Peer Review File

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Reviewer A:

This is a retrospective cohort study comparing intravenous and intragallbladder ICG injection for biliary visualization during laparoscopic cholecystectomy. The authors conclude that intraoperative gallbladder injection of ICG may be a more suitable option for patients with cirrhosis or fatty liver because of stronger background fluorescence in these patients. Therefore, the route of ICG administration should be tailored for individual patients.

My main criticism is with the interpretation of the study results and conclusions. The authors report that the CBD fluorescence visualization rates were 100% in the peripheral venous ICG injection group and 85.19% in the gallbladder ICG injection group. CBD fluorescence imaging could not be achieved through gallbladder ICG injection in patients with impacted stones. I would contend that CBD visualization with ICG is the most important factor for using ICG during cholecystectomy. Therefore, IV administration of ICG is clearly the superior technique. If ICG was injected into the gallbladder and the CBD could not be visualized, then IV injection of ICG would be needed regardless.

In addition, for patients with cirrhosis/fatty liver, the authors report that BLR>1 was 100% in the intragallbladder group and 0% in the IV group. The authors do not however comment on if the background fluorescence in the IV group was inhibitory to ICG use i.e. they could not identify the CBD in these cases. It is likely that the CBD can still be visualized despite the interference, making the background fluorescence of the liver a moot point.

Reply:

Dear review,

We appreciate your insightful critique and constructive feedback on our manuscript.

Prior to this study, both injection methods for cholecystectomy had been reported in the literature, with peripheral venous injection being more frequently discussed than intra-gallbladder injection. Numerous studies have been conducted on the appropriate timing and dosage of ICG for peripheral venous injection**[1-5]**. In their published research, they all attempted to address the same scientific question: how to achieve a good visualization of the extrahepatic biliary tract. Among the evaluation criteria for the visualization of the extrahepatic biliary tract, in addition to the fluorescence intensity of the common bile duct, the bile duct-to-liver fluorescence ratio (BLR) is also an important parameter. Given a certain fluorescence intensity, the higher the ratio, the more distinguishable the extrahepatic bile ducts are. In fact, in our clinical practice, we found it difficult to obtain good visualization of the extrahepatic biliary tract in patients with liver cirrhosis. Savvas et al **[6]**. pointed out a clinical limitation of near-infrared cholangiography (NIRC) poorly addressed so far is the accumulation of ICG in hepatic parenchyma shortly after the intravenous administration, which

produces a high background signal and obstructs safe biliary tree anatomy recognition. Subsequently, Liu et al **[7]**. studied the efficacy of direct intragallbladder ICG injection during LC in cases of severe inflammation, to limit liver parenchyma enhancement and to achieve sufficient biliary identification. Clara Gené et al **[8]**. point out NIRC with direct injection of ICG into the gallbladder is a feasible method that is not time-consuming; it does not require a different learning curve from standard laparoscopic cholecystectomies and has no major complications described so far.

Although both types of injection methods have been reported, and the results seem to indicate satisfactory outcomes, there are relatively few studies comparing these two approaches. Our research team believes that it is essential to objectively evaluate both injection methods. Therefore, we conducted this study to compare these two methods, which we deem highly necessary.

In our study, the extrahepatic biliary tract was visualized in 100% of the patients who underwent peripheral venous injection. However, when this method was applied to certain patients, the bile duct-to-liver fluorescence ratio (BLR) was notably low. Conversely, intra-gallbladder injection enabled the visualization of the extrahepatic biliary tract in only 85.19% of the cases, but it yielded a favorable BLR for the appropriate patients. Despite the lower rate of successful imaging with intra-gallbladder injection, it remains applicable to a subset of patients. Each method has its advantages. We do not dismiss any single injection method. Based on the results of our study, selecting different methods in accordance with the characteristics of the patients can maximize the advantages of each approach.

Changes in the text: No modifications.

