

Significance of background coloration in endoscopic detection of early esophageal squamous cell carcinoma

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ABSTRACT

Endoscopic diagnostics of early squamous cell carcinomas in laryngo-esophageal region have dramatically improved together with development of less invasive endoscopic treatment. It is essential for gastrointestinal endoscopists to detect the lesions when they are still endoscopically treatable especially in the region, of which surgical approach can still be extremely invasive.

Pioneers have found some notable fundamental alterations in early squamous cell carcinomas and created several classifications. Inoue proposed intra-papillary capillary (IPCL) classification. The classification focused on the microvascular change of the mucosal surface. One of the significances of this classification is that it clearly distinguished the lesions that require further pathological evaluation by categorizing diameter change of the IPCLs. On the other hand, Arima advocated about the alteration of microvessels as well as change of the vascular

arrangement in the area. Most recently, Japan Esophageal Society constructed the new classification uniting these two exemplary classifications as “Japanese Classification of Magnifying Endoscopy for Early Squamous Cell Carcinoma”. This classification was intended to be simple and easily applicable in general clinical practice.

Brownish color change between the IPCLs has reported to be one of the useful findings in distinguishing early squamous cell carcinoma from benign changes such as inflammatory change and low grade intraepithelial neoplasia (LGIN). Nevertheless, exact cause of this phenomenon remains unclear. We recently examined the association of color change with hemoglobin (Hb) in the cancer tissue, since NBI exclusively detects the wavelength of Hb in superficial vessels in the gastrointestinal tract. Present review article also describes our examination on the distinct finding in esophageal cancer, namely, “background coloration; BC”

Key words; background coloration, esophageal cancer, squamous cell carcinoma, NBI, Image enhanced endoscopy, magnifying endoscopy

INTRODUCTION

In Japan, squamous cell carcinoma (SCC) accounts for more than 95% of esophageal cancer. Although incidence of reflux esophagitis has increased, frequency of esophageal adenocarcinoma has remained immutable, accounting for only about 4% of esophageal cancer [1]. The prognosis of esophageal SCC that are found in the late stages remain exceedingly poor because of the aggressive nature of the tumor [2]. Proportion of superficial carcinomas among esophageal malignancies in the last 10 years has been approximately 30-35% (Japan Esophageal Society. Comprehensive registry of esophageal cancer in Japan. Available at <http://esophagus.jp> Last accessed 18 April 2007). Although early detection results in better prognosis, it is challenging to detect flat lesions with slight color change. However, it is exceedingly difficult to find the flat lesions with slight color change using conventional white light image endoscope, which can be treated using endoscopic mucosal resection (EMR) and endoscopic submucosal dissection (ESD) techniques.

Chromoendoscopy using iodine has been one of the most reliable diagnostic tools in detecting early SCC of the esophagus [3, 4]. It has been effectively utilized not only in detecting but also determining the local extent of the tumor. However, iodine spraying often induces unpleasant side effects such as chest discomfort, cough, and severe allergic reaction due to its high stimulation. Another issue to be mentioned is that detection of the early lesions can be complicated by surrounding severely inflamed mucosa resulted from patients' habit of drinking and smoking. Therefore, development of new technologies, which allows us to obtain diagnostic information without harming

patients, has been awaited. Recently, significant advancement has been made in the field of image enhanced endoscopy (IEE) such as magnifying endoscopy (ME), narrow band imaging (NBI), and Fuji Intelligent Chromo Endoscopy (FICE). These newly developed modalities enable us to obtain detailed information of gastrointestinal tract leading to more reliable evaluation that is consistent with pathological diagnosis.

Historically, several useful classifications, such as Inoue's intra-papillary capillary loop (IPCL) classification [5, 6] and Arima's microvascular patterns classification [7], have been proposed in Japan for diagnosis and evaluation of squamous cell carcinoma. However, these two major classifications have been thought to have some difficulties because of their complexity. Thus, Japan Esophageal Society decided to merge these two classifications into a simpler category that can easily be used in the general clinical practice. They introduced the Japanese Classification of Magnifying Endoscopy for Early Squamous Cell Carcinoma as comprehensive classification of superficial esophageal SCC [8]. Currently, new findings in addition to vascular structure, such as brownish color change of the epithelia between each IPCL, have been introduced in the esophageal diagnostics.

In this review article, we specifically focus on the endoscopic diagnosis of esophageal SCC.

