Comparison of Autofluorescence Imaging Endoscopic Findings with Pathologic Findings after Endoscopic Submucosal Dissection of Gastric Neoplasms

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Background/Aims: All epithelial cells emit autofluoresce, with tumor cells emitting weaker autofluorescence. We categorized patterns of autofluorescence imaging (AFI) and compared their clinical characteristics and pathology findings after endoscopic submucosal dissection. **Methods:** Twenty patients were enrolled, comprising 4 adenomas and 16 early gastric cancers. AFI findings were classified as follows: G0 (well-defined pink lesion on a green background with a clear interface over $\geq 50\%$ of its area), G1 (pink-green mottled lesion on a green background with a clear interface over $< 50\%$ of its area), P1 (pink-green mottled lesion on a purple background with a clear interface over $< 50\%$ of its area), and P2 (vague lesion on a purple background with a clear interface over $\leq 10\%$ of its area). **Results:** Most of the patients (80%) were male, and their median age was 62.4 years (range: 46-78 years). The lesion sizes by white-light mode, AFI mode, and pathology were 20.8 ± 13.1 , 22.8 ± 15.4 , and 20.0 ± 17.7 mm (mean $\pm$ SD), respectively. Sixteen cases of adenocarcinoma were classified as follows: G0 (n=10), G1 (n=2), P1 (n=2), and P2 (n=2). The G0 group has no p53 positive lesions, unlike the non-G0 group (p=0.044). All cases with the P1 and P2 patterns were of the gastric and intestinal types, respectively. **Conclusions:** AFI images of gastric tumors were categorized into four patterns that were useful for defining the resection margin in 87.5% of cases, with G0 being the most common pattern (62.5%). (*Gut and Liver* 2008;2:186-192)

Key Words: Autofluorescence imaging; Stomach neoplasms; Early gastric cancer; Endoscopic submucosal dissection

INTRODUCTION

Gastric cancer is the most common malignancy found with advancing age in Korean population. In Korea, endoscopic examination of the stomach is inexpensive and almost always included in health-checkups. Many endoscopic procedures to detect early gastric cancers (EGC) and to increase diagnostic accuracy have been designed and recently, new photodiagnostic techniques like autofluorescence and reflectance imaging (AFI) endoscopy have been developed.¹ A number of endogenous fluorophores have been identified, such as flavins (riboflavin, flavin mononucleotide and flavin dinucleotide), nicotinamide adenine dinucleotide, collagen, and pyridoxal 50-phosphate, which could be responsible for the observed differences in the autofluorescence spectra of normal and diseased tissues.² Porphyrin is concentrated in the mucosa and collagen and elastin are located primarily in the submucosa. Previous studies showed that the main source of normal tissue fluorescence is submucosal collagen fluorescing in the green range while gastrointestinal cancers have increased porphyrins fluorescing in the red range;^{3,4} the masking effect of the thickened mucosa overlaying the submucosal layer and the light-absorption effect by chromophores like hemoglobin are important factors in the reduced fluorescence of diseased tissues.^{3,5-7} The spectra of gastric adenoma⁵ and adenocarcinoma⁶ showed a lower total intensity, particularly in the green range ($\lambda = 480-570$ nm), compared to normal mucosa. The AFI endoscope was designed to catch this differential emission between the normal cells and tumor cells and to

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detect early stage of tumors. Normal epithelial cells appear as light green, which is a mixed pseudocolor of autofluorescence (green) and reflectance (red and blue) but the epithelial cells of adenoma and adenocarcinoma appear pink due to the decrement of autofluorescence.¹ Endoscopic examinations using autofluorescence aid in the detection of smaller and earlier malignant lesions such as EGC, which are not identified by routine white-light endoscopy (WLE).^{8,9} In theory, the AFI endoscope should outline the exact margins of the lesions, but in practice, it is not so reliable.^{2,8} Therefore, the authors investigated the autofluorescence patterns of adenoma and adenocarcinoma, categorized them, and compared their clinical characteristics and pathological findings after endoscopic submucosal dissection (ESD) in all groups.