Reference:

[1] Verbeek FP, Schaafsma BE, Tummers QR, van der Vorst JR, van der Made WJ, Baeten CI, et al. Optimization of near-infrared fluorescence cholangiography for open and laparoscopic surgery. Surg Endosc 2014;28:1076-82

[2] Zarrinpar A, Dutson EP, Mobley C, Busuttil RW, Lewis CE, Tillou A, et al. Intraoperative Laparoscopic Near-Infrared Fluorescence Cholangiography to Facilitate Anatomical Identification: When to Give Indocyanine Green and How Much. Surg Innov 2016;23:360-5

[3] Boogerd LSF, Handgraaf HJM, Huurman VAL, Lam HD, Mieog JSD, van der Made WJ, et al. The Best Approach for Laparoscopic Fluorescence Cholangiography: Overview of the Literature and Optimization of Dose and Dosing Time. Surg Innov 2017;24:386-96

[4] Tsutsui N, Yoshida M, Nakagawa H, Ito E, Iwase R, Suzuki N, et al. Optimal timing of preoperative indocyanine green administration for fluorescent cholangiography during laparoscopic cholecystectomy using the PINPOINT(R) Endoscopic Fluorescence Imaging System. Asian J Endosc Surg 2018;11:199-205

[5] Chen Q, Zhou R, Weng J, Lai Y, Liu H, Kuang J, et al. Extrahepatic biliary tract visualization using near-infrared fluorescence imaging with indocyanine green: optimization of dose and dosing time. Surg

Endosc 2021;35:5573-82

[6] Symeonidis S, Mantzoros I, Anestiadou E, Ioannidis O, Christidis P, Bitsianis S, et al. Near-infrared cholangiography with intragallbladder indocyanine green injection in minimally invasive cholecystectomy. World J Gastrointest Surg 2024;16:1017-29

[7] Liu YY, Liao CH, Diana M, Wang SY, Kong SH, Yeh CN, et al. Near-infrared cholecystocholangiography with direct intragallbladder indocyanine green injection: preliminary clinical results. Surg Endosc 2018;32:1506-14

[8] Gene Skrabec C, Pardo Aranda F, Espin F, Cremades M, Navines J, Zarate A*, et al.* Fluorescent cholangiography with direct injection of indocyanine green (ICG) into the gallbladder: a safety method to outline biliary anatomy. Langenbecks Arch Surg **2020**;405:827-32

What is one to do in a patient with cirrhosis/fatty liver and a gallbladder filled with stones? It seems to me that there are very few (if any) indications for gallbladder injection of ICG over IV administration, as this introduces unnecessary operative risk and increases operative time. The benefit of ICG is that it does not require additional operative steps and should decrease operative time compared to standard white light laparoscopy. While intragallbladder injection of ICG may enable bile duct visualization (and may improve visualization in some select cases), the reported reduction of background liver fluorescence likely does not outweigh the drawbacks in clinical practice.

Reply:

Dear review,

Your inquiry regarding the optimal approach for patients with cirrhosis or fatty liver and a gallbladder containing stones is indeed thought-provoking. It raises a crucial consideration in the application of ICG for bile duct visualization.

In conjunction with our study results presented in Table 5, we believe that the patient group you mentioned should primarily opt for the peripheral venous injection method. Concurrently, as depicted in Table 5, we describe that achieving satisfactory imaging effects with this patient group via peripheral venous injection may be challenging. A case-control study by Castagneto-Gissey et al. **[1]** investigated the fluorescence cholangiography effects of the two ICG injection methods and discovered that both methods facilitated the anatomical delineation of the Calot triangle for extrahepatic biliary structures. Compared to intravenous ICG injection, the intragallbladder ICG administration route reduced liver fluorescence, leading to an enhanced signal-to-noise ratio and increased contrast between bile ducts and liver tissue. Furthermore, they observed that peripheral venous administration was more effective in delineating the duodenum and common hepatic duct than gallbladder puncture administration. This study confirmed the efficacy of both injection methods for extrahepatic bile duct fluorescent imaging. However, the study did not address how to choose the injection method. Based on our research data, we believe that both methods have their advantages in achieving good imaging effects. Our Table 5 addresses the question of how to select the injection method. Of course, we

concur with your perspective that, in the majority of patients, the intravenous injection method is applicable compared to the gallbladder injection method.

We appreciate your scrutiny and the opportunity it provides to further elucidate our methodology and findings.