LUGOL CHROMOENDOSCOPY

Ability to detect early squamous neoplasia of the esophagus can be improved considerably by iodine staining during endoscopic examination, especially for high-risk

population such as over 50 year-old male with a history of alcohol and tobaccos abuse. Iodine unstained areas are usually indicate either inflammatory change, low-grade intraepithelial neoplasia (LGIN), high-grade intraepithelial neoplasia (HGIN), or invasive squamous cell carcinoma. Mori et al. analyzed the relationship between Lugol voiding area and histological feature [9]. Their study demonstrated that the staining intensity reflected well the thickness of the glycogen-containing cell layer in the lesion. Furthermore, sharpness of the margin after staining predicted whether the conversion from glycogen containing mucosa to non-containing cell layers were abrupt or gradually changed.

Pink-Color sign / Metallic-silver sign

Pink color sign (PCS) was first mentioned by Ohmori et al. as one of the useful findings for detecting of the early esophageal neoplasms among multiple Lugol voiding areas in the inflamed esophagus [10]. Shimizu et al. reported that HGIN could be identified as iodine-unstained areas that are more distinct and reddish than LGIN when the brown color of iodine solution in normal region has faded after the staining, because there is almost no remaining glycogen-containing epithelium in HGIN [3]. Recognition of PCS in the Lugol voiding area suggests the existence of carcinoma. With NBI, PCS is strongly emphasized and observed as shiny silver color change, which we call "metallic silver sign (MSS)".

INTRAPAPILLARY CAPILLARY LOOP (IPCL) CLASSIFICATION

Intrapapillary capillary loop (IPCL) in esophagus is usually observed as brown

dots under NBI observation. IPCL appears in the plane closely above the muscularis mucosae branched from the obliquely running vessels, which is observed as green vessels [11]. Inoue et al categorized the surface microvasculature of the esophagus into five groups according to the irregularity of shape and degree of each IPCL's dilation [5]. Type I represents normal or minimal change of IPCL. Type I and II usually lack clear boundary, corresponding to benign pathology including inflammatory change and LGIN. Type III is defined as IPCL with minimal change of caliber, which usually has clear boundary with background mucosa. Type III is thought to be borderline lesions including LGIN. In contrast, irregularly dilated IPCL with clear area formation is called IPCL type V, which corresponds to malignant pathology including HGIN with high probability. Type IV is the IPCL change in between type III and V. Type IV indicates benign change such as LGIN with the probability of approximately 50%. Therefore, the lesions that are categorized into type IV require consideration for removal, which enables further pathological evaluation. Type V is equivalent to definite malignant pathology including HGIN, which basically requires treatment including EMR and ESD. This classification simply offers the information of whether the pathological confirmation should be performed by differentiating type III and IV. Recently, Kaga et al. evaluated the detailed vascular structure pathologically and concluded that differences in vessels between IPCL type III and IV are highlighted by two factors; increased vessel caliber and prolongation of IPCLs toward the surface [12].

MICROVASCULAR PATTERNS OF SUPERFICIAL ESOPHAGEAL CANCERS BY

MAGNIFYING ENDOSCOPY

Arima et al classified the microvascular pattern into 4 categories according to the shape and irregularity of the surface microvessels[7]. Type 1 is characterized by thin, linear capillaries in the subepithelial papilla and was generally seen in normal mucosa. Type 2 is characterized by distended, dilated vessels, and the shape of capillaries in the subepithelial papilla was preserved. Type 2 is generally seen in inflammatory lesions. Type 3 is characterized by spiral vessels with an irregular caliber and crushed vessels with red spots, and the arrangement of the vessels was irregular. Type 3 is generally seen in T1a-EP or T1a-LPM cancers. Type 4 has 4 subcategories including multiple layered, irregularly branched, reticular, and avascular area (AVA). Type 4 is generally seen in cancers with T1a-MM or deeper invasion. AVAs and stretched type 4 vessels are seen in cancers with downward growth. They further subcategorized AVAs into 3 groups according to the size and reported that the size of AVAs was closely related to the depth of tumor invasion.

JAPANESE CLASSIFICATION OF MAGNIFYING ENDOSCOPY FOR EARLY SQUAMOUS CELL CARCINOMA (JAPAN ESOPHAGEAL SOCIETY) [8]

However, those two major classifications mentioned above have been thought to be somewhat complicated for global use. Therefore, Japan esophageal society decided to create a new simplified classification. The new classification is composed of two major criterias. One is shape and width of vessels and the other is size of AVA. The vessels observed by magnified endoscopy were classified into two groups, Type A and

B.