MATERIALS AND METHODS

From February to April 2006, 20 patients including 4 adenoma and 16 EGCs were consecutively enrolled out of the 27 patients who had undergone AFI examination. 7 cases were excluded because ESD was not performed. All of the lesions were pathologically confirmed as adenoma or adenocarcinoma by biopsy and clinically staged as EGC by computerized tomography and endoscopic ultrasonography (EUS) before the procedures. Patients were referred to our digestive disease center to remove the lesions endoscopically.

The prototype videoendoscope for AFI (XGIF-Q240FZ; Olympus Medical Systems Co., Tokyo, Japan) was used. All tumor margins of all lesions were thoroughly exam-

ined with the AFI-white light (WL) mode and AFI-autofluorescence (AF) mode by switching before ESD. All procedures were performed by one experienced endoscopist (JY Cho) and the AFI examination and the ESD procedures were captured with still images and recorded on digital video tapes.

After ESD, all of the resected specimens were stretched on the corkboard, pinned flat diagonally, and immersed in formalin. After fixation, they were cut along the shortest tumor margin into 2-mm wide strips. An experienced pathologist (SY Jin) determined the differentiation, lesion size and depth, and atrophic grade from 0 to 3 of the histologic specimen stained with hematoxylin and eosin (H&E) without knowledge of the endoscopic appearance. Immunohistochemical stains were performed to inves-

Table 1. Definition of AFI Findings of the Gastric Tumors

Pattern	Our definition
G0	Well-defined pink lesion, green background; clear interface $\geq 50\%$
G1	Pink-green mottled lesion, green background; clear interface $< 50\%$
P1	Pink-green mottled lesion, purple background; clear interface $< 50\%$
P2	Vague lesion on purple background; clear interface $\leq 10\%$

G or P, green or purple background color, respectively; 0, 1, 2, clear interface between lesion and surroundings $\geq 50\%$, $< 50\%$, and $\leq 10\%$, respectively.

AFI, autofluorescence imaging.

Fig. 1. Four AFI patterns (G0, G1, P1, and P2) of gastric adenocarcinoma from white-light mode and AFI-autofluorescence mode of AFI endoscopy and their respective histologic mappings of the resected specimen. Orange indicates the cancer area. The patterns are taken from Cases 6, 13, 12, and 11 in Table 2, respectively. AFI, autofluorescence imaging.

tigate the mucin phenotype, p53, and the vascular endothelial growth factor (VEGF) status.

After analyzing the captured pictures and videotapes, various AFI findings were categorized into 4 groups by the following definitions (Table 1, Fig. 1). The G, P represents a green and purple background color, respectively. The number 0, 1, 2 denotes a clear interface between the lesion and its surroundings that is $\geq 50\%$, $< 50\%$, and $\leq 10\%$, respectively. The maximum size of each lesions was estimated by comparing the thickness of the AFI endoscope. Three endoscopists each with over two years of gastrointestinal fellowship classified each AFI finding according to the above definition.

For statistical analysis, statistical software (SPSS for windows; SPSS, Inc., Chicago, USA) was used to perform the chi square test for categorical variables, the t-test for age and size comparison, and the Kappa values for inter-observer agreement of AFI findings. A calculated p value of less than 0.05 was regarded as significant.

RESULTS

Baseline characteristics of the patients and tumors are presented in Table 2. A total of 20 patients were enrolled and 16 patients (80%) were male. Median age was 62.4 years (46-78); 16 patients (80%) had adenocarcinoma and 4 patients (20%) had adenoma. In the adenocarcinoma

group, mean age was older (64 ± 7.4 vs. 53 ± 6.6 years, $p=0.016$) and the size on WLE was larger (22.8 ± 13.9 vs. 12.5 ± 2.9 years, $p=0.013$) than that of the adenoma group.

Three experienced observers (A, B, and C) blinded to the patients' data participated in the evaluation of the AFI patterns. The kappa value between A and B, A and C, and B and C were 0.570, 0.504, and 0.538, respectively. The level of inter-observer agreement was high for our definitions used for classifying AFI findings.

