

Dear Editors and Reviewers,

Thank you for reviewing our manuscript entitled "**Study of preoperative diagnostic modalities in Chinese patients with superficial esophageal squamous cell carcinoma**" (Manuscript NO.: 78103, Retrospective Study). The reviewers' comments are valuable and helped us revise and improve our manuscript. We have studied the comments carefully and made corrections that we hope will be met with approval. Revisions marked **in red** in the paper.

The main corrections in the paper and the responses to the reviewers' comments are as follows:

Reviewer #1:

1. Critique 1: How many lesions examined by NBI (Olympus endoscope) and how many lesions examined by BLI (Fujinon endoscope)? Is there a difference in accuracy between them?

Response 1: Thank you for these questions. NBI and BLI are used with similar frequency in our Center's daily practice. In our study, among 152 lesions in 148 patients, 84 lesions were observed by NBI, and 68 were observed by BLI; the accuracy ratings of NBI and BLI for determining invasion depth were 72.6% (50/68) and 73.5% (61/84) with $P=0.89$. Therefore, NBI and BLI possess similar accuracy.

As image-enhanced endoscopies taken by either NBI or BLI, and in combination with magnifying endoscopy, can observe the structure of the micro-vessels of the esophageal surface. Though similar, the devices were developed by two different companies. The latest retrospective studies compared the diagnostic performance of NBI and BLI for identifying the depth of invasion of superficial esophageal squamous cell carcinoma. The researchers concluded that NBI and BLI performed similarly^[1, 2], which is consistent with our findings.

However, our study compared the diagnostic performance between EUS and magnification combined with image-enhanced endoscopy. Therefore, our study classified NBI and BLI as a single group (ME-NBI/BLI) and did not examine accuracy separately.

2. Critique 2: also how many lesions were examined by radial EUS or mini-probes?

Response 2: Thank you for this question. In our study, 73 lesions were examined by radical EUS, and 79 by mini-probes. The relevant data are presented in Table 4. Therefore, we compared the accuracy of radical EUS and the miniature probe for determining lesion infiltration depth. The miniature probe was significantly more

accurate than radical EUS (82.3% vs. 49.3%, $P < 0.01$) for determining the infiltration depth of superficial esophageal squamous carcinoma. Relevant content was added to the Results and Discussion sections.

3. Critique 3: Still not clear how can EUS differentiate between the stage of MM/SM1, and SM2/SM3.

Response 3: The deficiency you raise is very meaningful. When the article was conceived, we reviewed a range of literature that focusing on how to group findings according to EUS; however, we failed to present this information clearly in the article. Based on your comments, the following text was added to the Methods section: **Specifically, lesions confined to the first and second layers were categorized as EP/LPM; lesions involving the third layer were MM/SM1; lesions that invaded the fourth layer were SM2/SM3.**

It was hard to truly distinguish between MM/SM1 and SM2/SM3 by current EUS, even using miniature, high-frequency probes. SM1 lesions were defined as micro-infiltrating into the submucosa, which were hard to observe by EUS. Therefore, in our study, once lesions invaded the submucosa and were observed by EUS, we grouped them as SM2/SM3; if lesions only invaded the MM layer, they were designated MM/SM1.

4. Critique 4: What will be the diagnostic accuracy of both techniques, ME and EUS, if added to each other? Would the accuracy will increase if compared to each modality alone? If so, you can recommend combination of both techniques in these patients.

Response 4: We attempted to calculate the diagnostic accuracy of ME with EUS but our results were less accurate than those obtained when each modality was examined alone. As a result, when we combined the finding of EUS and ME, we usually could determine only the layer of deepest infiltration by EUS or ME, resulting in overdiagnosis. EUS may carry a risk of overdiagnosis due to its limitation.

But interestingly, we found that of the 41 lesions misdiagnosed by ME-NBI/BLI, 24 were corrected by EUS (24/41, 58.5%). Therefore, how to “combine” the findings of these two modalities is a worthy subject that should be addressed. For pEP/LPM lesions, ME-NBI/BLI demonstrated better diagnostic efficiency than EUS; for pT1b-SM2/SM3 lesions, type B3 vessels were negative for 43.1% lesions, resulting in lower sensitivity than EUS. EUS appears to be a better modality for examining the invasion of the submucosa.

We therefore recommend combining EUS and ME for comprehensive preoperative diagnosis.

