Reviewer #1:

Anastomotic leakage is a common and serious complication after colorectal cancer operation, and poor blood supply is an important reason for the occurrence of it. The author used artificial intelligence to evaluate the ischemic state of colostomy marked by indocyanine green staining. The design idea of the study is good, the research content and data are very detailed, which has certain guiding significance for clinical. It is suggested that the author further elaborate the specific algorithm of artificial intelligence in order to promote peer learning.

➔ Thank you for your kind comment and encouragement. In the future, we will collect a sufficient number of data through clinical trials and multi-center studies so that AI can differentiate between various anastomotic complications and are less affected by the quality of the ICG curve.

Reviewer #2:

The authors reported the application of Artificial intelligence (AI) based real-time analysis microperfusion (AIRAM) to predict the risk of anastomotic complication in the patient with laparoscopic colorectal cancer surgery, which seems interesting and applicable. However, several issues should not be ignored:

1) The training data set consists of 10,000 ICG curves from 200 different locations in the ICG videos of 50 patients. However, a training set including 50 patients is rather small.

2) The study evaluates the risk of anastomotic complication in the patient with laparoscopic colorectal cancer surgery by laparoscopic colorectal cancer surgery, and how about the accuracy and consistency of anastomotic complication in conventional surgical.

3) The inclusion criteria of this study are patients who have sigmoid or rectal cancer. As we know, the right colonic carcinoma usually undergoes primary anastomosis, so how about the effectiveness of this model in evaluating the right colonic carcinoma.

4) Many factors can lead to anastomotic complications with hypoperfusion of blood acting as one of the risk factors. So, whether AIRAM is effective remains to be proven.

5) How does the author find 200 ROIs are the appropriate size?

6) Can this technology be used to predict the prognosis of anastomotic complications after gastrectomy and esophagectomy?

7) Can the authors make a comparison between his study with other microcirculation studies based on AI?

8) The clearance rate of indocyanine green is significantly affected by liver function, especially in the case of liver cirrhosis. Therefore, severe liver function impairment, such as liver cirrhosis, should be excluded.

9) How to divide training set and test set?

10) What is the "gold standard" for "risk-safe", "risk-intermediate", and "risk-dangerous"?

- ➔ 1) Thanks for the kind comments. This study included only a small number of patients from a single cohort. Since the number of patients in the training group was small sized, the AI process was hard enough to indicate perfusion with the high reliability. That is why we propose future multicenter studies and clinical trials to obtain a sufficient number of data. If AI is trained with enough data in the future, AIRAM will be able to classify various anastomotic complications and improve classification performance.
- ➔ 2) In conventional surgery, anastomotic complication is known to be around 10-25%. Surgeons generally regard 10% of the incidence of anastomotic complications as acceptable. If the operating surgeon predicts postoperative anastomotic complications, diverting ileostomy or end-colostomy may be performed. (However, when the incidence of complications is considered as 10%, the clinical prediction accuracy of anastomotic complications can be considered as 90%.) (Since the number of patients in this study is small, the accuracy of risk prediction of each quantitative blood flow analysis parameter is calculated as low level. However, it is expected that the accuracy of risk prediction can be improved in further studies with a larger number of patients.)
- ➔ 3) This study was limited to patients with sigmoid or rectal cancer. Actually, our research team is conducting another study in patients with right colonic carcinoma about optimal protocol of ICG fluorescence. When right hemicolectomy is performed, the left branch of the middle colic artery is usually preserved, so favorable perfusion conditions are maintained in most patients. And especially in Korea, the anastomotic complication is only about 1%. Therefore, right hemicolectomy was excluded from this study. However, hypoperfusion evaluation using ICG angiography could be also usefully applied to the right colon to evaluate perfusion status.