Table 5 Comparison of the two methods of ICG (Indocyanine Green) injection

Intravenous Injection via Peripheral Veins	Percutaneous Injection via Gallbladder
Indications: ● Gallbladder duct stone impaction ● Gallbladder filled with stones ● Gallbladder polyps ● Gallbladder atrophy ● Gallbladder polyps with potential malignancy ● Gallbladder adenomyomatosis	Indications: ● Patients with liver cirrhosis ● Patients with fatty liver ● Gallbladder polyps without potential malignancy ● Single and freely movable gallbladder stone ● Gallbladder adenomyomatosis ● No risk of substances in the gallbladder dropping into the common bile duct after injection (e.g., gallbladder sandy stone).
Preoperative intravenous injection of ICG may not achieve optimal contrast enhancement in the following patients: ● Patients with liver cirrhosis ● Patients with fatty liver	The following situations are not suitable for choosing this injection method: ● If the patient has variant accessory hepatic ducts or aberrant hepatic ducts, this method may not be suitable for fluorescence imaging ● Gallbladder duct stone impaction ● Gallbladder filled with stones ● Gallbladder polyps with potential malignancy ● Gallbladder atrophy.

Changes in the text: No modifications.

Reference:

[1] Castagneto-Gissey L, Russo MF, Iodice A, Casella-Mariolo J, Serao A, Picchetto A, et al. Intracholecystic versus Intravenous Indocyanine Green (ICG) Injection for Biliary Anatomy Evaluation by Fluorescent Cholangiography during Laparoscopic Cholecystectomy: A Case-Control Study. J Clin Med 2022;11

Reviewer B

Comment 1: *The study could yield better results and conclusion, if it was done in a prospective manner with randomization of patients between the two groups. With a retrospective study design, it is difficult to characterize the patients, especially the patients with cirrhosis and fatty liver. Were the patients diagnosed*

pre-operatively with liver disease or was it an incidental finding in all such cases. If diagnosed, pre-operatively, what was the grade of liver disease, because as per my understanding, the liver cirrhosis only hinders with the metabolism of indocyanine green in severe disease.

Reply:

Dear Reviewer,

We greatly appreciate your insightful comments and the constructive critique of our study's design. Your observations regarding the potential benefits of a prospective, randomized study are well-taken and provide valuable guidance for future research endeavors.

We acknowledge that a prospective study with randomization could potentially yield more controlled results and conclusions. The retrospective nature of our study indeed presents limitations in terms of patient characterization and the uniformity of diagnostic procedures applied prior to surgery.

In our study, the patients with cirrhosis and fatty liver were identified through a combination of pre-operative assessments and intra-operative findings.

The severity of liver disease can indeed affect the metabolism of Indocyanine green (ICG). In the context of cholecystectomy, it is standard clinical practice to assess liver function and the Child-Pugh score to rule out any contraindications for surgery. However, this assessment may not adequately reflect the metabolism of ICG. In fact, the most straightforward method is to measure the ICG retention rate at 15 minutes post-injection. Nevertheless, the generalizability of our research outcomes must be comprehensively considered. In most hospitals, the majority of patients do not undergo ICG 15-minute retention rate testing or liver fibrosis assessments prior to cholecystectomy. Instead, the presence of liver cirrhosis and fatty liver is typically determined by integrating preoperative diagnostic imaging results, such as CT, MRI, and ultrasound. These indicators serve as indirect references in the staging of liver disease.

Therefore, when preoperative considerations suggest the presence of liver cirrhosis or fatty liver, and in the absence of surgical contraindications, as well as meeting the conditions for intra-gallbladder injection as outlined in **Table 5**, intraoperative gallbladder injection can be contemplated. Since ICG injected into the gallbladder bypasses liver metabolism, this method does not necessitate consideration of the severity of liver disease grading. When the intra-gallbladder injection is not feasible, ICG injected intravenously shows a significant correlation between the severity of liver disease and the quality of visualization of the extrahepatic bile ducts. At present, there is no universally agreed-upon consensus regarding the optimal timing and dosage of ICG injection for patients in such cases. However, it is evident that once the dosage is determined, ICG administration should be scheduled significantly ahead of the surgical procedure. Our preliminary research indicates that an ICG dose of 10 mg should be administered 10 to 12 hours prior, even in patients without liver disease.