Type A is the vessels with mild or no atypia of IPCL. Type B is IPCL with atypia including dilatation, meandering, caliber change, and uneven form in each vessel. A lesion with type A strongly suggests intraepithelial neoplasia (IN), whereas type B indicates SCC. Type B was subclassified into 3 groups, and the invasion depth of B1, B2 and B3 was consistent with T1aEP or LPM, T1aMM or T1bSM1 and T1bSM2, respectively. In addition, AVA was divided into three groups according to the tumor size. AVA-small, AVA-middle, and AVA-large were defined as 0.5mm or less, between 0.5 and 3mm, and 3mm or larger, respectively. Accuracy of diagnosis using thus method was 90%. They concluded that the new classification of magnified endoscopy is simple and useful for diagnosis of invasion depth of esophageal SCC.

BACKGROUND COLORATION

We have reported the importance of the color change in the epithelia between IPCLs (background coloration; BC) in differentiating early squamous cell carcinoma in the esophagus from benign lesions including inflammatory changes [13]. The BC has been additionally noted as intravascular BC in the Japanese classification of magnifying endoscopy for early squamous cell carcinoma.

Ishihara et al. reported that both the background color change and dilated IPCL was important in diagnosing esophageal SCC among any other early changes such as brownish dots (dilated IPCL), tortuous IPCL, elongated IPCL, caliber change in IPCL, variety in IPCL shapes, demarcation line, and protrusion or depression [14]. Also,

Kanzaki et al statistically analyzed the cause of this color change. They reported that it may be related to thinning of the keratinous layer, caused by neoplastic cell proliferation and thinning of the epithelium [15]. However, NBI mechanically reflects the wavelength that is specific to hemoglobin (Hb). Therefore, we have come to conceive that the color change might be related to the extravascular Hb component in the cancer area.

- Evaluation of BC

After a brownish area (BA) in esophagus was found with NBI, the lesions were observed with NBI magnification in order to evaluate the presence of BC. The lesion was recorded as BC positive when distinct color change in the area between IPCLs was seen. When there was no color change in this area, the lesion was regarded as BC negative.

In our current study including 223 lesions of early pharyngo-esophageal SCC, 194 lesions (86.6%) were BC positive and only 3 lesions (2.5%) of them were pathologically diagnosed as benign (Table 1). Sensitivity, specificity and overall accuracy of BC in differentiating malignancy from benign pathology were 91.1%, 71.4%, and 89.4%, respectively.

- What makes the color change?

The cause of this phenomenon is still unclear. To begin with, NBI is a noble technique that enables us to exclusively identify the wavelength of Hb. Therefore, we speculated the involvement of intra- or extra vascular Hb in the cancer area. We applied immunohistochemical approach using anti-human Hb antibody to evaluate the correlation between BC and Hb component. Figure 1-c and d shows

immunopathological feature from both Hb positive cancerous area and negative surrounding non-cancerous area. Preliminary results revealed that there was a significant correlation between Hb-immunopositivity and pathology. Rate of correlation between BC and Hb positivity was as high as 80.9%. Interestingly, immuno-fluorescent image showed that Hb-positivity was mainly observed within cytosol of cancer cells outside of the nuclei with clear boundary (Figure 2-a, b).

Furthermore, real time polymerase chain reaction (RT-PCR) was performed to quantify the Hb- β expression of both SCC and surrounding non-cancerous mucosa. RT-PCR results revealed that Hb- β mRNA expression rate was three times as higher in cancer area as surrounding non-cancerous area ($p < 0.05$). In situ hybridization (ISH) of Hb- β mRNA also confirm the contribution of Hb- β expression in the cancer areas. These results support that the Hb might be produced inside of the cancer cells.

We also conducted immunostaining using anti-human CD68 antibody in order to evaluate the relevancy of macrophage in the cancer area, which may contain hemosiderin that might cause the color change (Figure 3. a-c). Very few macrophages were observed in the surface area of the lesion whereas immunopositivity for anti-human Hb antibody was clearly seen in the cancer area, which was confirmed with conventional H&E staining. This result suggests that the color change is independent from hemosiderin contained by macrophages in the cancer area.

The mechanism and the reason of Hb production in the cancer tissues are still obscure. However, many investigators have reported about the expression of Hb mRNA in various organs including both benign and malignancy [16-19]. Hypoxic environment

or oxidant stress may be a justifiable cause for this phenomenon. Also, as Kanzaki reported, thickness of the keratinous layer and optical mechanism could be the explanation. Further studies are mandatory to determine the exact cause of BC.

