

Peer Review File

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Reviewer A:

Comment 1: The author describes laparoscopic hepatectomy with near-infrared fluorescence for hepatolithiasis. This manuscript is an interesting and acceptable. This manuscript needs revision but may be accepted for publication. Most of the procedures studied with PSM were laparoscopic left hepatectomies. It seems that the NIF group had less blood loss, but the reason is unclear. Laparoscopic left hepatectomy in the WL group is also performed with MHV exposure, as in the NIF group, but which technique causes the difference in blood loss?

Response 1: We appreciate this valuable feedback. Following propensity score matching, laparoscopic left hepatectomy (LLH) was performed on 23/27 (85.19%) and 20/27 (74.07%) patients in the white light (WL) and near-infrared fluorescence (NIF) groups, respectively. The P value for the difference in intraoperative bleeding between the two groups was 0.052, which is close to, but not statistically significant (≤ 0.05). Given that the difference was not statistically significant, we did not explore these findings further. However, one of the reviewers commented that "It seems that the NIF group experienced less blood loss." We posit that NIF technology complements the traditional middle hepatic vein (MHV) exposure technique, that is, the procedures dovetail together well. Regarding the reviewer's question, if the difference in blood loss proves genuine with further study, there are two possible explanations for it. Firstly, exposing the MHV during LLH requires employment of techniques such as locating left and right hemihepatic ischemia lines or intraoperative ultrasound marking to guide identification of the MHV trunk. Traditionally, surgeons have chosen the location for separating the liver parenchyma subjectively on the basis of their experience. However, the boundary between the left and right hemi-livers is not standard. Thus, without the guidance of NIF technology, more thick blood vessels may be encountered during separation of the liver parenchyma, leading to increased bleeding. Secondly, during left hemihepatectomy for intrahepatic bile duct stones in the WL group, we noticed that many bile ducts or Glissonean pedicle structures of the left liver were markedly dilated, causing their terminals to cross the ventral plane of the MHV and enter the parenchyma of the preserved liver. This suggests that the traditional MHV exposure technique in white light mode may leave residual diseased bile ducts *in situ*. Our NIF technology, which uses biliary tracts for ICG fluorescence imaging, objectively demarcates the drainage area of the diseased bile ducts, making the duct structure encountered during the liver parenchyma separation process more accurate and predictable, and thus potentially reduce bleeding.

Changes to the text: Because the difference noted by the reviewer was not statistically significant, we have not revised the original manuscript regarding this point.

Reviewer B:

Comment 2: In this paper, the authors conducted an assessment of laparoscopic hepatectomy for hepatolithiasis using near-infrared fluorescence (NIF) guidance based on the 'biliary territory' concept. They presented preliminary evidence supporting the safety and efficacy of NIF guidance, revealing lower stone recurrence compared to traditional techniques. The authors' innovative

demarcation approach was noteworthy, providing a clever method for identifying liver areas with obstructed drainage. The utilization of the negative staining method in cases where positive staining failed suggests that the latter may not have been as consistent as anticipated. The authors found themselves unable to predict the failure before surgery, leading to hypothesized reasons for its inconsistency in certain patients. However, there remains uncertainty regarding the pathway from bile to hepatocytes, or whether it extends only to the bile canaliculi on a smaller scale, creating the impression of widespread parenchymal illumination. The authors themselves acknowledge this ambiguity, stating, "However, the mechanism of negative staining is not yet fully understood. Further basic science studies are warranted."

Response 2: We greatly appreciate the reviewer's comments on our manuscript. In some patients in whom preoperative magnetic resonance cholangiopancreatography suggested obvious bile duct stricture, we found that the success rate of normal staining was higher. But, as the reviewer has pointed out, in other cases we were unable to predict failure preoperatively. At this stage, the counter-staining technology can be used as a supplement to the NIF technique based on biliary territory, and does have very practical clinical value. The mechanism of negative staining will be the focus of our next study. In cases where negative staining is employed, we plan, with the patient's consent, to excise a small sample of liver parenchyma that retains fluorescence imaging, and then study it under a fluorescence microscope to determine the presence of ICG fluorescent molecules within the liver lobule structure. We also plan to conduct more animal experiments to clarify the relevant mechanisms.

Changes to the text: We have modified our Discussion section as advised (see Page 16, line 11-19)
