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ORIGINAL ARTICLE

Diagnostic value of autofluorescence imaging combined with narrow band imaging in intraepithelial neoplasia of Barrett's esophagus

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Summary

Purpose: *To evaluate the diagnostic values of Auto Fluorescence Imaging (AFI) combined with Narrow Band Imaging (NBI) in the diagnosis of the intraepithelial neoplasia of Barrett's esophagus (BE).*

Methods: *Seventy four suspicious BE intraepithelial lesions were assessed in 50 patients by AFI, who were further subjected to NBI mode to observe the changes of gastric mucosal capillaries and gastric pits. The corresponding lesions were biopsied for pathological examination.*

Results: *Among the 74 AFI-diagnosed cases of suspicious lesions, 44 (59.5%) were high-grade intraepithelial neoplasias (BE HGIN), while the remaining 30 cases (40.5%) were false-positive. The NBI-diagnostic results of these 44 BE HGIN lesions were as follows: 39 cases were confirmed and 5 were suspicious; among the 30 false-positive BE HGIN cases, NBI gave 7 false-positive cases. The false-positive rates decreased from 40.5% of AFI to 9.5% (7/74) of NBI-AFI ($p<0.05$). The positive predictive value of AFI in BE HGIN was 59.5% (44/74), while that of AFI-NBI combination was 84.8% (39/46; $p<0.05$).*

Conclusions: *The AFI-NBI combination technology could significantly improve ($p<0.05$) the detection rate of BE HGIN.*

Key words: *auto fluorescence imaging, Barrett's esophagus, high-grade intraepithelial neoplasia, narrow band imaging*

Introduction

In Barrett's esophagus (BE) the normal columnar epithelium is gradually replaced by squamous epithelium in the lower segment of esophagus [1], and this could be accompanied with or without intestinal metaplasia. If BE is accompanied with dysplasia, the risk of developing esophageal adenocarcinoma could be high, thus this condition is regarded as a precancerous lesion [2,3]. Conventional electronic endoscopy mainly relies on naked-eye observations under white light, and can find lesions with significant changes in shape or color (e.g. protruded lumps, depressed ulcer, etc.), while it is difficult to diagnose tiny and flat early cancerization and dysplasia. In recent years, with the applications of new optical technologies in the field of endoscopy, the targets of endoscopic observation have been gradually refined. The electronic-staining endoscope includes the AFI and NBI, which could not only overcome the time-consuming problem of conventional staining endoscopy, but also avoid the potential risks of chemical dyeing [4], and could help dis-

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tinguish the normal gastrointestinal mucosa from the lesion tissues, thus improving the detection rate of intraepithelial neoplasia and early cancer [5-8], thus helping a lot to get a successful optical biopsy [9].

In this study, the diagnostic value of AFI-NBI combination in BE intraepithelial neoplasia was investigated, and the results are reported below.

Methods

Patients

Fifty pathologically-confirmed BE patients from the Department of Digestion, Shanghai Pudong Hospital and the Affiliated Xinhua Hospital of Shanghai Jiatong University, from July 2012 to December 2013 were enrolled in this study. The inclusion criteria were as follows: 1) Patients with gastroscopic biopsy for suspicious BE and being followed-up thereafter; 2) positive pathological confirmation, while conventional endoscopy was negative or suspicious; 3) patients subjected to endoscopic BE treatment (argon plasma coagulation) and followed up. These 50 patients were subjected to AFI, and the 74 BE lesions found to be suspicious for intraepithelial neoplasia were subjected to further AFI-NBI combined examination with Olympus GIF FQ260Z electronic endoscope (Olympus EVIS LUCERA CV-260SL and CLV-260SL, Japan). Of these 50 patients, 22 were males and 28 females, aged 29-78 years (mean 41-42).

This study was conducted in accordance with the declaration of Helsinki and after approval from the Ethics Committee of Shanghai Pudong Hospital. Written informed consent was obtained from all participants.