ENDOCYTOSCOPY

Contact endoscopy was first described by Hamou in 1979 as a method of microhysteroscopy to examine the surface of the genital tract at high magnification [20]. Tada et al first described the ultra-high magnifying endoscope in the gastrointestinal tract in 1982 [21]. Conventional magnification endoscopy enables detailed evaluation of gastrointestinal mucosa. Furthermore, endocytoscopy (ECS) with ultra-high magnification allows in vivo observation of cellular atypia during routine endoscopic examination [22-24]. This novel technique opened the door to possibility of “optical biopsy”, which enables us to obtain pathological diagnosis without taking biopsies. It is predicted that the incidence of cardiovascular or cerebrovascular complications would significantly increase. Optical biopsy using endocytoscopy will be of great help in the point of saving time, money, and perioperative risk due to interruption of anti-coagulant therapy. Establishment of common diagnostic criteria of endocytoscopy image is essentially required.

CONCLUSION

Recent advancement in technology of image-enhanced endoscopy allows us to evaluate detailed surface structure of gastrointestinal tract. With the introduction of

newly constructed classification from the Japan Esophageal Society, diagnostics of esophageal SCC could be united and spread widely. Also, many studies for the purpose of illuminating the underlying mechanism and the significance of each finding have been conducted. It is of importance that we understand both the characteristics of the devices and the meaning of each finding comprehensively and precisely.

CONFLICT OF INTEREST

The authors declare that they have no conflict of interests in connection with this paper.

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Figure

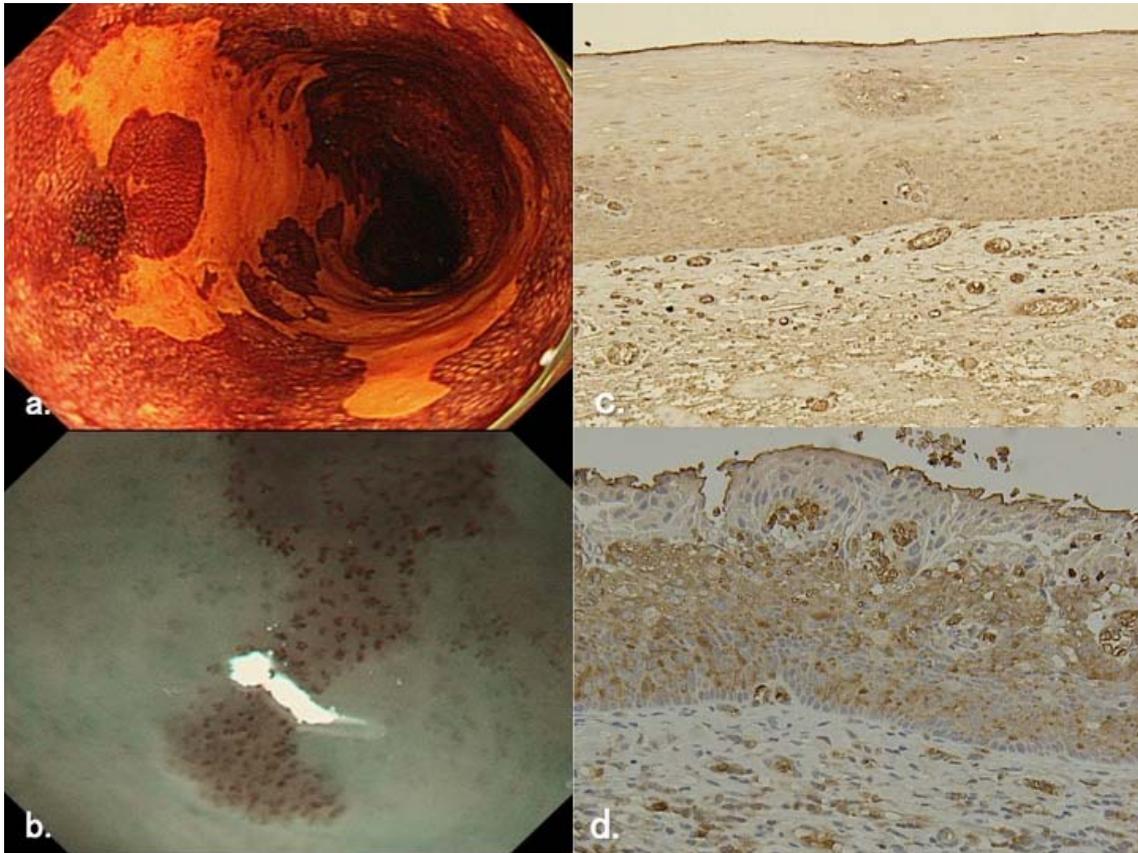


Figure 1

1-a. Iodine staining demonstrated well-demarcated slightly depressed lesion on the mid-esophagus.

