PEER REVIEW HISTORY

ARTICLE DETAILS

TITLE (PROVISIONAL)	Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study
AUTHORS	Gon, Hidetoshi; Komatsu, Shohei; Murakami, Sae; Kido, Masahiro; Tanaka, Motofumi; Kuramitsu, Kaori; Tsugawa, Daisuke; Awazu, Masahide; Toyama, Hirochika; Fukumoto, Takumi

VERSION 1 – REVIEW

REVIEWER	Thinzar Lwin University of California, San Diego
REVIEW RETURNED	24-Mar-2019

GENERAL COMMENTS	In this article titled “Real-time navigation during hepatectomy using fusion indocyanine green-fluorescence imaging: protocol for a prospective cohort study” by Gon et al, the authors outline a project to evaluate the efficacy of ICG-navigation for fluorescence-guided hepatectomies. The project proposal is straight forward, and the described protocol is reasonable. However, there are a number of details that should be addressed prior to proceeding with this study. 1. The primary endpoint is for concordance between pedicle ischemic border and fluorescence demarcation signal. If pedicle clamping already provides an approach to identifying hepatic segments, please expand discussion on the added value of using ICG. 2. While the study design is appropriate to answer the research question, the question to be answered by the primary endpoint is not novel and the value of ICG in perfusion has been established by many other studies. 3. Intraoperative videos will be evaluated by experienced surgeons to confirm identification of hepatic boundaries. Are these three surgeons also the same ones performing the surgeries or different ones? Will these surgeons use a systematic method to score the videos? 4. Intraoperative ultrasound (IOUS) is often used during hepatectomies to assist in hepatic segment identification based on duct and vascular anatomy. Will IOUS be used and if so, how will this impact the decision making step in setting anatomic resection margins during the study? 5. A fluorescence signal will be assessed during parenchymal dissection and this will be compared for concordance against pedicle ischemia. However, pedicle ischemia is used for setting surface boundaries to initiate resection, but not routinely

	used during parenchymal dissection. How will pedicle ischemia be assess at the cauterized surfaces? 6. The secondary endpoint is to evaluate the success and failure of identifying liver tumors. Liver lesions have differing fluorescence patterns based on their tumor biology (Completely enhancing, partially enhancing, or ring type fluorescence). Is success indicated by any fluorescence signal? What if the fluorescence signal is weak or partial? The authors could better define the criteria for a "successful" identification of a liver tumor. 7. While ICG is sensitive, it is not specific and not all lesions that fluoresce will be neoplasms. How will the authors plan to address this? Will other modalities such as concordance with pre-operative imaging, IOUS, clinical judgement or frozen section biopsies be used to determine lesions that need to be resected? 8. Additionally, this could lead to further foreseeable risk since these lesions with a falsely positive fluorescence signal could lead to resection of additional lesions that the surgeon would not have removed otherwise. This should be addressed in their manuscript.

REVIEWER	Yoshikuni Kawaguchi the University of Tokyo, Japan
REVIEW RETURNED	28-Mar-2019

GENERAL COMMENTS	The authors reported a study protocol about fluorescence imaging using indocyanine green (ICG) for identifying liver segments. Because of the lack of well-organized prospective studies in this field, the authors' study will provide useful data. Please address the following concerns/suggestions: Major and minor comments 1. Please describe a fluorescence imaging system which will be used in the study. 2. For identifying liver segments with fluorescence imaging, there are several methods as clarified by Kobayashi Y, et al. (J Surg Oncol. 2017). The authors seem to use "negative staining technique (systemic ICG injection after clamping the portal vein with interest)" in the report. However, the three references (references 7, 18, and 19) which were cited by the authors used the different method from the authors' study (i.e., transhepatic portal vein ICG injection). As such, this issue may be confusing for readers. Please further clarify the method for identifying liver segments in their study. 3. A dose of 0.5mg/kg body weight will be used for identifying liver segments. Could the authors add the explanation regarding how the authors determined the dose in the study? For example, the dose seems to be approximately 10 times greater than the amount of ICG used in the study of Kobayashi, et al. 4. The Method section (Page 11). The authors described that "we divided the time taken to perform parenchymal resection into three equal intervals…" This paragraph needs to be clarified because the three equal intervals for parenchymal transection can be identified after completing liver resection. For example, do they check videos after surgery? 5. The Method section (Page 11). The authors will report recurrence-free survival. However, they included patients with "liver tumor." That means they may include any type of liver tumor including benign tumor, primary liver cancer, and liver metastases. Please clarify this issue.

