rate of allergic reaction has been reported at 0.05% [1]. The same as the CT scan, we only ask the patient if they have a history of iodine allergy, instead of every patient should undergo iodine allergy testing. If the patient admits to having a history of iodine allergy, we will give up using ICG. None of the patients enrolled in the study had a history of iodine allergy.

Changes in the text: We added "ALL donors in ICG group denied a history of iodine allergy." in the methods. (see Page 6, line 147-148)

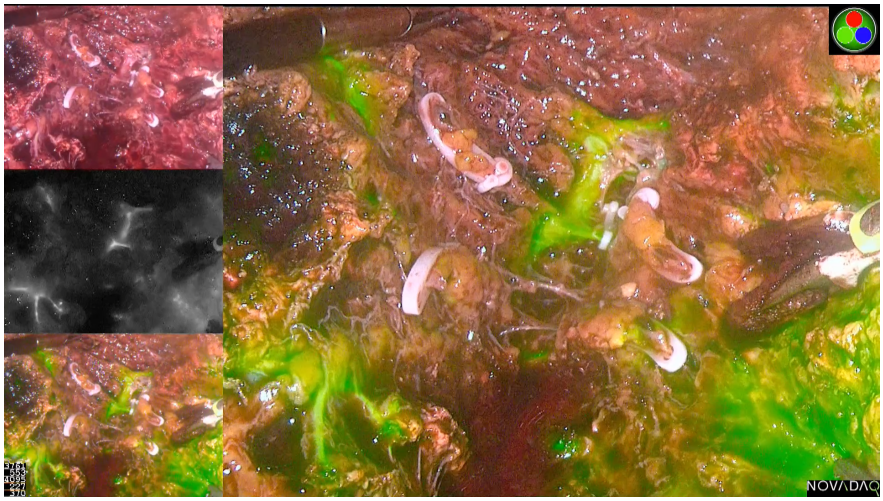
[1] Hope-Ross M, Yannuzzi LA, Gragoudas ES, et al. Adverse reactions due to indocyanine green. Ophthalmology. 1994;101:529-533.

Comment 2: Can the ICG cholangiography detect the bile duct branch from the Spiegel lobe?

Reply 2: Thank you for your question. In theory, ICG fluorescence cholangiography

can detect the bile duct branch from the Spiegel lobe. Because the intrahepatic bile ducts can be displayed very well by ICG cholangiography (see figure below). However, in LLLS, when the bile duct of the Spiegel lobe from the left hepatic duct was divided along with the liver parenchyma was transected, we did not intentionally display it. If bile leakage from the Spiegel lobe was detected by ICG fluorescence cholangiography during operation, the bile duct of the Spiegel lobe would be sutured.

Changes in the text: No changes.



Comment 3: The policies of authors regarding the Spiegel lobe in harvesting the graft of the left lateral section should be mentioned.

Reply 3: Thank you for your question. During the laparoscopic living donor left lateral sectionectomy, the left lateral lobe was harvested and the Spiegel lobe was reserved. As mentioned in most of the literatures [1,2], to increase the length of left hepatic artery and portal vein, the branches assigned to the Spiegel lobe were clipped and divided in advance. The bile duct of the Spiegel lobe from the left hepatic duct was also clipped and divided when the liver parenchyma was transected. However, no ischemic necrosis, bile leakage, or atrophy of the Spiegel lobe was occurred in all donors enrolled in our study.

Changes in the text: No changes.

[1] Scatton O, Katsanos G, Boillot O, et al. Pure laparoscopic left lateral sectionectomy in living donors: from innovation to development in France. Ann Surg. 2015,261(3):506-12.

[2] Kim KH, Jung DH, Park KM, et al. Comparison of open and laparoscopic live donor left lateral sectionectomy. Brit J Surg. 2011,98(9):1302-8.

Comment 4: Authors should explain in more detail why the best dose was 0.004mg/kg. Is there any difference between 0.005mg/kg and 0.004mg/kg?

Reply 4: It was found that the dosage of 0.05 ml/kg was too high, so it was diluted. At the beginning, it was diluted 50 times, however, it was found that the bile duct did not obviously displayed. And then it was diluted 25 times, the effect was still bad. Finally, it was found that 12.5 times diluted was the best choice. So the final dose was chosen as 0.004 mg/kg.

Changes in the text: No changes.

Comment 5: Authors should also explain whether the contrast of liver parenchyma and bile ducts is always best at 0.004mg/kg in different body mass index, or not.

Reply 5: The BMI of all cases which included in the study ranged from 13 to 36.7. Based on different BMI, we divided these cases into low BMI group (BMI<21.5) and high BMI group (BMI>21.5). The fluorescence intensity contrast value at different dose and observation time in different BMI groups were compared. There is no difference of the fluorescence intensity contrast value between the two groups, which indicated that the fluorescence intensity contrast value was not related to BMI (see table blow). It is always best at the dose of 0.004mg/kg 90min after ICG injection.

Changes in the text: No changes.

Table: Fluorescence intensity contrast value in different BMI groups:

	Low BMI<21.5(n=9)	High BMI>21.5(n=9)	P
0.05mg/kg 30min	-0.168	-0.154	0.66
0.05mg/kg 60min	-0.031	-0.041	0.48
0.05mg/kg 90min	0.075	0.081	0.62
	Low BMI<21.5(n=20)	High BMI>21.5(n=19)	P
0.004mg/kg 30min	-0.0098	-0.0078	0.87

0.004mg/kg 60min	0.065	0.064	0.89
0.004mg/kg 90min	0.101	0.094	0.47