1-b. Using NBI magnification, the color of the epithelia between each dilated IPCL in the lesion was altered into brownish color compare to surrounding whitish mucosa. This lesion was clearly positive for BC.

1-c. Hb immunostaining image of surrounding non-cancerous area. Immunopositivity for anti-human Hb antibody was negative.

1-d. Hb immunostaining image of cancer area. Immunopositivity was observed in as intense as Hb in the vessels.

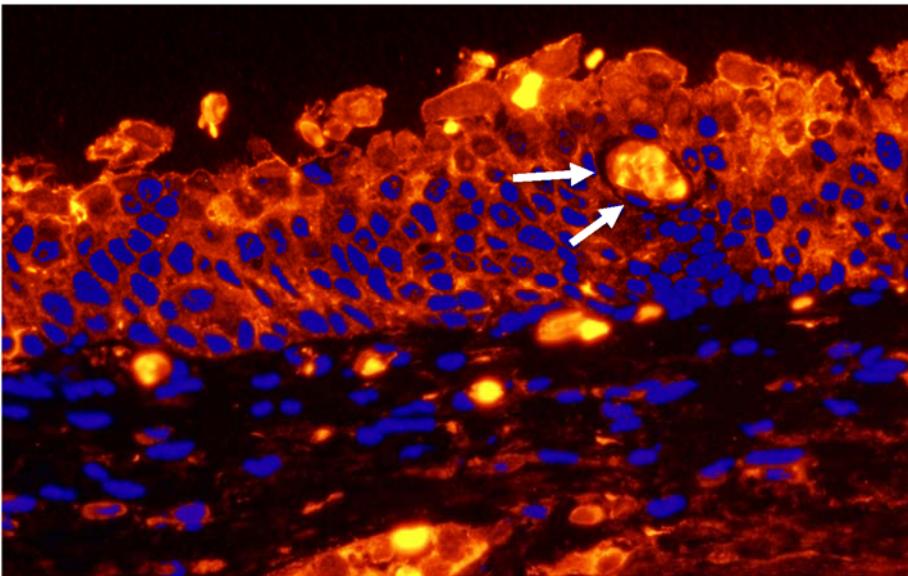
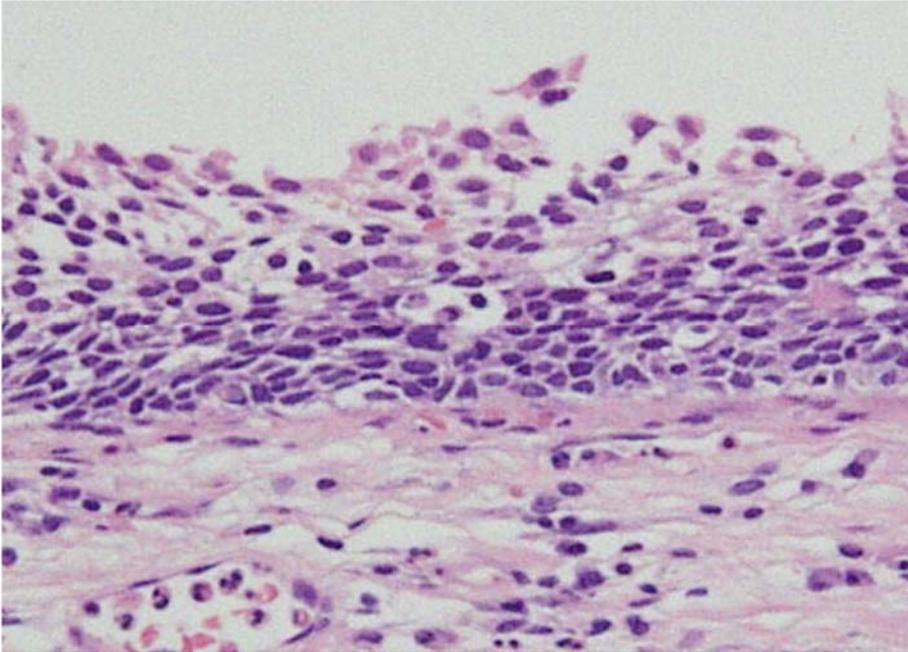


Figure 2-a. Conventional H&E staining revealed the T1a-EP esophageal squamous cell carcinoma.