AFI pattern analysis demonstrated 11 cases of G0, 5 cases of G1, 2 cases of P1, and 2 cases of P2 (Table 3). None of the variables including age, sex, size, gross shape, ulcer, depth on EUS, atrophy, location, cancer or adenoma, depth on pathological examination, p53, and VEGF different significantly among the AFI pattern groups. Subgroup analyses revealed the following results: 1) The number of tumors of the elevated type was not different among the AFI patterns ($p=0.10$); 2) There were significantly fewer gastric mucin phenotype tumors in the G0 pattern than in the P1 pattern ($p=0.015$); and 3) Adenocarcinoma tends to appear as the G0 pattern rather than as the G1 pattern ($p=0.063$).

To compare the most frequent and well-defined gastric neoplasms (G0) and the other neoplasms, we merged the pattern G1, P1, and P2 and renamed the result the non-G0 pattern (Table 4). The G0 pattern had 10 ad-

Table 2. Characteristics of the Patients with Gastric Adenocarcinomas and Adenomas

No	Age	Sex	AFI	Type	Ulcer	Location	Histology	Depth	Atrophy	IM	Mucin	p53	VEGF	Size1	Size2	Size3
1	59	M	G0	Ila	—	Antrum, GC	WD, LGA	M2	2	2	Mixed	—	—	20	20	10
2	70	M	G0	Ila	—	Angle, AW	WD, LGA	M2	3	3	Intestinal	—	—	10	10	10
3	67	M	G1	Ila	—	Antrum, PW	WD, HGA	M2	1	1	Mixed	—	—	20	20	3
4	47	M	G0	Ila	—	Angle, AW	WD, LGA	M2	1	1	Mixed	—	—	15	15	15
5	58	M	P2	Ila+c	—	Antrum, AW	WD, HGA	M2	1	2	Intestinal	+	—	5	5	5
6	64	M	G0	Ila+c	—	Antrum, GC	WD, LGA	M3	0	0	Intestinal	—	—	35	35	37
7	78	F	G0	Ilc	+	Antrum LC-PW	MD, HGA	M3	1	1	Mixed	—	—	50	70	72
8	63	M	P1	Ila	—	Mid body, PW	PD, HGA	SM	1	1	Gastric	+	+	30	30	32
9	62	M	G0	Ila	—	Antrum, LC	WD	M1	2	1	Mixed	—	—	10	10	8
10	69	M	G0	Ila	—	Cardia	CIS	M1	1	2	Intestinal	—	—	30	30	28
11	63	M	P2	Ilb	—	Low body, PW	WD, LGA	M3	3	3	Intestinal	—	—	30	30	30
12	61	M	P1	Ilc	—	Antrum, LC	MD, HGA	M3	1	2	Gastric	—	—	10	10	11
13	70	F	G1	Ilc	+	Antrum, GC	WD, HGA	M3	1	2	Mixed	+	—	15	20	17
14	63	M	G0	Ila	—	Angle	WD, LGA	M3	2	2	Mixed	—	—	50	50	52
15	76	M	G0	Ila+c	+	Antrum, LC	MD, HGA	SM	2	3	Null	—	—	25	25	22
16	60	M	G0	Ilb	—	Antrum, LC	WD	M1	1	1				10	20	3
17	53	M	G1	Ilc	—	Antrum, LC	LGD		2	1	Mixed	—	—	10	15	9
18	62	M	G1	Ila	—	Low body, GC	LGD		2	3	Gastric	—	—	10	10	6
19	46	F	G1	Ila	—	Antrum, GC	LGD		1	2	Intestinal	—	—	15	15	13
20	63	F	G0	Ila	—	Antrum, AW	HGD		2	2	Intestinal	—	—	15	15	16

VEGF, vascular endothelial growth factor; LC/GC, lesser/greater curvature; AW/PW, anterior/posterior wall; WD/MD/PD, well/moderate/poorly differentiated adenocarcinoma; LGA/HGA, low/high grade atypia; CIS, carcinoma in situ; LGD/HGD, low/high grade dysplasia; IM, intestinal metaplasia; Size1/2/3, on White-light endoscopy/Autofluorescence imaging/Pathology.