Device and specific operation

This study used the electronic endoscope Olympus GIF FQ260Z, which could smoothly switch the 3 modes (WLI, AFI and ME -NBI) of detection simply through a button on the handle. The AFI system uses blue light at a wavelength of 395-475 nm, as well as green and red light at a wavelength of 540-560 nm and 600-620 nm as the excitation source, and a filter, which allows the light to pass only within a wavelength range of 400-625 nm, was installed in front of the charge-coupled device (CCD) fluorescence endoscope, thus the blue light is cutoff and blocked. The CCD collects the generated autofluorescence and reflects the green and red light to construct the fluorescence images. NBI imaging system uses the narrow band interference blue/green filter, which produces a 30 nm narrow band blue-green light, with the center wavelength as 415 nm and 540 nm as the light source for the lighting. It can clearly show the submucosal microvascular morphology.

The endoscopic observation was performed by one endoscopic doctor. After the patient was positioned under the scope, the endoscope passed through the esophagus and stomach, and entered the descending duodenum, followed by slowly moving back for observation. The profiles of gastroesophageal junction and squamocolumnar junction were carefully examined. Conventional white light imaging (WLI) was firstly performed. The suspicious lesions were rinsed with saline, and then the AFI was conducted to observe the color changes of suspicious lesions. The observation plane depth was adjusted to make the image clear. The AFI images were acquired and stored for the fluorescence diagnosis of suspicious lesions. Then, NBI with zooming function was performed to observe the shape and structure of gastric mucosal microvessels and pits. The mainly blue narrow band light could clearly show the mucosal surface and shapes of mucosal glandular opening. Furthermore, the optical property of haemoglobin could absorb the blue light strongly [10]. The diagnosis was made according to the form and structure changes of the gastric pit and mucosal capillaries. The diagnosis of BE intraepithelial neoplasia was divided as highly suspicious, suspicious and negative. After rinsing the coloring agent, biopsy of the mucosa in the examined sites was performed in NBI amplification mode, and then the histopathological examination was carried out by the same pathologist who was blinded to the results of endoscopic diagnosis.

Classification of gastric pits

According to the classification criteria of Sakaki et al. [11], the gastric pits were divided into the following 6 basic types: A type: dot-like; B type: short rod-like, the pits are deep, might have branches and bending, although significantly less than C type; C type: the pits are prolonged, tortuous, with significantly increased bending and branches, which might be connected to each other and dendritic; D type: plaque-like, grid-like, with cobblestone-like changes; E type: the pits appear as villous-like and finger-like protrusions, and intestinal villi-like changes; F type: disordered or no structure [12].

Observation indicators

The esophageal mucosal pathological changes were graded as inflammation, intestinal metaplasia, low-grade intraepithelial neoplasia (LGIN) and high-grade intraepithelial neoplasia (HGIN). Lesions with overlap of histopathologic diagnosis were incorporated into high-grade pathological change. According to the results of pathological diagnosis, the positive predictive value (PPV) and false-positive rate of AFI and AFI-NBI for BE HGIN were determined.

Statistics

Statistical analyses were performed using SPSS 13.0 statistical software (SPSS Inc., Chicago, III, USA). Comparisons were carried out using Student's t-test and chi-square test. Two groups of independent sam-

Table 1. Diagnosis of 74 AFI-suspicious BE HGIN cases by AFI-NBI

Pathology	*Total cases N (%)*	*Highly suspicious N (%)*	*Suspicious N (%)*	*Negative N (%)*
HGIN positive	44 (100)	39 (52.7)	5 (6.76)	0
HGIN negative	30 (100)	7 (9.46)	0	23 (31.08)

BE: Barett's esophagus, HGIN: high grade intraepithelial neoplasia

Table 2. Diagnosis of 74 BE-suspicious HGIN lesions by NBI

Pathology	*Cases*	*Highly suspicious N (%)*	*Suspicious N (%)*	*Negative N (%)*
Inflammation	7	0	0	7 (9.46)
Intestinal metaplasia	18	4 (5.45)	0	14 (18.91)
LGIN	5	3 (4.05)	0	2 (2.70)
HGIN	44	39 (52.7)	5 (6.76)	0

BE: Barett's esophagus, LGIN: low grade intraepithelial neoplasia, HGIN: high grade intraepithelial neoplasia

ples were analyzed using the Wilcoxon rank sum test, and multiple groups of samples were analyzed using Kruskal-Wallis test. P<0.05 was considered as statistically significant.

