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"Simplified criteria for diagnosing superficial esophageal squamous neoplasms using NBI magnifying endoscopy"

Dear Editor:

We appreciate your constructive and thoughtful comments on our original article. We have revised the manuscript according to the reviewers' questions and comments, as described in the following point-by-point responses.

We hope the revised manuscript is now acceptable for publication in your journal.

Yours sincerely,

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Point-by-point response:

The relevant additions or corrections to the manuscript as detailed below are indicated in red and underlined typeface in the manuscript.

#1. Table 3. Please show the odds ratio and 95% confidence interval of the other parameters. In addition, presence or absence of each four criteria by dividing the esophagitis, LGIN, and ESCC should be described.

We have edited Table 3. The odds ratio was calculated by using univariate analysis, and we have added the following sentences to the Materials and Methods and Results sections.

Abstract

Results

In the univariate analysis, proliferation, tortuosity, change in caliber, and VS were significantly associated with SESCC ($P < 0.01$). The combination of VS and proliferation was statistically extracted from the 6 NBI-ME findings by using the stepwise logistic regression model.

~~The odds ratios of VS and proliferation for SESCC was 4.8 (95% confidence interval; 1.7 – 13.4) and 4.0 (95% confidence interval; 1.1 – 15.1), respectively.~~

Main text

Materials and Methods

To assess the relationship between each NBI-ME finding and the diagnosis of SESCC, the Pearson χ^2 (chi-square) test was used for comparisons of variables in a univariate analysis. We did not perform a multivariate analysis.

Results

In the univariate analysis (Table 3), proliferation, tortuosity, change in caliber, and VS were significantly associated with SESCC ($P < 0.01$). The combination of VS and proliferation was statistically extracted from the 6 NBI-ME findings by using a stepwise logistic regression model. We defined the combination of VS and proliferation as a simplified "dyad criteria" for the NBI-ME diagnosis of SESCC.

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~~and proliferation for SESCC was 4.8 (95% CI; 1.7 – 13.4) and 4.0 (95% CI; 1.1 – 15.1), respectively. We defined the combination of VS and proliferation as a simplified "dyad criteria" for the NBI-ME diagnosis of SESCC.~~

Table 3. The results of the univariate analysis of the NBI-ME findings for the diagnosis of SESCC

NBI-ME findings	Esophagitis (n = 8)	LGIN (n = 27)	SESCC (n = 54)	Odds ratio (95% CI)	*P*
IBC; n (%)	1 (13)	19 (70)	45 (83)	3.3 (1.2–8.9)	NS
Proliferation; n (%)	3 (38)	21 (78)	50 (93)	5.0 (1.4–17.5)	<0.01
Dilatation; n (%)	3 (38)	20 (74)	48 (89)	3.6 (1.2–11.1)	NS
Tortuosity; n (%)	2 (25)	14 (52)	44 (81)	5.2 (2.0–13.6)	<0.01
Change in caliber; n (%)	2 (25)	6 (22)	31 (57)	4.5 (1.7–11.8)	<0.01
Various shapes; n (%)	3 (38)	14 (52)	46 (85)	5.4 (2.0–14.8)	<0.01

SESCC: superficial esophageal squamous cell carcinoma; NBI-ME: narrow-band imaging combined with magnifying endoscopy; LGIN: low-grade intraepithelial neoplasia; IBC: intervascular background coloration: NS; not

significant

#2. Table 2. 10 cases of M3 or deeper cancer were included. Tumor vasculature (neovasculature) might be observed in these cases. Could authors observe IPCLs inside the cancer lesion in all these 10 cases?

We observed IPCL-like abnormal microvessels in 4 of the 10 cases of M3 or deeper cancers. The other 6 cases had abnormal microvessels without loop-like formation. Please refer to our response to comment 5.

#3. Proliferation of the IPCL has been proven from the aspect of the immunohistochemistry. Authors should refer the following manuscript and discuss this issue in discussion section if possible.

Kubota Y, Kaneko K, Konishi K et al. The onset of angiogenesis in a multistep process of esophageal squamous cell carcinoma. Front Biosci 2009 ;14:3872-8.

Kumagai Y, Sobajima J, Higashi M et al. Angiogenesis in Superficial Esophageal Squamous Cell Carcinoma: Assessment of Microvessel Density

Based on Immunostaining for CD34 and CD105. Jpn J Clin Oncol. 2014 Jun;44(6):526-33.

We have added the following sentences to the Discussion section to show the evidence of proliferation of IPCL because immunohistochemistry had already proven the angiogenesis.

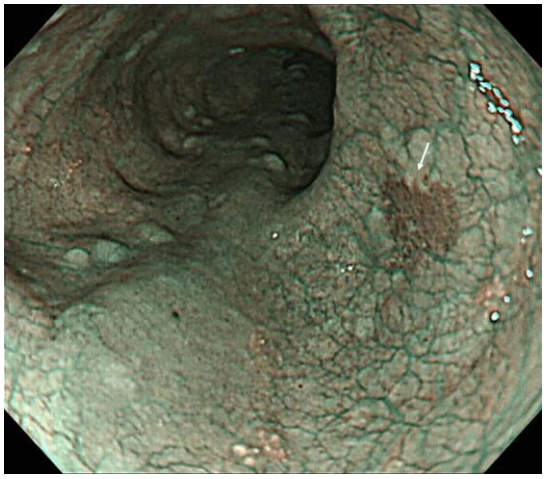
Kumagai et al. already indicated that the microvessel density in SESCC gradually increased in proportion to the depth by using immunohistochemical staining with CD34 and CD105[24]. Their study will support our result that proliferation of IPCLs is one of two highly suggestive NBI-ME findings to diagnose SESCC.