	6. Similar to the comment above, the authors will examine the level of alpha-fetoprotein. Will the authors test this value for patients without hepatocellular carcinoma? 7. The Method section (Page 14-15). The use of logistic regression analysis needs to be refined. First, what is the meaning of "the success or failure of the ICG fluorescence imaging system?" Does this mean the success or failure for identifying liver segments or liver tumors or both? Second, the authors may perform the logistic regression analysis to predict the success or failure of fluorescence imaging for some purposes. Is it appropriate to include variables which will not be available before applying fluorescence imaging (i.e., operative time, blood loss, and postoperative complications)? 8. The Introduction section (Page 7). The authors described that "Also, the potential utility of this approach…" The sentence mentioned bile leakage, but the reference 15 is about fluorescence cholangiography for laparoscopic cholecystectomy. As such, this sentence needs to be modified to be concordant with the citation or the reference 15 may be removed.

VERSION 1 – AUTHOR RESPONSE

Reviewer #1

1. The primary endpoint is for concordance between pedicle ischemic border and fluorescence demarcation signal. If pedicle clamping already provides an approach to identifying hepatic segments, please expand discussion on the added value of using ICG.

Response: As reviewer #1 commented, the pedicle clamping technique is an established method to identify hepatic segments on the surface of liver. We aim to analyse whether the demarcation line determined by the conventional pedicle clamping method is consistent with the signal indicated by fusion indocyanine green (ICG)-fluorescence imaging in order to evaluate the efficacy of this method in a prospectively designed cohort. Moreover, this imaging approach has the potential to enable us to identify the border between ischaemic and non-ischaemic areas during parenchymal resection, which cannot be detected by the conventional pedicle clamping method. We consider this to be an added value of this approach. We have added the following sentence to the ETHICS AND DISSEMINATION section to clarify the added value of this study (page 17, line 2): "Whereas the conventional pedicle clamping method can only detect hepatic boundaries from the hepatic surface, the ICG-fluorescence imaging system can detect both the hepatic surface and transection surface during parenchymal resection."

2. While the study design is appropriate to answer the research question, the question to be answered by the primary endpoint is not novel and the value of ICG in perfusion has been established by many other studies.

Response: The fusion ICG-fluorescence imaging is a technologically established method, but further investigation is needed to determine the efficacy and safety of this system for clinical use. In this study, we evaluate the efficacy and safety of hepatectomy using ICG-fluorescence imaging systems by analysing the association between the success rate of identifying hepatic segments and clinical outcomes, including liver functional indicators, postoperative complications, and recurrence-free survival time. In addition, because of the lack of well-organized prospective studies in this field, our study has the potential to provide useful data.

3. Intraoperative videos will be evaluated by experienced surgeons to confirm identification of hepatic boundaries. Are these three surgeons also the same ones performing the surgeries or different ones? Will these surgeons use a systematic method to score the videos?

Response: The three surgeons who will evaluate the identification of hepatic boundaries in the additional expert panel are different from those who perform the surgeries. We modified the concordant sentence as follows (page 12, line 18): “The entire surgical procedure, including ICG-fluorescence imaging, will be digitally recorded and analysed by an additional expert panel consisting of three highly experienced surgeons, different from those performing the surgeries, to confirm the identification of hepatic segment boundaries.” instead of “The entire surgical procedure, including ICG-fluorescence imaging, will be digitally recorded and analyzed by an additional expert panel consisting of three highly experienced surgeons to confirm the identification of hepatic segment boundaries.”

As for the method to assess the videos, we evaluated the identification of hepatic segments in two points as described in the Outcome measures section. In particular, as for hepatic transection surface, we consider successful identification of hepatic boundaries when we can detect the boundaries for more than 80% of the process during parenchymal resection. Our description in the original manuscript is inadequate; we have modified the concordant sentence in the Outcome measurement section as follows (page 11, line 10): “Identification of hepatic segments is considered successful when we can identify the hepatic segments for more than 80 % of the process during parenchymal resection at more than two intervals.” instead of “Identification of hepatic segments is considered successful when we can identify the hepatic segments at more than two intervals.”

4. Intraoperative ultrasound (IOUS) is often used during hepatectomies to assist in hepatic segment identification based on duct and vascular anatomy. Will IOUS be used and if so, how will this impact the decision making step in setting anatomic resection margins during the study?