Table 5 Comparison of the two methods of ICG (Indocyanine Green) injection

Intravenous Injection via Peripheral Veins	Percutaneous Injection via Gallbladder
Indications: ● Gallbladder duct stone impaction	Indications: ● Patients with liver cirrhosis

● Gallbladder filled with stones ● Gallbladder polyps ● Gallbladder atrophy ● Gallbladder polyps with potential malignancy ● Gallbladder adenomyomatosis	● Patients with fatty liver ● Gallbladder polyps without potential malignancy ● Single and freely movable gallbladder stone ● Gallbladder adenomyomatosis ● No risk of substances in the gallbladder dropping into the common bile duct after injection (e.g., gallbladder sandy stone).
Preoperative intravenous injection of ICG may not achieve optimal contrast enhancement in the following patients: ● Patients with liver cirrhosis ● Patients with fatty liver	The following situations are not suitable for choosing this injection method: ● If the patient has variant accessory hepatic ducts or aberrant hepatic ducts, this method may not be suitable for fluorescence imaging ● Gallbladder duct stone impaction ● Gallbladder filled with stones ● Gallbladder polyps with potential malignancy ● Gallbladder atrophy.

We concur that future research employing a prospective, randomized approach would be highly beneficial. Our study serves as a foundation for further investigation and we look forward to contributing to the ongoing discourse in this field.

Changes in the text: No modifications.

Reference:

[1] Chen Q, Zhou R, Weng J, Lai Y, Liu H, Kuang J, et al. Extrahepatic biliary tract visualization using near-infrared fluorescence imaging with indocyanine green: optimization of dose and dosing time. Surg Endosc 2021;35:5573-82

Comment 2: *If the study shows that intragallbladder injection is a better method of cholangiography in patients with cirrhosis, should we establish a cut-off criteria with a pre-operative fibroscan, to see beyond what levels will the method be useful?*

Reply:

Dear review,

This is an excellent question. We believe that the selection of the injection method should be based on a comprehensive consideration of patient factors, including but not limited to liver cirrhosis. According to our research data, patients with appropriate liver cirrhosis can indeed achieve satisfactory imaging of the extrahepatic bile ducts through intra-gallbladder injection of ICG. Given that ICG does not undergo hepatic metabolism after intra-gallbladder injection, the factor of liver fibrosis does not influence the imaging outcomes obtained via this injection route. In other words, provided that patients lack the high-risk factors for intra-gallbladder injection **(Table 5)**, ICG injection via the gallbladder can be applied regardless of the degree

of liver fibrosis.

Liver fibrosis can affect the imaging of the extrahepatic bile ducts following peripheral venous injection. With varying degrees of liver fibrosis, the 15-minute retention rate of ICG in the bloodstream post-intravenous injection is not uniform, Theoretically, as long as the liver retains the capability to excrete ICG, selecting an appropriate timing for preoperative ICG injection can yield a BLR greater than 1. Should the liver lack the capacity to excrete ICG, it would be prudent to consider the presence of severe liver dysfunction and complete biliary obstruction in the patient. If such conditions are present, the patient may no longer be a suitable candidate for cholecystectomy or surgery, and potentially neither method of ICG injection would be appropriate.

In conclusion, the necessity to explore the critical threshold of liver fibrosis to guide the method of ICG injection is a question that warrants thorough consideration.

Changes in the text: No modifications.

Comment 3: *What other factors, other than cirrhosis or fatty liver disease, can impede ICG metabolism?*
Reply:
Dear Reviewer,
Thank you for your insightful question. It is well established that following intravenous injection, indocyanine green (ICG) is taken up by hepatocytes and almost entirely excreted into the bile **[1, 2]**. Additionally, ICG exhibits fluorescence upon stimulation by near-infrared light (700-900 nm), emitting light at a wavelength of approximately 830 nm post-injection. In our previous studies **[3]**, we observed variations in the intensity of biliary fluorescence imaging after administering the same dose of ICG at different time points, suggesting that the excreted ICG does not engage in the enterohepatic circulation. In clinical practice, a standard dose of ICG (0.5 mg/kg) is administered via a peripheral vein **[4]**, and it is generally accepted that within approximately 30 minutes post-injection, the liver uptakes more than 98% of ICG, initiating its hepatic elimination phase **[5]**. Consequently, any factor that compromises liver function or leads to bile duct obstruction has the potential to influence the metabolism and clearance of ICG.
We appreciate your consideration and look forward to your feedback.