Results

General information

According to the inclusion criteria, 74 lesions found in 50 patients were observed with AFI and magnifying endoscopy (ME) -NBI mode, and 44 BE HGIN cases were confirmed by pathological examination, while 30 cases were characterized as false positive. The false positive lesions included 7 cases of inflammation, 18 cases of intestinal metaplasia, and 5 cases of low-grade intraepithelial neoplasia (LGIN). The NBI diagnosis for the 44 BE HGIN cases was: 39 cases were positive, and 5 cases were suspicious; among the 30 false-positive BE HGIN cases, 7 cases were proved to be false positive by NBI (Tables 1 and 2).

Positive predictive value and false positive rate of AFI in the BE HGIN diagnosis

Among the 74 AFI-diagnosed suspicious lesions, 44 were diagnosed as BE HGIN, while 30 lesions were false positive. The false positive rate of AFI in BE HGIN was 40.5% (30/74), and the positive predictive value was 59.5% (44/74; p<0.05).

Positive predictive value and false positive rate of AFI-NBI in the BE HGIN diagnosis

The diagnosis of AFI-NBI in these 44 BE HGIN lesions was that 39 cases were positive and 5 were suspicious. Among the 30 false positive BE HGIN lesions, the false positive diagnosis by NBI was 7 cases. The positive predictive value of AFI-NBI was 84.8% (39/46), which was much higher than that of AFI alone in BE HGIN (59.5%) (p<0.05). The 24 AFI-suspected BE HGIN lesions clearly exhibited capillary structures and regular gastric pits' structures in NBI, which indicated they were not BE HGIN changes (Figure 1). Therefore, the false positive rate dropped from 40.5% (30/74) to 14.9% (7/74) (p<0.05). AFI-NBI diagnosed 7 cases as false positive, among which 3 cases were LGIN, and 4 cases were intestinal metaplasia.

Fluorescence endoscopy

Under AFI mode, the normal gastric mucosa showed green fluorescence, while mucosa with erosion, inflammation and benign ulcer was light purple, the one with bleeding dots was dark red, that with malignant lesions was dark red or deep red, the white fur that covered the ulcer surface was still white, with clear distinction from the surrounding normal mucosa. The mucosal blood vessels also exhibited vascular network with clear boundaries in the AFI mode (Figure 1).

AFI-NBI combination diagnosis

The AFI-NBI combination could diagnose HGIN much more accurately. Under white light, some lesions only showed anabrotic and rough surface and it was difficult to determine whether they were benign or malignant. In the AFI mode, the lesions showed abnormal red changes that could improve the detection rate of biopsy. In the NBI mode, the lesions' outlines were clearly visi-

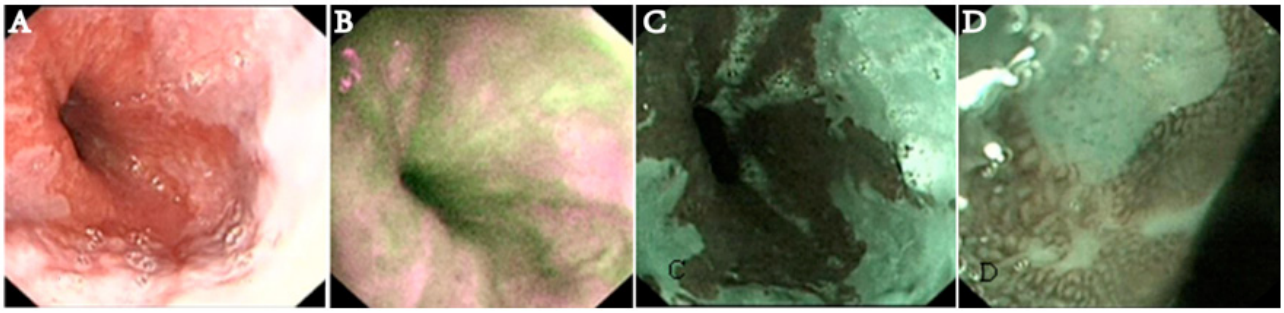

Figure 1. Images of suspicious BE HGIN under WLI, AFI, NBI and ME -NBI. **A:** No obvious suspicious lesions under WLI; **B:** AFI shows existence of suspicious HGIN; **C**, **D:** NBI exhibits regular shapes of mucosal vessels and gastric pits in which pathology confirmed that there were no BE HGIN. For abbreviations see text.