5. Critique 5: What about the diagnostic accuracy of “lifting sign” during ER?

Response 5: Classical ER uses a submucosal injection to form a water cushion. The “lifting sign” indicates the lesion is confined to the mucosal layer and the mucosal and muscular layers can be separated by submucosal injection. This can increase the success rate of ER and reduce the occurrence of adverse events like perforation and bleeding. At our center, patients are comprehensively evaluated before ER, including a gastroscope combined with ME, EUS, and whole-body enhanced CT. ER is only considered for patients with a relatively clear diagnosis. Consequently, more than 90% of patients with lifting signs are included in this group. Absent of a lifting sign is not considered an absolute contraindication to ER, and we also performed diagnostic ER in these patients. Therefore these patients may be subjected to subsequent radiotherapy or esophagectomy if can not be cured by ER. Approximately two such patients were included in our study, but we did not disclose them further in the article.

Previous studies performed by our center have proved submucosal saline injection can improve the accuracy of preoperative diagnosis by distinguishing between mucosal and submucosal lesions under EUS in patients with esophageal squamous cell carcinoma (ESCC). This might help avoid unnecessary esophagectomy and diagnostic ER^[3,4].

6. Critique 6: You should mention that EUS has the advantage of detecting and even sampling local lymph nodes not seen by CT or MRI.

Response 6: Thank you for this valuable suggestion. We revised a portion of the Discussion, as follows: ...EUS can determine the presence of malignant regional lymph nodes with better sensitivity than CT and PET-CT^[5] and can sample the suspected lymph nodes to gain pathological confirmation.

7. Critique 7: Still English editing is needed.

Response 7: We apologize for the poor language of our manuscript. We have worked on both language and readability and invited native English speakers for language corrections. We really hope that the flow and language level have been substantially improved.

Reviewer 2#

1. & 2. Critiques 1 and 2: How many endoscopists participate in this study? What is their experience with magnifying endoscopy and EUS?

Responses 1 and 2: We agree that some details were not well clarified. For example, six endoscopists participated in this study, but we failed to mention that. Participating endoscopists were divided into junior and senior groups according to seniority.

Specifically, a senior endoscopist held the title of Associate Professor or higher and had at least 12 years of experience in endoscopy. Junior endoscopists had the title of attending physician or higher, with more than 6 years of experience in endoscopy. Residents and trainees did not participate in this study. In Chinese tertiary hospitals, most endoscopists are proficient in ME and EUS examinations, and our center is the top oncology specialty hospital in China. The revised manuscript elaborates on the qualifications of the endoscopists in method section : **Six certified and experienced endoscopists at our center performed all these examinations. The involved endoscopists were divided into junior and senior groups according to their seniority. The senior endoscopist is defined as titled of Associate Professor or higher with at least 12 years of experience in endoscopy. The junior endoscopist is defined as having a title of attending physician or above, with more than 6 years of experience in endoscopy. Residents and trainees did not participate in this study.**

3. Critique 3: The authors used NBI or BLI for ME. They are not the same. Is there any difference in accuracy between those two systems?

Response 3: Please refer to our response to Reviewer 1, Critique 1. NBI and BLI are both powerful tools for characterizing lesions. Both are image-enhanced endoscopies, that observe micro-vessel esophageal surface structure but manufactured by two different companies. The point of our study was to compare the diagnostic efficiency between ME and EUS so that we can classify NBI and BLI into the same group as ME-NBI/BLI.

In our study, among 152 lesions in 148 patients, 84 lesions were observed by NBI, and 68 were observed by BLI; the accuracy ratings of NBI and BLI for determining invasion depth were 72.6% (50/68) and 73.5% (61/84) with $P=0.89$. Therefore, NBI and BLI possess similar accuracy. This result was not included in the article because it wasn't the focus of our study. Moreover, some retrospective studies have concluded that NBI and BLI play similar and useful roles in identifying the invasion depth of superficial esophageal squamous cell carcinoma ^[1, 2].

4. Critique 4: In real-life practice, one endoscopist typically prefers one system (NBI or BLI). What is the endoscopists' preference in the authors' endoscopy center?

Response 4: Luckily, our center is equipped with both systems: NBI from Olympus Corporation and BLI from Fujifilm Corporation. NBI and BLI are used with similar frequency in our Center's daily practice. Endoscopists are required to use both systems and all are proficient in NBI and BLI.

5. Critique 5: Because of the retrospective design, some of the ME and EUS was performed by junior endoscopists. What is the definition of “junior”? Did it include “trainee”? Did it affect the diagnostic accuracy?

Response 5: As stated in the revised manuscript, Section of Examination procedure: Six certified and experienced endoscopists at our center performed all these examinations. The involved endoscopists were divided into junior and senior groups according to their seniority. The senior endoscopist is defined as titled of Associate Professor or higher with at least 12 years of experience in endoscopy. The junior endoscopist is defined as having a title of attending physician or above, with more than 6 years of experience in endoscopy. Residents and trainees did not participate in this study.