Figure 2-b. Immunofluorescent image (blue; nuclei. yellow- brown; anti-human Hb antibody). Intra vascular RBCs are intensely positive (white arrow). Similarly, SCC cells are positive in the cancer cells as well, especially in the supposed cytoplasm around the nuclei that are stained blue.

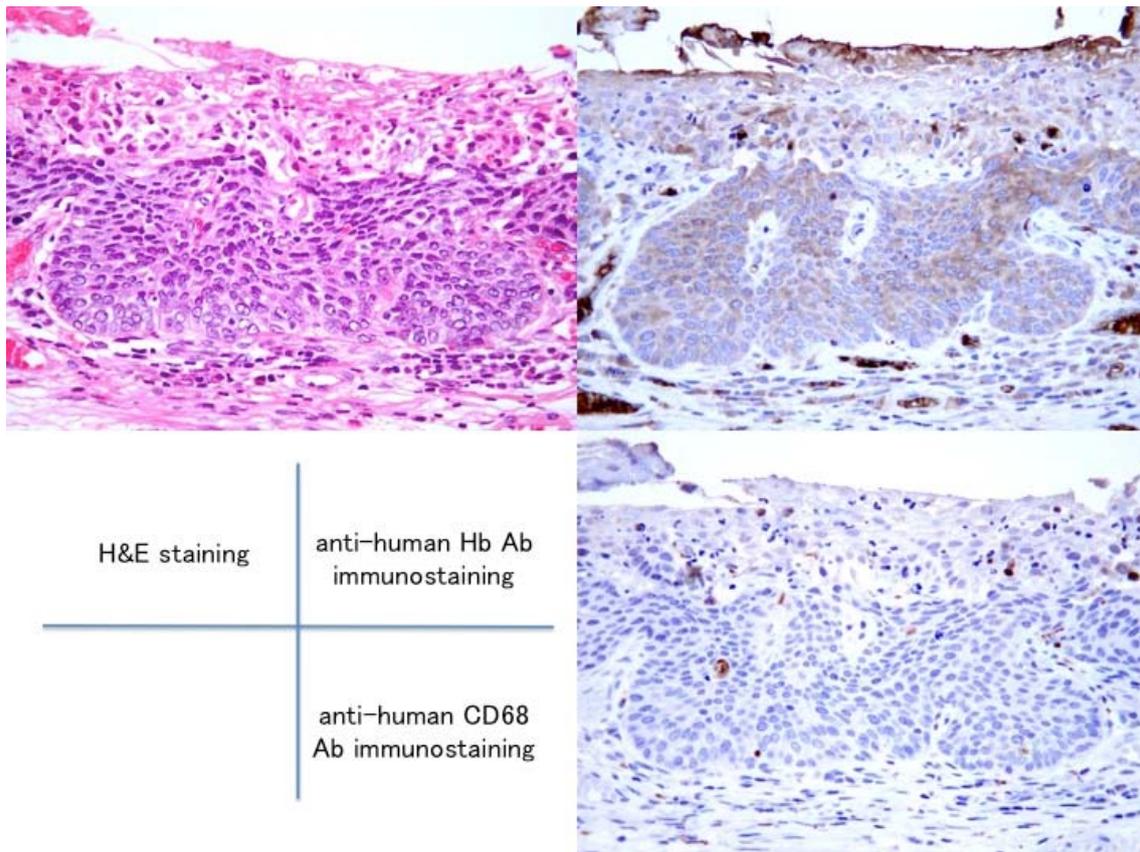


Figure 3. Immunohistological image using anti-human CD68 antibody. Immunostaining showed strong positivity consistent with cancer area, which was confirmed by conventional H&E staining. On the other hand, immunopositivity for anti-human CD68 antibody was only slightly seen in the surface area of the lesion.

Table 1. Correlation between BC positivity and pathology.

Overall accuracy in diagnosing malignancies (HGIN/SCC) from benign change was 90.1% (10+191/223). (Sensitivity; 191/219=91.0%, 10/13=Specificity; 77.0%, Positive predictive Value; 191/194=98.5%, Negative predictive value; 10/29=34.5%)

	n	Inf./LGIN	HGIN/SCC
BC(-)	29	10 (43.9%)	19(56.1%)
BC(+)	194	3 (2.5%)	191(97.5%)
total	223	13	210