Changes in the text: No modifications.

Reference:

[1] Mordon S, Devoisselle JM, Soulie-Begu S, Desmettre T. Indocyanine green: physicochemical factors affecting its fluorescence in vivo. Microvasc Res 1998;55:146-52
[2] Cherrick GR, Stein SW, Leevy CM, Davidson CS. Indocyanine green: observations on its physical properties, plasma decay, and hepatic extraction. J Clin Invest 1960;39:592-600

[3] Chen Q, Zhou R, Weng J, Lai Y, Liu H, Kuang J, et al. Extrahepatic biliary tract visualization using near-infrared fluorescence imaging with indocyanine green: optimization of dose and dosing time. Surg Endosc 2021;35:5573-82
[4] Sakka SG. Assessment of liver perfusion and function by indocyanine green in the perioperative setting and in critically ill patients. J Clin Monit Comput 2018;32:787-96
[5] Meijer DK, Weert B, Vermeer GA. Pharmacokinetics of biliary excretion in man. VI. Indocyanine green. Eur J Clin Pharmacol 1988;35:295-303

Comment 4: *The intragallbladder injection of ICG is a technically challenging procedure especially for trainee surgeons and residents. It increases the time and complexity of a procedure which can be avoided without any added much advantage.*

Reply:

Dear Reviewer,

We acknowledge that the intra-gallbladder injection indeed introduces additional procedural steps compared to the relatively simpler and more convenient intravenous injection. The qualifications required for surgeons to perform laparoscopic cholecystectomy (LC) differ across various countries and regions. In China, it is attending surgeons with seniority, not trainee surgeons or residents, who are qualified to perform LC. For these senior surgeons, executing an intra-gallbladder injection of ICG is a relatively straightforward procedure, which our research team can accomplish in approximately 5 minutes. Our statistical data reveal a statistically significant difference in operation time between the two groups; however, the actual difference is minimal: the operation time for the intra-gallbladder injection group is 66.78±9.88 minutes, compared to 60.38±9.35 minutes for the peripheral intravenous injection group.

We acknowledge that the intra-gallbladder injection may not be suitable for all patients; however, in select cases, it provides superior imaging quality. Our study evaluates the conditions under which the intra-gallbladder injection of ICG is advantageous. This is part of our effort to contribute to a more nuanced understanding of ICG usage in LC, supporting clinical decision-making. We recognize that the choice of injection method should be based on a careful assessment of individual patient condition.

We trust that this response effectively addresses your concerns.

Changes in the text: No modifications.

Comment 5: *The study says that there was a statistically significant difference between the two groups in terms of pre-operative diagnosis. Kindly explain.*

Reply:

We greatly appreciate your insightful comments and the constructive critique of our study's design. Your observations regarding the potential benefits of a prospective, randomized study are well-taken and provide valuable guidance for future research endeavors.

We acknowledge that a prospective study with randomization could potentially yield more controlled results and conclusions. The retrospective nature of our study indeed presents limitations in terms of patient characterization and the uniformity of diagnostic procedures applied prior to surgery.
Our preoperative disease diagnosis is divided into four main categories: gallbladder polyps, Single stone of the gallbladder, Multiple stones in the gallbladder, and gallbladder adenomyosis. This diagnostic classification minimizes interference with subsequent research. Our investigation revealed that patients with gallbladder atrophy, sediment-like gallstones, fully occupied gallstones, or impacted stones in the gallbladder duct or neck, as well as those with cirrhosis or fatty liver, were found to have an impact on the results.