Diagnostic accuracy did not vary significantly according to endoscopist seniority for ME or EUS. We therefore assume that endoscopist seniority did not affect diagnostic accuracy. This conclusion has been presented in 3.3 *Clinicopathological factors that influence diagnostic accuracy*. The relevant data are shown in Table 4.

6. Critique 6: There are misspellings in this manuscript. (Ex. Table 2 NE-NBI/BLI) Please carefully check before submission.

Response 6: The manuscript has been thoroughly re-edited by a native English speaker.

7. Critique 7: This study compared ME and EUS, but the diagnosis of the depth of esophageal cancer should be ME in the standard guideline. The additional EUS will help or not is up to the ME expertise of endoscopists. EUS could not be better than ME, but EUS with ME could be better.

Response 7: our results are in agreement with your point. The addition of EUS is controversial. Considering of this, we revised the abstract and introduction in article. Our study found that EUS has comparable accuracy with NBI and can compensate for deficiencies inherent to NBI in some cases. For example, Type B3 vessels were negative for 43.1% of the pT1b-SM2/SM3 lesions^[6], leading to low sensitivity; in such a situation, EUS can be a useful tool to provide supplementary information about lesion depth. Besides, compared with foreign peers, most Chinese endoscopists are proficient in ME-NBI/BLI and EUS examinations, and the examination cost in China is relatively low. We recommend that preoperative diagnosis of SESCC be conducted based on the finding of WLI and ME-NBI/BLI. EUS can be added after patient consent in China, preferably utilizing a high-frequency miniature probe or miniature probe combined with conventional radical EUS.

We hope this revised manuscript has addressed all your comments and suggestions. We appreciate reviewers' efforts and hope these corrections will meet with approval. Once again, thank you very much for your comments and suggestion.

Sincerely,

Chun-Yu Huang

Reference:

- 1 Ueda T, Dohi O, Naito Y, Yoshida T, Azuma Y, Ishida T, Matsumura S, Kitae H, Takayama S, Mizuno N, Nakano T, Iwai N, Hirose R, Inoue K, Yoshida N, Kamada K, Uchiyama K, Ishikawa T, Takagi T, Konishi H, Nishimura A, Kishimoto M, Itoh Y. Diagnostic performance of magnifying blue laser imaging versus magnifying narrow-band imaging for identifying the depth of invasion of superficial esophageal squamous cell carcinoma. *Diseases of the esophagus : official journal of the International Society for Diseases of the Esophagus*. 2021: [PMID: 32691042 10.1093/dote/daaa078: 10.1093/dote/daaa078]
- 2 Hatta W, Koike T, Ogata Y, Kondo Y, Ara N, Uno K, Asano N, Imatani A, Masamune A. Comparison of Magnifying Endoscopy with Blue Light Imaging and Narrow Band Imaging for Determining the Invasion Depth of Superficial Esophageal Squamous Cell Carcinoma by the Japanese Esophageal Society's Intrapapillary Capillary Loop Classification. *Diagnostics (Basel, Switzerland)*. 2021: [PMID: 34829288 10.3390/diagnostics11111941: 10.3390/diagnostics11111941]
- 3 He LJ, Xie C, Wang ZX, Li Y, Xiao YT, Gao XY, Shan HB, Luo LN, Chen LM, Luo GY, Yang P, Zeng SC, Xu GL, Li JJ. Submucosal Saline Injection Followed by Endoscopic Ultrasound versus Endoscopic Ultrasound Only for Distinguishing between T1a and T1b Esophageal Cancer. *Clin Cancer Res*. 2020: 384 [PMID: 31615934 10.1158/1078-0432.CCR-19-1722: 10.1158/1078-0432.CCR-19-1722]
- 4 Li JJ, Shan HB, Xu GL, He LJ, Xia JC. Submucosal saline solution injection combined with endosonography for distinguishing between stages T1a and T1b of early esophageal cancer. *Gastrointestinal endoscopy*. 2013: 159 [PMID: 23261111 10.1016/j.gie.2012.08.028: 10.1016/j.gie.2012.08.028]
- 5 van Vliet EP, Heijenbrok-Kal MH, Hunink MG, Kuipers EJ, Siersema PD. Staging investigations for oesophageal cancer: a meta-analysis. *Br J Cancer*. 2008: 547 [PMID: 18212745 10.1038/sj.bjc.6604200: 10.1038/sj.bjc.6604200]
- 6 Ikeda H, Inoue H, Sato H, Sato C, Onimaru M, Yamaguchi N, Hamatani S, Kudo S. Usefulness of a New Classification by the Japan Esophageal Society to Predict the Depth of Invasion of Esophageal Cancer: Type B3 Vessels. *Stomach and Intestine (Tokyo)*. 2014: 186,