Response: The fusion ICG-fluorescence imaging system cannot detect the three-dimensional location of the intrahepatic Glissonian pedicle and hepatic vein. To identify the location of these structures, we use IOUS.

5. A fluorescence signal will be assessed during parenchymal dissection and this will be compared for concordance against pedicle ischemia. However, pedicle ischemia is used for setting surface boundaries to initiate resection, but not routinely used during parenchymal dissection. How will pedicle ischemia be assess at the cauterized surfaces?

Response: In this study, we observe the liver surface and assess whether the demarcation line determined by fusion ICG-fluorescence imaging is consistent with the line determined by pedicle clamping. We do not plan to assess pedicle ischaemia during parenchymal resection because, as reviewer #1 commented, the conventional pedicle clamping technique cannot detect the boundaries of the hepatic segment during parenchymal resection. In contrast, fusion ICG-fluorescence imaging has the potential to assess the boundaries of the hepatic segment during parenchymal resection. We consider this to be one of the advantages of the fusion ICG-fluorescence imaging system.

6. The secondary endpoint is to evaluate the success and failure of identifying liver tumors. Liver lesions have differing fluorescence patterns based on their tumor biology (Completely enhancing, partially enhancing, or ring type fluorescence). Is success indicated by any fluorescence signal? What if the fluorescence signal is weak or partial? The authors could better define the criteria for a “successful” identification of a liver tumor.

Response: I appreciate the reviewer’s appropriate advice. We evaluate the success of identifying liver tumours when any isolated fluorescence signals are detected, and also consider liver tumours

identified by other modalities, including preoperative imaging and IOUS, and finally confirm the diagnosis by pathological examination. We do not diagnose liver tumours based solely on fusion ICG-fluorescence imaging. Of course, the fluorescence pattern is considered according to the preoperative diagnosis because liver lesions have differing fluorescence patterns based on their tumour biology. Our description in the original manuscript is inadequate; we added following sentence with a concordant reference into the secondary endpoints section (page 11, line 19): “Successful identification of liver tumours is determined when any isolated fluorescence signals are detected, also considering liver tumours diagnosed by other modalities, including preoperative imaging and IOUS, and finally confirmed by pathological examination. The fluorescence pattern is considered according to the preoperative diagnosis because liver lesions have differing fluorescence patterns on the basis of their tumour biology.25” The added reference is as follows: Abo T, Nanashima A, Tobinaga S, et al. Usefulness of intraoperative diagnosis of hepatic tumors located at the liver surface and hepatic segmental visualization using indocyanine green-photodynamic eye imaging. Eur J Surg Oncol. 2015;41:257-64.

7. While ICG is sensitive, it is not specific and not all lesions that fluoresce will be neoplasms. How will the authors plan to address this? Will other modalities such as concordance with pre-operative imaging, IOUS, clinical judgement or frozen section biopsies be used to determine lesions that need to be resected?

Response: I appreciate the reviewer’s advice. As described in the comment for #6, we comprehensively evaluate whether any isolated fluorescence signal detected by fusion ICG-fluorescence imaging are lesions that need to be resected. If we identify lesions with isolated fluorescence signal detected by fusion-fluorescence imaging that were not identified by preoperative imaging, we evaluate the lesions by intraoperative ultrasound sonography, and if necessary, frozen section biopsies are performed to determine if additional hepatectomy is required. We have added the following sentence to the secondary endpoints section (page 12, line 5): “If we identify lesions with isolated fluorescence signal on fusion-fluorescence imaging that were not identified by preoperative imaging, we evaluate the lesions by intraoperative ultrasound sonography, and, if necessary, frozen section biopsies are performed to determine whether additional hepatectomy is required.”

8. Additionally, this could lead to further foreseeable risk since these lesions with a falsely positive fluorescence signal could lead to resection of additional lesions that the surgeon would not have removed otherwise. This should be addressed in their manuscript.

Response: I appreciate the reviewer’s appropriate advice. As described in the comment for #6 and #7, we evaluate any isolated fluorescence signals detected by fusion ICG-fluorescence imaging comprehensively using several modalities, including preoperative imaging, IOUS, or intraoperative frozen section biopsies to avoid unnecessary hepatectomy.

Reviewer #2

Major and minor comments

1. Please describe a fluorescence imaging system which will be used in the study.

Response: The fluorescence imaging system used in this study is PINPOINT (Stryker Japan K.K.). We have added the name of instrument in the Intervention section as follows (Page 9, line 11): “Intraoperatively, we will initially observe the hepatic surface using a fusion ICG-fluorescence imaging system (PINPOINT, Stryker Japan K.K.) to detect liver tumours.”