Comment 6: *As proved in the given study as well, better bile duct fluorescence and a shorter duration of surgery were both seen in the peripheral ICG injection group. Pancreatitis is a dreaded complication after cholecystectomy, and if the procedure of intragallbladder injection increases the chances of this happening, the cost to patient, the hospital stay, co- morbidities, all will increase.*

Reply:

Dear Reviewer,

We are deeply grateful for your meticulous inquiries. It is indeed the case that some patients can achieve satisfactory extrahepatic bile duct imaging with peripheral intravenous injection of ICG, coupled with shorter operative times. In our study, one patient who received ICG injection via the gallbladder experienced mild pancreatitis postoperatively. As you rightly pointed out, pancreatitis can prolong hospital stays and increase hospitalization costs. Based on the number of cases in our study, we cannot conclude that the peripheral intravenous injection of ICG significantly increases the risk of pancreatitis as a complication. However, we should pay attention to this issue. As summarized in Table 5, for patients with sediment-like stones in the gallbladder, we recommend peripheral intravenous injection of ICG. In the discussion section of our manuscript, we have also made the following clarification:

“Our analysis suggests that the intraoperative displacement of sediment-like calculi into the common bile duct might have contributed to this incidence of pancreatitis. Consequently, for patients with impacted gallbladder stones or sediment-like gallbladder stones, preoperative ICG injection is recommended. Conversely, for patients with cirrhosis or fatty liver, an intraoperative gallbladder ICG injection could enhance discernibility for better visualization.”

This approach ensures that the method of ICG injection is tailored to the specific conditions and needs of the patient, aiming to balance the risks and benefits for optimal surgical outcomes.

Changes in the text: No modifications.

Comment 7: *I would like to appreciate the author for two of the conclusions they have drawn in the end: Not to use the intragallbladder injection method in patients with multiple stones, impacted stones or sediment*

like calculi. Not to use the intragallbladder method in patients with potentially malignant gall bladder polyps. In old age patients with acute/chronic cholecystitis, when the underlying risk of incidental carcinoma of gall bladder is present, would the intragallbladder method if ICG cholangiography still be preferable. If not, then how to ascertain which patients to use this method in and in which patients not.

Reply:

Dear Reviewer,

We greatly appreciate your recognition of our research findings. The issue you mentioned regarding these patients is indeed very interesting. Based on Table 5, we recommend that these patients receive peripheral intravenous injection of ICG preoperatively. This approach ensures that the method of ICG injection is tailored to the specific conditions and needs of the patient, aiming to balance the risks and benefits for optimal surgical outcomes.

Changes in the text: No modifications.

Comment 8: *Since, the study establishes the intragallbladder method to have a superior bile duct to liver contrast ratio, further work is needed in this field to establish a criteria where this method could actually be useful.*

Reply:

Dear Reviewer,

First and foremost, we would like to express our sincere gratitude for your insightful comments and thorough review of our study. Your observation regarding the superior bile duct to liver contrast ratio achieved through the intragallbladder method, and the suggestion for further research to establish criteria for its practical utility, is both pertinent and constructive.

In response to your feedback, we concur that additional research is warranted to delineate the specific criteria under which the intragallbladder method could be advantageous in clinical practice.

Once again, we appreciate your valuable input and look forward to your continued guidance and support in our future research endeavors.

Changes in the text: No modifications.

Reviewer C:

Comment 1:

First, the authors need to indicate the clinical outcomes of interest in the title in this study.

Reply:

Dear Reviewer,

We greatly appreciate your valuable feedback on our manuscript titled "A comparative analysis of indocyanine green administration: peripheral intravenous injection versus intragallbladder injection for laparoscopic cholecystectomy—a retrospective cohort study." Your suggestion to clarify the clinical outcomes of interest in the title is well-received and will help to better communicate the focus of our research.

In response to your recommendation, we have revised the title to more accurately reflect the clinical outcomes assessed in our study. The revised title is as follows:

“Intragallbladder versus Intravenous Indocyanine Green (ICG) Injection for Enhanced Bile Duct Visualization by Fluorescent Cholangiography during Laparoscopic Cholecystectomy: A Retrospective Cohort Study”

We thank you once again for your constructive comments, which have helped us to improve the presentation of our research.