  After ligation of inferior mesenteric artery in left sided colons such as sigmoid or rectal cancer, collateral blood flow decreases and hypoperfusion of anastomotic sites may occur

in about 10-20% of patients. This perfusion properties are considered to be one of the reasons for the higher anastomotic complication in anterior resection than in right hemicolectomy

➔ 4) Anastomotic complication is associated with a variety of causes as well as hypoperfusion. Various causes such as advanced stage, male patient, preoperative concurrent chemoradiation therapy (CCRT), anastomosis level close to anus, smoking, DM, steroids, etc. are related to anastomotic complications. Since intraoperative angiography has not been easy to date, surgeons could not easily access perfusion status of the anastomosis site using objective methods. The authors applied the NIR camera system to colorectal cancer surgery and experienced more than 300 intraoperative angiography. The anastomotic complications decreased from 10% to 2% at the period of applying intraoperative ICG angiography. However, it was never easy to visually detect the abnormality of the change pattern of the ICG angiography image in 10-20 seconds. Through quantitative analysis, the authors were able to understand the approximate change pattern of ICG fluorescence intensity and use it in the intraoperative decision process. Therefore, if AI could be applied in the real-time quantitative analysis process of perfusion status in the colorectal surgery, it is expected that the anastomotic complications will also decrease especially in the inexperienced surgeon's operations. In order to prove an effectiveness of AIRAM, a well-designed prospective multicenter study is urgently needed in the near future.

➔ 5) Acquire 200 ICG curves from one ICG video. If the ICG curve is obtained from one pixel, the quality of the ICG curve is degraded due to the camera shake and low-light noise. To alleviate this problem, the ROI is set up and the ICG curve is acquired with the average brightness inside the ROI. The appropriate size of the ROI is determined by the quality of the ICG video. If the ICG video is shaken and shot dark, the ROI should be increased. In the opposite case, the ROI needs to be small to increase the spatial resolution. The size of the ROI used in this study is 5 by 5 pixels because ICG video has less shake.

➔ 6) It is expected that microcirculation analysis using AI can be applied to various organs. Especially, in the gastric conduit for bariatric surgery or esophageal cancer, maintenance of blood flow in the gastric conduit is important for successful anastomosis. (If the algorithm and the risk rabelling of the specific organ are set and undergo the training process, it is expected that it can be applied to various organ surgery. Therefore, the researchers intend to expand this study so that it can be applied to various surgeries.

➔ 7) In this study, the authors could not find any other studies comparable to the AI-based microcirculation analysis that the authors designed. However, recently, AI is actively used in medical device development. And fluorescence imaging technology using a near-infrared

camera system enabled surgeons to perform intraoperative angiography. Therefore, as attempted in this study, it is possible to utilize a technology that can analyze microcirculation in real time using artificial intelligence in the operating room. The authors hope that these technological advances will reduce patient complications and improve surgical safety. And it would be a great honor if this paper could spread the beginning of a new transformation in the field of artificial intelligence microcirculation.

➔ 8) Thanks for the great comment.

As you commented on, ICG is metabolized in the liver, so it may be safe not to use it during surgery in patients with poor liver function such as liver cirrhosis. In this study, all patients were evaluated using laboratory liver function test panel before surgery, and liver function was normal in all patients.

In particular, since the removal rate of serum ICG could be decrease in patients with cirrhosis, ICG washout may be delayed in the venous phase during fluorescence perfusion analysis, and there is a high possibility that quantitative analysis of ICG perfusion may be incorrect. Therefore, it is expected that the operator must be careful for application of ICG fluorescence perfusion test in patients with cirrhosis.

➔ 9) In AI learning, to prevent overfitting and validate meaningfully, we used a cross validation method that does not train all patient data. Some of the patient data is used for testing. Patient data consists of a large number of normal patient data and a small number of complication risk patient data. Since patient data are not of the same distribution, some of the normal patient data were divided into a test set and some of the complication risk patient data were divided into a test set. The criteria for selecting test data from the data were randomly selected.

➔ 10) The risk of anastomotic complications in the clustered ICG curve pattern was evaluated using the complication rate. The patterns with more than 50% complications were evaluated as risk-dangerous, the patterns with more than 10% and less than 50% complications were evaluated as risk-intermediate, and the patterns with less than 10% complications were evaluated as risk-safe.