2. For identifying liver segments with fluorescence imaging, there are several methods as clarified by Kobayashi Y, et al. (J Surg Oncol. 2017). The authors seem to use “negative staining technique

(systemic ICG injection after clamping the portal vein with interest)" in the report. However, the three references (references 7, 18, and 19) which were cited by the authors used the different method from the authors' study (i.e., transhepatic portal vein ICG injection). As such, this issue may be confusing for readers. Please further clarify the method for identifying liver segments in their study.

Response: I appreciate the reviewer's appropriate advice. To clarify the method for identifying liver segments in our study, we have added a sentence with a concordant reference as follows (page 9, line 12): "Among several methods for identifying liver segments with fluorescence imaging, we will use the negative staining technique to identify the liver segments in this study.23" The added reference is as follows: Kobayashi Y, Kawaguchi Y, Kobayashi K, et al. Portal vein territory identification using indocyanine green fluorescence imaging: Technical details and short-term outcomes. J Surg Oncol. 2017;116:921-31.

3. A dose of 0.5mg/kg body weight will be used for identifying liver segments. Could the authors add the explanation regarding how the authors determined the dose in the study? For example, the dose seems to be approximately 10 times greater than the amount of ICG used in the study of Kobayashi, et al.

Response: To identifying liver segments by the negative staining method, Uchiyama K and colleagues used ICG at a dose of 0.5mg/kg body weight. We have added this report as a reference in the Intervention section (page 9, line 16). The added reference is as follows: Uchiyama K, Ueno M, Ozawa S, et al. Combined intraoperative use of contrast-enhanced ultrasonography imaging using a sonazoid and fluorescence navigation system with indocyanine green during anatomical hepatectomy. Langenbecks Arch Surg. 2011;396:1101-7.

4. The Method section (Page 11). The authors described that "we divided the time taken to perform parenchymal resection into three equal intervals…" This paragraph needs to be clarified because the three equal intervals for parenchymal transection can be identified after completing liver resection. For example, do they check videos after surgery?

Response: I am sorry that the description is ambiguous. As described in the original manuscript (Page 12, line 4), the entire surgical procedure, including ICG-fluorescence imaging, is digitally recorded and analysed by an additional expert panel to confirm the identification of hepatic segment boundaries after surgery. Since the expression was ambiguous, we changed the sentence as follow (page 11, line 8): "We divide the time taken to perform parenchymal resection into three equal intervals by reviewing the recorded videos after surgery, and the identification of hepatic segment boundaries is evaluated at each interval." instead of "We divide the time taken to perform parenchymal resection into three equal intervals, and the identification of hepatic segment boundaries is evaluated at each interval."

5. The Method section (Page 11). The authors will report recurrence-free survival. However, they included patients with "liver tumor." That means they may include any type of liver tumor including benign tumor, primary liver cancer, and liver metastases. Please clarify this issue.

Response: I appreciate the reviewer's appropriate advice. In our study, "liver tumour" includes primary liver cancer and liver metastases. Since the expression was ambiguous, we have added following sentence in the secondary endpoints section (page 12, line 8): "The recurrence-free survival is analysed for each liver tumour, including primary liver cancer and liver metastases."

6. Similar to the comment above, the authors will examine the level of alpha-fetoprotein. Will the authors test this value for patients without hepatocellular carcinoma?

Response: I appreciate the reviewer's appropriate advice. As you mentioned, we used inappropriate wording in the original manuscript. We should use a more generalized word because "liver tumour" includes both primary liver cancer and liver metastases. We have used "serum tumour maker levels depending on the type of liver tumour" instead of "serum AFP level" (page 13, line 10).

7. The Method section (Page 14-15). The use of logistic regression analysis needs to be refined. First, what is the meaning of "the success or failure of the ICG fluorescence imaging system?" Does this mean the success or failure for identifying liver segments or liver tumors or both? Second, the authors may perform the logistic regression analysis to predict the success or failure of fluorescence imaging for some purposes. Is it appropriate to include variables which will not be available before applying fluorescence imaging (i.e., operative time, blood loss, and postoperative complications)?