Changes in the text:
Original title: A comparative analysis of indocyanine green administration: peripheral intravenous injection versus intragallbladder injection for laparoscopic cholecystectomy—a retrospective cohort study.
Revised title: Intragallbladder versus Intravenous Indocyanine Green (ICG) Injection for Enhanced Bile Duct Visualization by Fluorescent Cholangiography during Laparoscopic Cholecystectomy: A Retrospective Cohort Study

Comment 2:
Second, the abstract is inadequate. The background needs to indicate the controversy regarding the efficacy outcomes of peripheral intravenous injection versus intragallbladder injection and what the potential clinical contribution of this study was. The methods need to describe the assessment of baseline clinical factors, inclusion criteria, and measurements of the parameters and complications. The results need to briefly present the baseline clinical characteristics of the two groups and their baseline comparability. The conclusion needs more detailed comments for the clinical implications of the findings.
Reply:
Dear Reviewer,
We extend our heartfelt thanks for the insightful and constructive feedback you have provided on our manuscript. Your suggestions for enhancing the clarity and depth of our abstract have been carefully considered and incorporated.

We have made the necessary revisions to the background, methods, and results sections of our abstract as per your instructions. However, we have decided not to alter the conclusions section for the following reasons: The core objective of applying indocyanine green (ICG) in conjunction with near-infrared fluorescence during cholecystectomy is to enhance the visibility of the extrahepatic bile ducts. Therefore, the outcome measures assessing the bile duct visualization are the most critical indicators, which we believe have been adequately

presented in our conclusions.

We are pleased to have addressed the concerns raised, resulting in a more robust abstract. We are sincerely grateful for your valuable feedback and the opportunity to enhance our submission.

Changes in the text:

Abstract

Background: Iatrogenic bile duct injuries (BDIs) prevention during laparoscopic cholecystectomy (LC) relies on meticulous anatomical dissections through direct visualization. Near-infrared fluorescence (NIRF) with indocyanine green (ICG) improves the visualization of extrahepatic biliary structures. Although ICG can be administered either intravenously or intragallbladder, there remains uncertainty regarding the optimal method for different patient populations. This study sought to assess the suitability of each method for specific patient groups.

Methods: Between October 2021 and May 2022, 59 consecutive patients underwent fluorescence-guided LC at West China Hospital of Sichuan University. Among them, 32 patients received an intravenous injection of ICG (10 mg) 10 to 12 hours prior to surgery (Group A: the intravenous group), while 27 patients received an intragallbladder injection of ICG (10 mg) (Group B: the intragallbladder group). Baseline clinical factors, inclusion criteria, and measurements of parameters and complications were assessed. Data were retrospectively collected and analyzed to evaluate the comparability of the two groups and the clinical outcomes. ~~Data on parameters and complications were collected and analysed retrospectively.~~

Results: Group A and B included 32 patients (18 males, 14 females), and 27 patients (13 men, 14 women), respectively. In our Statistical analysis, significant differences were observed in preoperative diagnoses between the two groups (p=0.041), but the majority of other baseline clinical factors were comparable. Notably, no statistically significant differences were found in complication rates. ~~Statistically significant differences were not observed between the two groups in terms of complication rates, cirrhosis, fatty liver, and most of the other parameters.~~ However, Group A had a shorter operative time (60.38±9.35 vs. 66.78±9.88 min, p=0.01) and superior bile duct fluorescence (p=0.04) than Group B. Interestingly, fluorescence was not observed in impacted gallbladder stones in Group B. Additionally, patients with cirrhosis (p=0.008) and fatty liver (p=0.005) in Group B had higher common bile duct-to-liver ratios than those in Group A.

Comment 3:

Third, in the introduction, it is not adequate to describe the lack of comparisons between peripheral intravenous injection and intragallbladder injection as the rationale of this study. The authors need to explain whether the two approaches have different indications for patients or all patients could be administered either one. This is important, if the two have different indications, the current comparisons are not necessary. The authors also need to present the controversy regarding the efficacy outcomes of the two approaches and explain the potential clinical significance of this study.

Reply:

Dear Reviewer,

We appreciate your insightful comments and acknowledge the need for a clearer rationale for our study as suggested in your review. Your guidance on enhancing the introduction has been invaluable, and we have made the following revised.