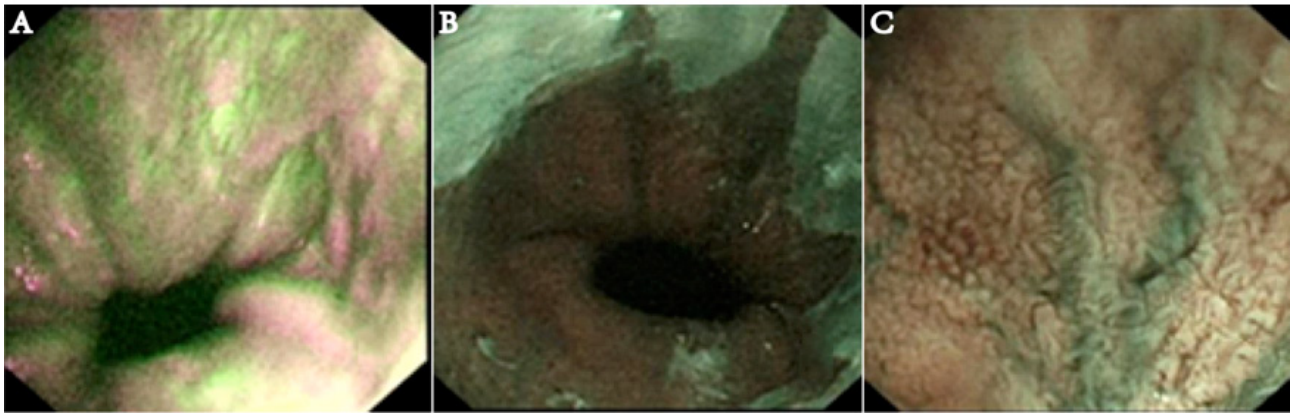

Figure 2. Images of suspicious BE HGIN under AFI, NBI and ME-NBI. **A:** AFI shows existence of suspicious BE HGIN; **B**, **C:** NBI exhibits disordered mucosal vessels and irregular gastric pits' shapes in which pathology confirmed the existence of BE HGIN. For abbreviations see text.

ble, with depression on the surface, irregular gastric pit structures were obvious when amplified, the microvascular network was disordered, thus the total picture was highly suspected of being BE HGIN (Figure 2). The relative pathological examination confirmed the existence of BE HGIN, thus it could be said that the AFI-NBI combination could improve the detection rate of BE HGIN and the diagnostic accuracy of benign and malignant lesions.

Relationship of gastric pits' basic forms and histology

The 18 cases with intestinal metaplasia appeared mainly as C, D and E type under the endoscope, among which 66.7% were of the D-type; 5 cases of LGIN showed mild depression, bumps or flat lesions, associated with disordered or disappeared gastric pits, among which 80% were of E-type. The important feature of BE HGIN was the pleomorphism of gastric pits, mainly as F-type, and the shapes of small pits were various, irregular and dendritic (Table 3).

Discussion

When WLI reveals that the lesions display roughness of gastric mucosa, erosion, plaques, abnormal color, slight ample or depression, the AFI mode should be performed for the observation. When the excitated light shines the submucosa, the generated strong fluorescence would become reduced and weakened after meeting the abnormal lesions (e.g. abnormal aggregation or mucosal thickening of superficial vessels), so these subtle changes would be converted into color information, making such subtle differences more sharp between normal mucosa and lesions [13,14]. After recording the diagnostic results of fluorescence endoscopy, the diagnostic procedure was switched

Table 3. Relationship of gastric pit basic forms and histology

Group	*Cases* *N*	*A* *N (%)*	*B* *N (%)*	*C* *N (%)*	*D* *N (%)*	*E* *N (%)*	*F* *N (%)*
Gastritis	7	5 (6.8)	2 (2.7)	0	0	0	0
Intestinal metaplasia	18	0	0	5 (6.8)	12 (16.2)	1 (1.4)	0
LGIN	5	0	0	0	1 (1.4)	4 (5.4)	1 (1.4)
HGIN	44	0	0	0	5 (6.8)	12 (16.2)	27 (36.5)

A type: dot-like; B type: short red-like, the pits are deep, might have branches and bending, although significantly less than C type; C type: the pits are prolonged, tortuous, with significantly increased bending and branches which might be connected to each other and dendritic; D type: plaque-like, grid-like, with cobblestone-like changes; E type: pits appear as villous-like and finger-like protrusions, and intestinal villi-like changes; F type: disordered or no structure

to ME-NBI mode because this modality could significantly improve the contrast of intrapapillary capillary loop and subcutaneous capillaries, and focus mainly on the microglandular and fine capillary morphologies on the gastric mucosal surface, thus it could guide more precisely to getting biopsy [15], as well as to improve the positive rate of the lesions [15].