Response: I appreciate the reviewer's appropriate advice. First, we will perform logistic regression analysis in association with the success or failure of identifying liver segments using the ICG fluorescence imaging system, not for liver tumour. Second, with regard to the statistical analysis, we will perform logistic regression analysis to evaluate the association between the proportion of the successful cases for identifying liver segments and clinical variables including operative time, blood loss, and postoperative complications. This analysis potentially clarifies the efficacy of the ICG fluorescence imaging system in terms of clinical factors, including operative procedure. We do not plan to perform logistic regression analysis to predict the success or failure of identifying liver segment using the ICG fluorescence imaging system. Since the expression was ambiguous, we have modified the sentence as follows (page 15, line 15):" We will perform logistic regression analysis regarding the success or failure of identifying liver segments using the ICG fluorescence imaging system. The following factors will be included in the model to evaluate the association between the proportion of successful cases of liver segment identification and clinical variables: age, gender, body mass index, viral infection, Child-Pugh classification, cirrhosis, tumour size, tumour number, tumour location, type of hepatectomy, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio, prothrombin time, platelet count), operative time, blood loss, rate of postoperative complications, and recurrence-free time." instead of "We will perform logistic regression analysis of the success or failure of the ICG fluorescence imaging system. The following factors will be included in the model: age, gender, body mass index, viral infection, Child-Pugh classification, cirrhosis, tumour size, tumour number, tumour location, type of hepatectomy, liver function indicators (alanine transaminase, albumin, total bilirubin, international normalized ratio and prothrombin time, platelet count), operative time, blood loss, rate of postoperative complications, and recurrence-free time."

8. The Introduction section (Page 7). The authors described that "Also, the potential utility of this approach…" The sentence mentioned bile leakage, but the reference 15 is about fluorescence cholangiography for laparoscopic cholecystectomy. As such, this sentence needs to be modified to be concordant with the citation or the reference 15 may be removed.

Response: I appreciate the reviewer's appropriate advice. I modified this sentence to be concordant with reference 15 as follows (page 7, line 8): "Also, the potential utility of this approach to identify liver tumours and hepatic segment boundaries, as well as to detect the bile duct tree intraoperatively, has recently been demonstrated." instead of "Also, the potential utility of this approach to identify liver tumours and hepatic segment boundaries, as well as to detect intraoperative bile leakage has recently been demonstrated."

VERSION 2 – REVIEW

REVIEWER	Thinzar Lwin University of California, San Diego
REVIEW RETURNED	02-Jun-2019

GENERAL COMMENTS	The authors have proposed a thorough and systematic approach to evaluate the use of of ICG in demarcating liver segments during hepatectomy. They have edited the manuscript to reflect some major clarifications needed to improve the overall work. Some minor points of clarification are needed for the primary end point. The authors state "Identification of hepatic segments is considered successful when we can identify the hepatic segments for more than 80% of the process during parenchymal resection at more than two intervals." 1. Please clarify what it means by "identify the hepatic segments" and state objective measures for identification. 2. How will the video be captured? Overhead light fixture mounted camera? Helmet mounted camera? Would static image capture at intermittent intervals be useful? This work will be valuable and information from the secondary (more interesting) endpoints will be valuable to those working in the field of hepatobiliary fluorescence-guided surgery.

REVIEWER	Yoshikuni Kawaguchi the University of Tokyo, Japan
REVIEW RETURNED	20-May-2019

GENERAL COMMENTS	The authors revised their protocol report and improved their manuscript. The manuscript would be accepted after clarifying the following issues. Major and minor comments: 1. The method of multivariable logistic regression model needs to be modified. The purpose of the model is to “predict” the success of identifying liver segments using ICG-fluorescence imaging. As such, the model should consist of variables which are available “before” using ICG-fluorescence imaging. Operative time, blood loss, postoperative complications, and recurrence-free time seem to be inappropriate to “predict” the success of ICG-fluorescence imaging because these variables will be available “after” performing the technique. 2. Another issue of multivariable logistic regression model in the protocol is overfitting. The authors include 20 variables in the model. However, the number of variables largely exceeded the “ten events per variable” rule (Peduzzi P et al. J Clin Epidemiol 1995) because the number of event is estimated as being 78 (98 [participants] × 0.8 [success rate]). To ensure the accuracy of the regression estimates, 200 events (success of ICG-fluorescence imaging) will be required. The authors should reduce the number of variables in the model because the sample size has been already targeted for 110 patients. 3. Page 9. The information of Stryker should be as follows: PINPOINT; Stryker, Kalamazoo, MI, US.