Table 3. Characteristics of the Patients and Gastric Neoplasms according to the AFI Patterns

Variables	G0 (n=11)	G1 (n=5)	P1 (n=2)	P2 (n=2)	p-value
Mean age	63±10 (46-78)	61±8 (53-70)	62±1 (61-63)	60±8 (58-63)	0.97
Sex (female/male)	2/9	2/3	0/2	0/2	0.58
Size on WLE	24.5±15.1	14.0±4.2	20.0±14.1	17.5±17.7	0.53
Size on AFI	27.3±18.5	16.0±4.2	20.0±14.1	17.5±17.7	0.57
Size on pathologic map	24.8±21.2	9.6±5.6	21.5±14.8	17.5±17.7	0.49
Shape (IIa, IIa+IIc/IIb, IIc)	10/1	3/2	1/1	1/1	0.10*
Ulcer (positive/negative)	2/9	1/4	0/2	0/2	0.44
EUS (mucosa/submucosa)	8/1	5/0	2/0	1/0	0.43
Atrophy grade (0&1/2&3)	4/7	3/2	2/0	1/1	0.27*
IM grade (0&1/2&3)	5/6	2/3	1/1	0/2	0.37*
Site (antrum/non-antrum)	7/4	4/1	1/1	1/1	0.73
Pathology (cancer/adenoma)	10/1[†]	2/3[†]	2/0	2/0	1.00
Depth (mucosa/submucosa)	10/1	5/0	1/1	2/0	0.72
Mucin (gastric/non-gastric)	0/10[‡]	1/4	2/0[‡]	0/2	0.11
p53 (positive/negative)	0/10	1/4	1/1	1/1	0.14
VEGF (positive/negative)	0/10	0/5	1/1	0/2	0.23

*Gross shape, atrophy, and IM was not different among AFI patterns (p=0.10, 0.27, 0.37).

[†]Adenocarcinoma tends to appeared as G0 pattern rather than as G1 pattern (p=0.063).

[‡]Non-gastric mucin phenotype was significantly higher in G0 pattern than P1 pattern (p=0.015).

WLE, white light endoscopy; AFI, autofluorescence imaging; EUS, endoscopic ultrasonography; VEGF, vascular endothelial growth factor.

Table 4. Comparison of Well-defined Tumors (G0) and Ill-defined Neoplasms (G1, P1, and P2)

Variables	G0 (n=11)	Non G0 (n=9)	p-value
Mean age	63.1±10.3 (46-78)	61.1±5.7 (53-70)	0.61
Sex (female/male)	2/9	2/7	1.00
Size on WLE	24.6±15.1	16.1±8.9	0.16
Size on AFI	27.3±18.5	17.2±8.7	0.16
Size on pathologic map	24.8±21.2	14.0±10.6	0.18
Shape (IIa, IIa+c/IIb, IIc)	10/1	5/4	0.13*
Ulcer (positive/negative)	2/9	1/8	1.00
EUS (mucosa/submucosa)	8/1	8/0	1.00
Atrophy grade (0&1/2&3)	4/7	6/3	0.37*
IM grade (0&1/2&3)	5/6	3/6	0.67*
Site (antrum/non-antrum)	7/4	6/3	1.00
Pathology (cancer/adenoma)	10/1	6/3	0.29[†]
Depth (mucosa/submucosa)	10/1	8/1	1.00
Mucin (gastric/non-gastric)	0/10	3/6	0.09[‡]
p53 (positive/negative)	0/10	3/6	0.09[§]
VEGF (positive/negative)	0/10	1/8	0.47

*Gross shape, atrophy, and IM was not different among AFI patterns (p=0.13, 0.37, 0.67).

[†]Adenocarcinomas and adenomas were not different between two groups (p=0.29).

[‡]Gastric mucin phenotype was lower in G0 pattern than in non-G0 pattern (p=0.09).

[§]p53 positive tumors were lower in G0 pattern than in non-G0 pattern (p=0.09).