24) Kumagai Y, Sobajima J, Higashi M, Ishiguro T, Fukuchi M, Ishibashi K, Baba H, Mochiki E, Yakabi K, Kawano T, Tamaru J, Ishida H. Angiogenesis in superficial esophageal squamous cell carcinoma: assessment of microvessel density based on immunostaining for CD34 and CD105. *Jpn J Clin Oncol* 2014; **44**: 526-533 [PMID: 24748644 DOI:10.1093/jjco/hyu039 [doi]]

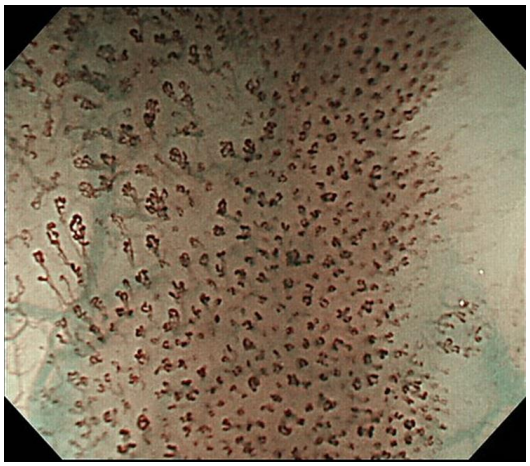
#4. Figure 2B: This picture is very beautiful. However, the magnification power is low, and IPCLs are observed as dots. Thus, it is difficult to recognize the "variety of shape" for the readers. Please show another picture that clearly demonstrate the "variety of shape" of the IPCLs.

We have changed the cases with those with pictures with high magnification.

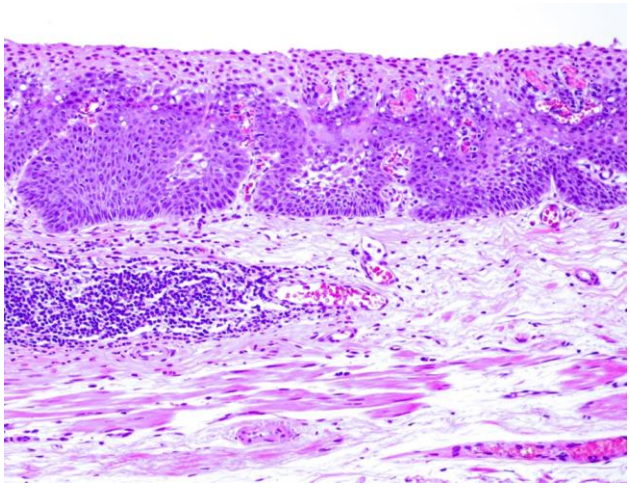
Figure 2. A representative case of superficial squamous cell carcinoma.



A) On non-magnifying NBI endoscopy, the lesion demonstrated a well-demarcated brownish area.



B) The lesion has all six of the diagnostic findings obtained by using NBI-ME.



C) Histology from endoscopic submucosal dissection showing squamous cell carcinoma invading up to the lamina propria mucosae.

#5. Authors should introduce the outline of morphological changes of the microvasculature from normal squamous epithelium through invasive cancer in introduction section.

To address this comment, we have added the following sentences to the Introduction section to explain the outline of the morphological changes of the microvasculature from the normal squamous epithelium through the invasive cancer.

In the normal esophageal mucosa, loop-like vessels arise from the subepithelial capillary network beneath the epithelium. These microvessels are inside the epithelial papillae and named "intrapapillary capillary loops (IPCLs)"[6]. The IPCLs or abnormal microvessels in the superficial layer of the lesion are clearly visualized on magnifying endoscopy in combination with NBI.

SESCC lesions have abnormal microvessels with severe irregularity. The severe irregularity is defined as having morphological changes as follows: "dilation," "tortuosity," "change in caliber," and "various shapes"[6, 10]. When SCC invades up to the lamina propria mucosae, the abnormal microvessels with

severe irregularity show a loop-like formation. When the tumor invasion reaches the muscularis mucosae or shallowly invades the submucosae, abnormal microvessels show no loop-like formation that has a stretched and markedly elongated transformation. The tumor deeply invading the submucosae has abnormal vessels with severe dilation and green color. Thus, the identification of IPCLs and abnormal microvessels can contribute to accurate diagnosis and reduced false-positive results in the diagnosis of SESCC[6, 8].

~~Microvascular structures on the lesion's surface (e.g., intrapapillary capillary loops [IPCLs]) are clearly visualized using magnifying endoscopy in combination with NBI [6]. The identification of IPCL can contribute to accurate diagnosis and reduced false-positive results in the diagnosis of SESCC [6,8].~~