VERSION 2 – AUTHOR RESPONSE

Reviewer #1

The authors have proposed a thorough and systematic approach to evaluate the use of of ICG in demarcating liver segments during hepatectomy. They have edited the manuscript to reflect some major clarifications needed to improve the overall work. Some minor points of clarification are needed for the primary end point. The authors state "Identification of hepatic segments is considered successful when we can identify the hepatic segments for more than 80% of the process during parenchymal resection at more than two intervals."

1. Please clarify what it means by "identify the hepatic segments" and state objective measures for identification.

Response: I apologize for the ambiguous description. In this study, the hepatic segments are considered as the border of fluorescing and non-fluorescing areas observed by a fusion ICG-fluorescence imaging system as described in the primary endpoint section (page 11, line 6). Identification of hepatic segments is considered successful when we can identify the hepatic segments for more than 80% of the transected surface area , which we described as “80% of the process” in the original manuscript. We have modified the applicable sentence as follows (page 11, line 11): “Identification of hepatic segments is considered successful when we can identify the hepatic segments in more than 80% of the transected area during parenchymal resection at more than two intervals.” instead of “Identification of hepatic segments is considered successful when we can identify the hepatic segments for more than 80% of the process during parenchymal resection at more than two intervals.”

2. How will the video be captured? Overhead light fixture mounted camera? Helmet mounted camera? Would static image capture at intermittent intervals be useful?

Response: I am sorry that the description is ambiguous. We will check the demarcation as continuously as possible during parenchymal resection. When we perform an open hepatectomy, the video will be captured by another surgeon using the scope of a fusion ICG-fluorescence imaging system. When we perform a laparoscopic hepatectomy, we can check the ICG-fluorescence images through the laparoscope. We have modified applicable sentence as follows (page 9, line 18): “The demarcation will also be checked as continuously as possible during parenchymal resection.” instead of “The demarcation will also be checked at appropriate intervals during parenchymal resection.”

Reviewer #2

Major and minor comments

1. The method of multivariable logistic regression model needs to be modified. The purpose of the model is to “predict” the success of identifying liver segments using ICG-fluorescence imaging. As such, the model should consist of variables which are available “before” using ICG-fluorescence imaging. Operative time, blood loss, postoperative complications, and recurrence-free time seem to be inappropriate to “predict” the success of ICG-fluorescence imaging because these variables will be available “after” performing the technique.

Response: I appreciate your kind comment. We were mistaken about the description of the analysis method. Our plan is to determine the effect of successfully identifying liver segments using ICG-fluorescence imaging on recurrence-free survival, not to predict the success of identifying the liver segments using ICG-fluorescence imaging. We have already included the recurrence-free survival as one of the secondary endpoints, so we have added the sentence about the statistical method into secondary outcomes section as follows (page 15, line 12): “We will estimate the

recurrence-free survival by the Kaplan Meier method. The recurrence-free survival will also be analysed by univariate COX proportional hazard model for each clinical variable. Multivariate Cox proportional hazard models will be adopted to analyse the risk factors of recurrence-free survival. The following variables will be included in the multivariate model: the success or failure of identifying liver segments using the ICG fluorescence imaging system and other variables for which the p-value is under 0.05 in the univariate analysis.", and we have deleted the exploratory analysis section in the original manuscript.

2. Another issue of multivariable logistic regression model in the protocol is overfitting. The authors include 20 variables in the model. However, the number of variables largely exceeded the "ten events per variable" rule (Peduzzi P et al. J Clin Epidemiol 1995) because the number of event is estimated as being 78 (98 [participants] × 0.8 [success rate]). To ensure the accuracy of the regression estimates, 200 events (success of ICG-fluorescence imaging) will be required. The authors should reduce the number of variables in the model because the sample size has been already targeted for 110 patients.

Response: I appreciate your constructive comment. As you mentioned, 20 variables for 110 patients is too many. In the multivariate analysis, we will only include the factors for which the p-value is under 0.05 in the univariate analysis, and the success or failure of identifying liver segments using ICG fluorescence imaging. Our revision in response to this comment is included as the last sentence of our answer for comment 1.

3. Page 9. The information of Stryker should be as follows: PINPOINT; Stryker, Kalamazoo, MI, US.

Response: I appreciate your appropriate advice. We have corrected the information regarding Stryker according to your advice (page 9, line 11).

VERSION 3 – REVIEW

REVIEWER	Yoshikuni Kawaguchi the University of Tokyo, Japan
REVIEW RETURNED	09-Jul-2019

GENERAL COMMENTS	The authors have clarified issues raised by the reviewer. Congratulations to the authors.