EUS, endoscopic ultrasonography; VEGF, vascular endothelial growth factor; WLE, white light endoscopy; AFI, autofluorescence imaging.

enocarcinomas and 1 adenoma and the non-G0 pattern included 6 adenocarcinomas and 3 adenomas (p=0.285). The number of tumors of the elevated types (IIa, IIa+IIc) was higher in the G0 pattern than in the non-G0 pattern (p=0.127). The G0 pattern included less gastric mucin phenotype tumors (p=0.09) and less p53-positive tumors (p=0.09). Other variables did not differ significantly between the groups.

Among the 16 cases of adenocarcinomas, there were 10 cases of G0, 2 cases of G1, 2 cases of P1, and 2 cases of P2 (Table 5, Fig. 2). None of the variables including gross shape, atrophy grade, and intestinal metaplasia were significantly different among the AFI patterns except for p53 status (p=0.04). Subgroup analyses demonstrated the following results: 1) Elevated lesions tended to be more frequent in the G0 pattern than in the non-G0 pattern (p=0.118); 2) The G0 pattern had significantly fewer gastric mucin phenotype cancers than the P1 pattern (p=0.018); and 3) The G0 pattern included significantly fewer p53-positive tumors than the non-G0 group (p=0.04). Mucosal cancers were composed of 9 cases of G0, 2 cases of G1, 1 case of P1, and 2 cases of P2; 2 adenocarcinomas invaded to the submucosa which consisted of 1 case of G0 and 1 case of P1 (Case 15 and Case 8). Well-to-moderately differentiated adenocarcinomas showed 10 cases of G0, 2 cases of G1, 1 case of P1, and 2 cases of P2. One case of the poorly differentiated type had a P1 pattern and was located on the midbody with the IIa shape (Case 8).

Table 5. Characteristics of the Patients and Gastric Cancer according to AFI Patterns

Variables	G0 (n=10)	G1 (n=2)	P1 (n=2)	P2 (n=2)	p-value
Mean age	65±9 (47-78)	69±2 (67-70)	62±1 (61-63)	61±4 (47-78)	0.75
Sex (female/male)	1/9	1/1	0/2	0/2	0.74
Size on WLE	25.5±15.5	17.5±3.5	20.0±14.1	17.5±17.7	0.83
Size on AFI	28.5±19.0	20.0±0.0	20.0±14.1	17.5±17.7	0.79
Size on pathologic map	25.7±22.2	10.0±9.9	21.5±14.8	17.5±17.7	0.78
Shape (IIa, IIa+IIc/IIb, IIc)	9/1	1/1	1/1	1/1	0.12*
Ulcer (positive/negative)	2/8	1/1	0/2	0/2	0.48
EUS (mucosa/submucosa)	7/1	2/0	2/0	1/0	0.48
Atrophy grade (0&1/2&3)	4/6	2/0	2/0	1/1	0.31*
IM grade (0&1/2&3)	5/5	1/1	1/1	0/2	0.31*
Site (antrum/non-antrum)	6/4	2/0	1/1	1/1	0.82
Depth (mucosa/submucosa)	9/1	2/0	1/1	2/0	0.74[†]
Mucin (gastric/non-gastric)	0/9[‡]	0/2	2/0[‡]	0/2	0.11
p53 (positive/negative)	0/9	1/1	1/1	1/1	0.04[§]
VEGF (positive/negative)	0/9	0/2	1/1	0/2	0.28

*Gross shape, atrophy, and IM was not different among the AFI patterns ($p=0.12, 0.31, 0.31$).

[†]Depth of invasion was not different among the AFI patterns ($p=0.74$).

[‡]Gastric mucin phenotype was significantly lower in G0 pattern than in P1 pattern ($p=0.018$).

[§]p53 positive tumors were significantly different among four patterns ($p=0.04$); significantly lower in G0 pattern than in non-G0 pattern ($p=0.04$).

WLE, white light endoscopy; AFI, autofluorescence imaging; EUS, endoscopic ultrasonography; VEGF, vascular endothelial growth factor.

Fig. 2. Diagram of the four AFI patterns and their respective frequencies in gastric adenocarcinoma.