Changes in the text:

Although existing literature has documented favorable results in visualizing the extrahepatic bile duct for each method, there remains uncertainty regarding the optimal method for different patient populations. Furthermore, ~~Currently,~~ there is a lack of comparative studies on the two ICG administration routes. The aim of this retrospective study is to explore the optimal ICG administration route for patients who underwent LC using NIRF.

Comment 4:

Fourth, in the methodology, please describe the sample size estimation procedures, inclusion criteria, details of the assessment of the outcomes, and follow up procedures. In statistics, please describe the test of the baseline comparability between the two groups and the adjustment procedures when the two groups were not comparable. Please ensure P<0.05 is two-sided. One methodology concern is the small sample size, which would result in unstable and misleading findings.

Reply:

Dear Reviewer,

We acknowledge the significance of sample size determination, yet it is essential to recognize that the scope of sample size in retrospective studies is influenced by various factors. Unlike prospective studies, where sample size can be predetermined, retrospective cohort studies are typically based on existing data or historical records, meaning the sample size is often dictated by the available data. Additionally, the calculation of sample size is contingent upon the primary clinical outcome measures. In this study, we did not designate a primary outcome measure; instead, we analyzed multiple outcomes related to the visualization of the extrahepatic bile duct. Moreover, as there were no similar reports published prior to our study, we lacked reference literature to calculate the necessary sample size for the outcomes of interest. Consequently, we included all eligible patients in our analysis to the best of our ability.

In the methods section, we have detailed our inclusion criteria to encompass all patients who underwent laparoscopic cholecystectomy utilizing indocyanine green (ICG) in conjunction with near-infrared fluorescence imaging.

Regarding the statistical section, we believe our documentation meets the requirements you have set forth. We have taken all types of variables into account, including continuous and categorical variables. Here, we reattach the main body of our statistical methods for your review.

“Statistical analysis was performed using IBM SPSS 19.0 (SPSS Inc., Chicago, Illinois, USA). The normally distributed continuous variables were expressed as mean ± standard deviation (SD) and were analyzed using the independent sample t-test. The non-normally distributed continuous variables were expressed as median (interquartile range), and group differences were compared using the Mann-Whitney U test. The categorical variables are expressed as frequency (n, %), and were analyzed using the chi-square or Fisher’s exact test. All

the tests were two-sided, with a significance level set at P<0.05."

Changes in the text: No modifications.

Comment 5:

Finally, please review and cite several related papers: 1. Tian F, Cao L, Chen J, Zheng S, Li J. 3D laparoscopic anatomical hepatectomy guided by 2D real-time indocyanine green fluorescence imaging for hepatocellular carcinoma. Hepatobiliary Surg Nutr 2024;13(3):494-499. doi: 10.21037/hbsn-22-587. 2. Asti E, Bernardi D, Andreatta E, Conti A, Carmignani L, Bonavina L. Laparoscopic management of colovesical fistula secondary to sigmoid diverticulitis: case report and the role of intraoperative indocyanine-green fluorescence. J Vis Surg 2023;9:9. 3. Gyoda Y, Mise Y, Terasawa M, Ichida H, Mizuno T, Yoshioka R, Imamura H, Saiura A. Narrative review of fluorescence imaging-guided liver surgery. Laparosc Surg 2021;5:33.

Reply:

Dear Reviewer,

We sincerely appreciate the articles you have recommended, showcasing the extensive applications of indocyanine green (ICG) combined with near-infrared fluorescence in diverse medical domains. However, after meticulous consideration, we have concluded that these references may not be appropriate for citation in our paper. Below are our reasons:

1) Articles 1 and 3 focus on the application of ICG in liver surgery, providing valuable perspectives that, while insightful, are not directly relevant to the emphasis of our research.
2) Article 2 presents the application of ICG in a case of colovesical fistula secondary to sigmoid diverticulitis. Although it is an intriguing application, it falls outside the scope of our study.

We trust these clarifications shed light on our stance and extend our gratitude for your esteemed feedback and support for our research endeavors.

Changes in the text: No modifications.