In recent years, an increasing number of authors have studied the value of fluorescence endoscopy in the BE diagnosis. Kara et al. [16] applied fluorescence endoscopy and conventional endoscopy to inspect 60 BE patients, and detected 20 cases of early cancer or severe dysplasia, among which 6 cases were detected by fluorescence endoscopy alone, and 14 cases were found with obvious abnormalities by conventional endoscopy and fluorescence endoscopy. This study found that the positive predictive value of AFI-NBI combination was 84.8% (39/46), much higher than that of AFI alone (59.5%) in the diagnosis of BE HGIN. In parallel, the false positive rate decreased from 40.5 to 14.9%, similar to another Kara's et al. study [17], which combined AFI and NBI to find the BE dysplasia and early cancerous lesions. Among the 19 false positive lesions, 14 exhibited basically normal mucosal pit patterns under NBI, so the false positive rate was decreased from 40 to 10%. This was similar to the Curvers et al. study [18].

The diagnostic value of AFI-NBI combination in precancerous lesions and early gastric cancer was higher compared to simple fluorescence endoscopy, because the current fluorescence endoscope still lacks considerable sensitivity in autofluorescence generated by the tissues, and the image quality still needs to be improved. Furthermore, it is often affected by some confounding factors, such as mucosal thickening edema etc [19]. Our study found that, under the AFI mode, the normal mucosa was green, while the inflammation, erosion, depression and ulcers exhibited purple or dark red changes. Yet, during the research, some parts were found as suspicious red lesions, which exhibited no obvious changes of gastric pits and microvessels under the NBI mode, a finding consistent with the Singh et al. study [20]. NBI could increase the sensitivity and specificity, while a meta analysis came to the conclusion that NBI could accurately diagnose BE accompanied with atypical hyperplasia [21]. And this might be related to: (1) the necrotic tissues, mucus and bile attached to the stomach, thus interfering with the autofluorescence information; and (2) possible shaded areas caused by geometric factors when constructing the autofluorescence images. These shaded areas might be likely to be misdiagnosed as abnormal mucosa. A study reported that because of the difference between the molecular structures of normal tissues and tumor tissues, the characteristics of their fluorescence spectrum were easy to be distinguished, so the combination of fluorescence endoscopy and fluorescence spectroscopy could significantly improve the diagnostic reliability and sensitivity of fluorescence endoscopy [18]. The autofluorescence characteristics represented the biochemical structure of tissues, and the changes of tissues' biochemical structure was the main cause of the changes of the autofluorescence features. The characteristics of the emission spectrum of tumor tissues and normal tissues could thus be exploited to distinguish the malignant tumors.

This study found that the AFI-NBI combination technology could identify BE HGIN, thus improving the diagnostic reliability and sensitivity over AFI. By observing the morphological changes of gastric pits and microvessels, ME-NBI could better distinguish the small lesions [18]. This research studied 5 cases of LGIN, which all appeared as mild depressions, bumps or flat lesions, accompanied with disordered or disappeared gastric pits' structures, and 80% of them were of E type. The main characteristic of BE HGIN was the pleomorphism of gastric pits, exhibited as F type,

along with the irregular and dendritic shapes.

In summary, the advantages of AFI-NBI combination-guided biopsy were as follows: the combination could reduce the numbers of biopsies. The objectivity was good and not restricted by the doctor's experience. The sensitivity and specificity in BE HGIN diagnosis were high, thus increasing significantly the positive rate of biopsies. In addition, this most relevant information was obtained from a small-sample clinical study. The reliability and clinical value of this technology still needs further large-scale randomized controlled studies for verification of the present results.

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