AFI, autofluorescence imaging.

Mean size of the lesion by WLE, AFI, and pathologic map was 20.8 ± 13.1 , 22.8 ± 15.4 , and 20.0 ± 17.7 mm respectively. AFI overestimated the lesion size as compared to the pathological size ($p=0.04$). All of the lesion sizes between WLE and AFI were similar except for in one case (Case 7); the lesions were located on the lesser curvature and posterior wall of the antrum by WLE but the margin was extended up to an angle according to AFI endoscopy (Fig. 3A). One cancer missed by AFI examination (Case 5) was due to diffuse inflammation and fibrosis around the lesion (Fig. 3B). The margin of another flat elevated adenoma (Case 18) was extended on AFI but was pathologically similar to the WLE finding.

DISCUSSION

When tissues are illuminated with short wavelength visible light (blue) or ultraviolet light, most of the light is reflected, absorbed, and scattered; a faint autofluorescence light of a longer wavelength is emitted.⁷ This tissue autofluorescence is known to be affected by the following factors: 1) the tissue architecture (mucosal thickening or loss of layered structures); 2) the light absorption and scattering properties of each layer, particularly hemoglobin in the capillary networks; 3) the distribution and concentration of fluorophores in the different layers; 4) the biochemical microenvironment of the tissue which may alter the fluorescence yield or spectral shape; and 5) the metabolic status of the tissue (e.g., NADH is only fluorescent in the reduced form).⁷

In our study, we categorized AFI findings of gastric tumors into 4 patterns and found that G0 was the most frequent in gastric tumors (55%) followed by a less clearly margined pattern (G1: 25%, P1: 10%) and a vaguely margined pattern (P2: 10%). Therefore, AFI was useful for deciding the resection margins for ESD in 90% of gastric tumors except for the 10% of cases with the P2 pattern.

AFI patterns (except P2) aided in determining the margin of EGC in 85.7% of mucosal cancers, 100% of submucosal cancers (1 case of G0 and 1 case of P1), 86.7% of well-to-moderately differentiated cancers, and 100% of poorly differentiated cancers (1 case of P1). Earlier stud-

Fig. 3. Interesting cases of AFI endoscopy. (A) A carcinoma appeared to be localized in lesser curvature and posterior wall side of antrum on WLE, but AFI extended the lesion up to the angle that was confirmed by the pathologic specimen (Case 7). (B) WLE and AFI did not distinguish small carcinoma due to the presence of fibrosis (Case 5). (C) The margin of a small and elevated adenoma was extended on AFI but was pathologically similar to the WLE finding (Case 18).

WLE, white light endoscopy; AFI, autofluorescence imaging.

ies using autofluorescence endoscopy (LIFE-GI) demonstrated that the detection rate of EGC increased significantly as the invasion became deeper, as the mucosal thickness exceeded 1.5 times that of the surrounding normal mucosa, and with a histologic differentiation of well to moderate.⁸ Detection rates were 57.5% for extension to the mucosa, 74.3% for extension to the submucosa, 82% for well-to-moderate differentiated cancer, and 61% for poorly differentiated cancer and signet ring cell cancer.⁸

Among the 16 gastric adenocarcinomas, 62.5% of the lesions showed a clear margin (G0) and the border was confirmed with the pathologic map; 25% were cancers with a less clear margin (G1, P1); and 12.5% showed a vague margin (P2). This data was similar to the previous AFI result; 62%, 12%, 21%, and 5% respectively.¹⁰ In 87.5% of the cases, the extension of cancer was correctly detected which was similar to the previous report (85%).² Pattern analysis on LIFE-GI revealed that 64% appeared as dark red, 30% had a mixed pattern of dark red and white fluorescence, and 6% could not be detected; the color images correlated well to the histologic type and to fibrosis within the cancer lesion.² Well differentiated gastric lesions were dark red on LIFE-GI but highly fibrotic and some non-differentiated lesions had a whitish appearance.¹¹ Mucin phenotype was notable that all P1 lesions (2) were the gastric type and all P2 lesions (2) were the intestinal type.

The G0 pattern included only differentiated EGCs with mucosal invasion with one exception (Case 15) and the

P1 pattern was composed of differentiated adenocarcinoma with mucosal invasion and focal poorly-differentiated adenocarcinoma with submucosal invasion (Case 8), while all tumors showing the G1 and P2 patterns were differentiated EGCs with mucosal invasion. This result is conflicts with the previous result where all cancers that appeared as dark red were differentiated adenocarcinoma and the cancers of mixed pattern consisted of undifferentiated adenocarcinoma with fibrosis (70%) and differentiated adenocarcinoma with submucosal invasion and/or fibrosis (30%).

A Japanese study divided the AFI patterns into 4 groups and explained them in association with pathologic findings without a clear definition of AFI patterns: 1) A green background was associated with atrophy and a clearly margined lesion was typical of differentiated type IIa cancers; 2) A mixed lesion on the green background represents differentiated type IIc cancer; 3) A mixed lesion on the purple background was undifferentiated cancer in the backgrounds of the fundic gland; and 4) A vague lesion was differentiated cancer in the backgrounds of the fundic gland or severe inflammation.¹⁰ In our study, we arbitrarily chose 50% and 10% for dividing each pattern for easy and objective classification in practice. While 90% of the G0 pattern were elevated type, only one of the two G1 pattern lesions was differentiated type IIc cancer. One of the two P1 pattern lesions was moderate-to-poorly differentiated submucosal cancer located in the mid-body and the other one was differentiated mucosal cancer located at the antrum just below the gastric angle. One of the two

P2 pattern lesions was differentiated mucosal cancer located in the lower body and the other one was differentiated mucosal cancer with submucosal fibrosis located at the antrum just below the gastric angle. A green background (G0 and G1) was not more significantly associated with moderate or severe atrophy than a pink background (P1 and P2) ($p=0.59$). Therefore, we found agreement with the Japanese study but not in atrophy grade. This difference may be partly explained by the small sample size and the presence of only one case of poorly differentiated cancer.

The size of lesions on AFI was significantly larger than the lesion size on the pathologic map in our study ($p=0.04$, especially in Cases 3 and 16). The former was associated with severe intestinal metaplasia and the latter with a recurrent EGC after ESD with a fibrotic background. Thus, overestimation by AFI may be related to the status of fibrosis and intestinal metaplasia.

In Case 7, the border of EGC was extended to an angle on AFI rather than localized to the antrum as shown on WLE; the extension was confirmed by histology after ESD. Tatsumi *et al.* detected well-differentiated flat mucosal cancer that was not evident on the WLE images during AFI examination for ESD follow-up.¹² Although it is not a common occurrence, AFI examination may be helpful for early detection of EGC and accurate targeting for ESD in selected cases.

In Case 5, AFI could not determine the accurate location of the lesion due to diffuse inflammation and fibrosis. This false negative finding was known to be associated with severe atrophy, increased amount of submucosal collagen (signet-ring cell cancer), the extremely low intensity of the red fluorescence of the tumor tissue,³ overlying mucosal thickness, and hemoglobin content.⁵ Therefore, AFI endoscopy and WLE maybe complementary tools to each others.

At the authors' center, the mucin phenotype, p53, and VEGF status are always checked after ESD with permission for follow-up and compared it with AFI pattern. Although no G0 pattern included p53-positive lesions, there were only 3 p53-positive lesions in non-G0 pattern.

In conclusion, AFI images of gastric tumors can be classified into 4 patterns and were useful for defining the resection margin for ESD in 87.5% of the cases of EGC. G0 was the most common pattern (62.5%). All of the G0 pattern lesions demonstrated negative p53 and all of the P1 pattern lesions (2) showed gastric mucin type; all of the P2 pattern lesions were intestinal mucin type. These result indicate that more large-scale, controlled, and prospective studies are needed to investigate the photodiagnostic characteristics of gastric cancers. Although AFI is convenient

for the endoscopist because it highlights subtle lesions without magnifying or dye spraying, the endoscopist should be aware of its limitations and regard it as an adjunct techniques for diagnosis like chromoendoscopy.

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